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ASSESSMENT OF MALARIA PARASITEMIA AND GENOTYPE RELATIONSHIP OF PATIENTS ATTENDING TERTIARY HEALTH CARE CENTRE IN KATSINA STATE, NIGERIA

[1]Habiba, Z., [2]Abdullahi, K., [1]and [3*]Aminu, A.

[1]Department of Biology; Umaru Musa Yar'adua University Katsina, Katsina state Nigeria
[2]Department of Biological Sciences Federal University Gusau, Zamfara state Nigeria
[3]Department of Biotechnology Modibbo Adama University Yola, Adamawa State, Nigeria

[*]Email: *aminuabdubawa71@gmail.com*

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ABSTRACT

Malaria parasitemia and genotype of patients attending Tertiary Medical Center, in Katsina State was assessed. A total of 400 samples were collected from consented patients through venipuncture techniques. The blood samples were processed within 3 to 6 hours of collection. Using blood samples Infection status and genotype were screened using standard techniques. Their demographic characteristics were determined using questionnaires. Chi-square test was used to determine the degree of relationship between malarial parasite infection and genotype. Out of 400 consented patients examined, 193 patients (48.3%) were affected by malaria at varying degree of parasitemia. According to the gender, the prevalence of malaria was found to be (48.3%) among female respondents compared to their male counterparts with 48.1%($P>0.05$). The results further showed that the subjects with normal genotypes are significantly more infected compared to those with S- gene. The occurrence of malarial infection among the participants with normal (AA) genotype is comparatively higher (53.8%) compared to the carrier (AS) participants with 41.7% and those with sickle cell disease (SS) having the least with 33.3%. Conclusively people with AS and SS genotype have more genetic advantage toward resistance to Malarial parasite infection. However, more scientific reasons require further elucidation.

Keywords: Malaria, *Plasmodium falciparum*, Parasitemia, Genotype, Gender.

Background

Human malaria is a mosquito borne infectious disease caused by parasitic protozoa belonging to the genus *Plasmodium*. Malaria is a life-threatening disease that are transmitted to people through the bites of infected female Anopheles mosquitoes (Auta *et al.,* 2016). In the African continent, where *Plasmodium falciparum* is the most prevalent human malaria parasite species, anaemia is responsible for about half of the malaria-related deaths (Juan *et al.,* 2011). *Plasmodium falciparum* is the most virulent form of human malaria and kills at least one million children annually (Ibekwe *et al.,* 2009). Malaria occurs mostly in poor tropical and subtropical areas of the world. In many of the countries affected by malaria, it is a leading cause of illness and death. In areas with high transmission rates, the most vulnerable groups are young children, who have not developed immunity to malaria yet, and pregnant women, whose immunity has been decreased by pregnancy (Runka *et al*, 2016). Malaria is still a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world (WHO, 2015). About 97% of Nigerian population are at risk of malarial infection with the remaining 3% of the population living in the malaria free highlands (WHO, 2015). There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. This compares with 215,000 deaths per year from HIV/AIDS in Nigeria (RBM Fact Sheet Report, 2016).

Sickle cell anaemia is an inherited blood cell disorder; characterized with episode of chronic anaemia and pain. In the disorder, haemoglobin molecules in the red blood cells which carry oxygen throughout the body are defective. Those defective molecules cause some of the red blood cells to form a sickle shape. The abnormal shaped blood cells are the source of anemia and pain. Sickle cell anemia is common among people whose ancestors come from sub–Saharan Africa and other parts of Spanish speaking regions of the world (Aidoo *et al*., 2002). Presence of sickle cells in a person is believed to provide resistance to malaria parasite for people living in areas which are endemic. This is clear from the fact that in the African lowlands, where malaria is endemic, the incidence of individuals with sickle cells is high, as compared to the African highlands (Aidoo *et al.*, 2002). Abu-Zeid *et al.,* (2002) reported that sickle trait (Hb^{AS}) provides a survival advantage over people with normal hemoglobin (Hb^{AA}) as well as people with sickle cell disease (Hb^{SS}) in regions where malaria is endemic. Sickle cell trait provides neither absolute protection nor invulnerability to the disease. Rather, people (particularly children) infected with *Plasmodium falciparum* are more likely to survive the acute illness if they have sickle cell trait (Abu-Zeid *et al*., 2002). Therefore, the people with sickle cell trait are more likely to reach reproductive age and pass their genes on to the next generation (Williams *et al.*, 2005).

This research was aimed at investigating the intensity of malarial parasite infection and Genotype among patient in the study area with hope data generated to assist while designing malaria controlprograms.

Materials and Methods

Study Area

The study area is Katsina metropolis. Katsina is a major town and a Local Government Area in Northern Nigeria, and it is the capital of Katsina state. Katsina main town is located between latitude 12°59’ North of the equator and longitude

07°36' East of Greenwich meridian. As a local government area LGA, Katsina shares boundaries with Kaita local government to the North, Jibia local government to the Northwest, Batagarawa local government to the South and Rimi local government to the Southeast. Katsina LGA covers an area or landmass of 143 km^2(Muhammad, 2011).

Study Population

The population of Katsina Local Government as at 2015 is 394 800 people (NPC 2015). The predominant tribes are the Hausa and Fulani (n=296100,75%), with internal migrants comprising mainly the Igbos, Yoruba and Nupes (n= 59115, 15%), and external migrants from Niger Republic (n=39410, 10%) (Muhammad, 2011).

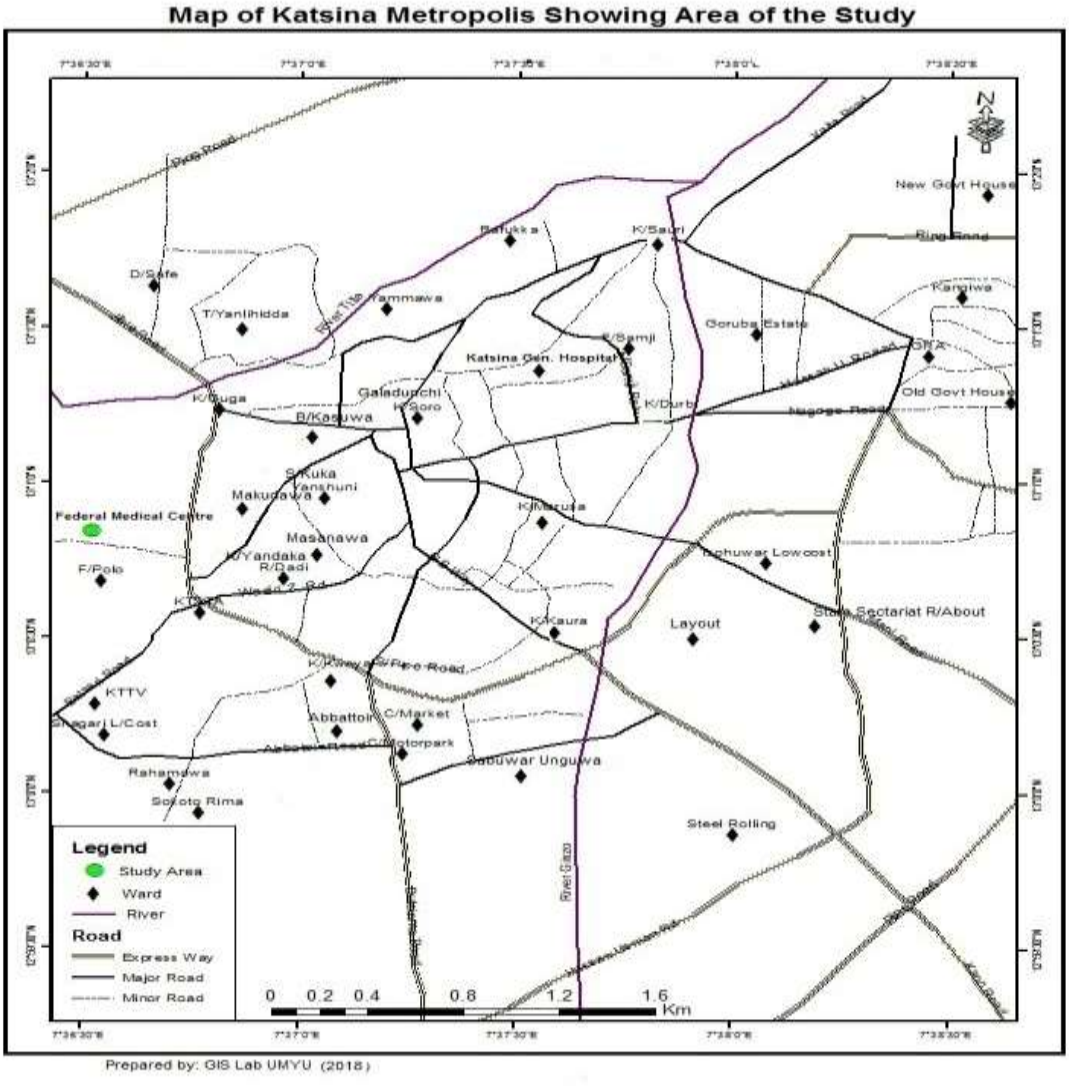


Figure1: Map of Katsina metropolis showing the study area (Source: GIS Lab UMYU, 2018)

Sample Size

The sample size for this study was calculated in accordance with WHO guidelines (Lwanga and Lemeshow, 1991). The formula used for the calculation of the sample size is as follows:

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

Where:

- n = sample size
- Z = Z statistic for a level of confidence (95% confidence interval = 1.96) was used for this study.
- P = expected prevalence or proportion. The value of **"P"** used for this study was derived from another research (Auta *et al.*, 2016) which showed 35% prevalence (0.35).
- d = precision or Margin of Error = 5% or 0.05.

Based on the formula, a total of 400 respondents were required for this survey.

Ethical Approval

Prior to the commencement of the research, an application letter was tendered by the Department of Biology, UMYU to the Federal Medical Center Katsina requesting for ethical approval. The ethical approval was given for the commencement of the research. Informed consent was sought from each patient (respondent) before sample collection. For the under aged (0-5 years), permission was sought and gotten for them from their parents before the collection of samples and data.

Blood Sample Collection

Using sterile needle and syringes, two millimeters (2 ml) of venous blood was collected from the subjects with the assistance of the Laboratory technologists (Simon-Oke *et al*., 2016). Each blood sample collected was immediately dispensed into ethylene- diamine-tetra-acetic acid (EDTA) labeled appropriately (Simon-Oke *et al*., 2016). Relevant data including age and gender were obtained from the respondents at the end of collection to aid the epidemiological studies.

Thick Blood Film

Thick blood film was prepared by using a standard procedure described by (Cheesbrough2009). To standardize the amount of blood used for each sample, a plastic bulb pipette was used for collecting and for placing a blood drop at a center of completely cleaned grease-free microscope slide. A small drop 5mm of blood was added at the center of the slide and a larger drop about 15mm was added to the right. The drop was then spread evenly over a specified area of the slide. The prepared slide film was then allowed to air-dry with the slide kept in a horizontal position. (Simon-Oke *et al*., 2016).

Thin Blood film

Using the same procedure described above (section 3.4.2) thin blood films was prepared by placing a drop of blood on the end of a clean dry slide (Simon-Oke *et al*., 2016). A clean smooth edged of the spreader was used to draw back the drop of blood and allow the blood to extend along the edge of the spreader. (Simon-Oke *et al*., 2016) By holding the spreader at an angle of about 30^o, the drop of blood spreads to make a film about 40–50 mm in length (two thirds of the slide) (Cheesbrough, 2009). The film was immediately air dried by waving the slide back and front and then fixed for few minutes in absolute methanol. (Cheesbrough, 2009).

Staining of Thick and Thin Blood Films

Following fixation using absolute methanol the blood films was stained using Field Stain as described by Cheesbrough (2009).

Microscopic Examination, Parasite Identification and Report

A drop of immersion oil was placed to an area of the film, and the prepared slides were each mounted on microscope stage. The films were focused first at a lower magnification ×100objective lens. Malaria parasites and malaria pigment with the aid

of standard malaria parasite flowchart, box texts, and colour plates (Cheesbrough, 2009). Thin blood films were used to confirm the *Plasmodium* species. The malaria scoring was based on the following

1. 1–10 Parasites per 100 high power fields (Scanty)……..+
2. 11–100 Parasites per 100 high power fields (Moderate)…….++
3. 1–10 in every high power field (High)……+++
4. More than 10 in every high power field (Very high) …….++++

Blood Genotyping

Cellulose acetate paper electrophoresis technique was employed for Haemoglobin Genotyping of the participants as described by Lugosand polit (2018). Each sample was diluted using haemolysate to obtain haemoglobin concentration of approximately 10g/100ml. Using applicator, the haemolysed blood sample were placed on the cellulose acetate paper. Electrophoresis in Tris buffer solution was run for 15-20 minutes at 350v. Haemolysed blood samples of known Hb^{AS} and AC were used as control.

Statistical Analysis

Numerical data obtained from the research were analyzed using GraphPad version 5.0 for windows. The data collected for this research are mostly numerical in nature hence there is no need for transformation of the data. Using chi-squire test relationship between malaria and genotype among patients attending FMC Katsina were analysed at 95 % level of significance.P-values that are found to be less than 0.05 ($P<0.05$) were considered significant at 95% confidence limit.

Results

Distribution of Malaria Parasitemia among Respondents

The occurrence of malaria parasitemia among 193 infected individuals varies with very high parasitemia (++++) having highest percentage of occurrence (51.8%). The high parasitemia (+++) was recorded in 23.3% of the infected individuals while moderate parasitemia (++) was recorded in 17.1% of the infected respondents. Lastly, scanty parasitemia (+) was observed in only 7.8% of the infected participants. The detailed result is presented on Figure 1 below.

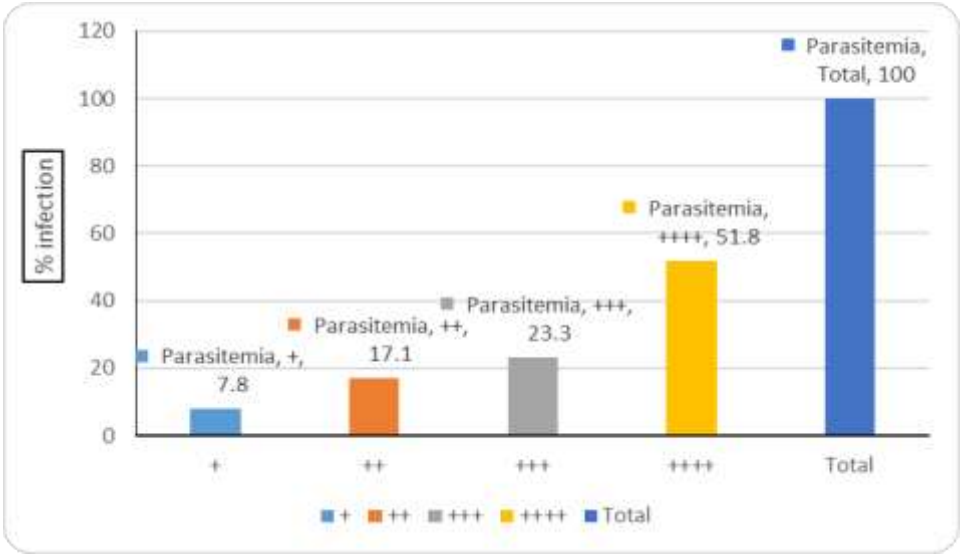


Figure 1: Malaria Parasetemia among infected patients

Table 1: Overall Prevalence of Malaria and Gender

Gender	No of Examined patients (%)	No. Positive (%)	No. Negative (%)	P-Value
Male	158 (39.5)	76 (48.1)	82 (51.9)	0.9616
Female	242 (60.5)	117 (48.3)	125 (51.7)	
Total	400 (100.0)	193 (48.3)	207 (51.7)	

$\chi 2 = 0.0023$, df = 1, ($P>0.05$)

Prevalence of malaria in relation to gender

From the total of 400 patients examined, 193 patients (48.3%) were affected by malaria at varying degree of parasetemia. The prevalence of malaria infection was found to be similar in females (48.3%) and their male counterparts with 48.1% (Table 1).According to the gender, the result of our findings did not reveal any statistical association between malaria infection and gender (P>0.05).

Prevalence of Malaria by Age Group

The result did not show any statistically significant difference among the age groups (P>0.05). Patients within the age group of >40 years were found to have the highest prevalence of infection by malaria parasites (57.9%). Similarly, patients within the age group of 18-40 years had the least prevalence of 41.8%.

Table 2: Prevalence of Malaria Infection by Age Group

Age Group (Years)	No of Examined patients (%)	No. Positive (%)	No. Negative (%)	P-value
< 1	9 (2.3)	5 (55.6)	4 (44.4)	0.1595
1-5	61 (15.2)	33 (54.1)	28 (45.9)	
6-17	96 (24.0)	51 (53.1)	45 (46.9)	
18-40	196 (49.0)	82 (41.8)	114 (58.2)	
>40	38 (9.5)	22 (57.9)	16 (42.1)	
Total	**400 (100.0)**	**193 (48.3)**	**207 (51.7)**	

$\chi 2 = 6.586$, df = 4, (P> 0.05)

Prevalence of malaria by genotype

Result on prevalence of malarial parasite infection by genotype as depicted on Table 1; showed that the subjects with normal genotypes are significantly more infected compared to those with S- gene. The occurrence of malarial infection among the participants with normal (AA) genotype is comparatively higher (53.8%) compared to the carrier (AS) participants with 41.7% and those with sickle cell disease (SS) having 33.3%. The prevalence of malarial infection by genotype among the participants in this survey, revealed a significant association between genotype and infection (P<0.05). In other words, the results showed that malarial prevalence is lower in AS and much lower in SS compared to AA. The percentage variation between AA and AS is higher compared to that between AS and SS.

Table 3: Prevalence of Malaria by Genotype of Respondent

Genotype	No of Examined patients	No. Positive (%)	No. Negative (%)	P- value
AA	247	133 (53.8)	114 (46.2)	0.0112*
AS	108	45 (41.7)	63 (58.3)	
SS	45	15 (33.3)	30 (66.7)	
Total	400	193 (48.3)	207 (51.7)	

$\chi 2$= 8.983, df =2, (P< 0.05)

Discussion

This research was conducted to study the relationship between malarial parasite and demographic factors among some population of patients attending Federal Medical Center Katsina State. The overall occurrence of malaria parasitemia among 193 infected individuals varies with highest parasitemia (+++) recorded in 23.3% of the infected individuals while moderate parasitemia (++) was recorded in 17.1% of the infected respondents. Lastly, scanty parasitemia (+) was observed in only 7.8% of the infected participants respectively. The findings of the research based on gender revealed that male and female had similar prevalence of malarial infection. This finding contradicts with that of Otajevwo, (2013) who reported (m=45.5%, f= 54.5%) more female are infected than male. However, more prevalence in female may probably be due to their lifestyle and early morning household responsibilities which may exposed them to more mosquitoes bites and consequently to malaria infection than male.

Moreover, regarding malarial parasite infection and genotype of the participant findings revealed that; malaria was prevalent in all genotypes, but the prevalence differs among the different haemoglobin genotypes with Hb^{AA} (53.8%) having a slightly higher prevalence of malaria infection, followed by the genotype Hb^{AS} (41.7%) and the least prevalence was recorded Hb^{SS} (33.3%) genotype respectively the variation in the malarial parasite infection observed among the genotypes was in agreement with findings of Rowe *et al.,* (1997) which shows that malaria infection spreads across all the genotype with varying degrees. WHO reported that the prevalence of malaria infection was less among subjects with HbA^{SS} and Hb^{AA} than among those of Hb^{AA}.

The mechanism by which Hb^{AS} protects against malaria has been the subject of speculation for several years (Williams *et al.,* 2003). The findings of this research regarding the relationships between malaria infection and Hb^{AS} as well as Hb^{SS} are in-line with most recent research findings. The findings of the research showed that the protective mechanism against *Plasmodium falciparum* by Hb^{AS} carriers is highly significant when compared with Hb^{AA} and increased in $Hb^{SS.}$ respectively. According to Haematol and Hebbel (2003), establishing relationship between malarial parasite, Hb and genotype is difficult because immunity to malaria is hard to measure. To date, no single immune response has been described that reliably predicts protective immunity. As a result, immunity to malaria is usually defined as the ability to control new infections to a level at which they fail to reach a clinical threshold (Polley.*et al.,* 2004). Most of the cohort studies out whether Hb^{AS} carriers and Hb^{SS} sickles involves a significant immune response most of the findings reported to date, involve repeated cross-sectional sampling, so how might sickle genes result in the accelerated acquisition of malaria-specific immunity. A number of mechanisms have been proposed; In common with other red cell genetic defects, enhanced phagocytosis of Hb^{AS} erythrocytes infected with ring stage *P. falciparum* has been demonstrated in vitro, Haematol and Hebbel (2003), a process that appears to be mediated by a mechanism essentially similar to that involved in the phagocytosis of senescent or damaged normal erythrocytes. Experimental data suggest that this process is initiated by enhanced oxidant damage to the erythrocyte membrane and that this leads to the aggregation of band 3 protein and the binding of autologous IgG and complement (Haematol and Hebbel, 2003), a mechanism

similar to that previously proposed for thalassemia (Ayi *et al.,* 2004). It therefore seems plausible that enhanced immunity could be mediated by the accelerated acquisition of antibodies to alter host's antigens expressed on the parasite infected red cell surface, such as band 3 protein (Ayi *et al.,* 2004).

On the other hand, parasite-derived proteins such as the variant surface antigen *P. falciparum* erythrocyte membrane protein-1 might represent an alternative target, a hypothesis supported by the raised titers of antibodies directed towards variant antigens seen in HbAS children. The resistance of carriers of the sickle cell gene to malaria can be linked directly to a single abnormal protein. Under in-vitro conditions in which this variant hemoglobin behaves much like the normal variant; there is no inhibition of parasite growth and multiplication. Under deoxygenating conditions, which affect directly and profoundly the physical nature of HbSS, the parasites are severely injured (Fairhurst *et al*., 2005). At the same time, cyanate, an agent that functions exclusively to limit the abnormal functioning of this variant, protects, within those limits, the parasites from destruction. Thus, it can be concluded that the reversible physical state of HbSS mediates the resistance observed in vitro and that a sole erythrocytic mechanism is sufficient for resistance in-vivo. While other mechanisms involving no erythrocytes cells might in fact operate, they do not appear to be necessary (Fairhurst *et al*., 2005). Morphological evidence allows one to hypothesize that two mechanisms of killing operate in vitro (Fairhurst *et al*., 2005).

The complete disappearance of parasites from cultures of SS erythrocytes indicates a lytic process. This might originate from physical disruption of the parasite membranes by paracrystal line, HbS needles and the subsequent liberation of digestive enzymes. Deoxygenated SS red cells have been shown to be densely packed with these Hb^{SS} aggregates according to Fairhurst *et al.,* (2005), leaving little room for intracellular parasites. Mechanical disruption, however, is not believed to be the subsequent liberation of digestive enzymes. Deoxygenated SS red cells have been shown to be densely packed with these H^{bS} aggregates, leaving little room for intracellular parasites (Fairhurst., *et al.,* 2005).

The finding from the study showed that malaria prevalence has weaker relationship with demographic factors and that AS and SS individuals benefit a genetic advantage over AA individuals in relation to Malarial parasite infection. Environmental hygiene should be encouraged in order to reduce the breeding chances of female Anopheline mosquito which is the potential vector of the malarial parasite.

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