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A Probe into Protective Association of Haemoglobin Genotypes with Malaria Parasitaemia among Students of a University in Western Delta, Nigeria

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Authors' contributions

Every aspect of this work carried out by author TOE was closely supervised by author FDO in the University laboratory. This also included the write up.

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ABSTRACT

Aim: This work was designed to investigate any possible protective association of haemoglobin genotypes with malaria parasitaemia among students of a private University in Western Delta.
Study Design: Whole blood samples were obtained from a cross section of students by simple random sampling method. Collected blood samples were dispensed into ethylene-diamine-tetra-acetic acid (EDTA) containers which were appropriately labelled. Collected blood samples were tested for haemoglobin genotypes and malaria parasites by standard methods. Data obtained were statistically analysed.
Place and Duration of Study: The study was carried out in the Microbiology and Biotechnology laboratory of Western Delta University, Oghara, Nigeria between May, 2013 to October, 2013.
Methods: Venous blood (2ml volume) was obtained by venipuncture from 360 symptomatic and

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asymptomatic students made of 150(41.7%) males and 210(58.3%) females with a mean age of 23.5±8.5 years. Malaria parasite screening was done by both *Plasmodium falciparum* antigen rapid (Micropoint, USA) test and Giemsa staining. Haemoglobin genotyping was done by a modified cellulose acetate membrane electrophoresis (CAME) method.

Results: A total of 225(70.8%) students were infected with trophozoites and other forms of *P. falciparum* parasites of which 53.3% and 44.7% were infected females and males respectively. There was significant association between sex and malaria infection rates ($P<0.05$). Two hundred and forty (66.7%), 99(27.5%) and 21(5.8%) students were of HbAA, HbAS, and HbSS genotypes. Sixty five (18.1%) HbAA male and 99(27.5%) HbAA female students were infected with malaria parasites. Thirty nine (10.8%) and 21(5.8%) male HbAS and female HbAS students respectively were infected with malaria parasites. Eight (2.2%) and 7(1.9%) male HbSS and female HbSS students respectively were infected. Malaria infection rates among types were HbAA (45.6%), HbAS (16.7%) and HbSS (4.2%). Age groups 21-25 and 15-20 had the highest malaria infection rates and population of HbAA, HbAS and HbSS students while 41-45 group had the least. Chi square analysis showed that there was no significant association between male and female HbAA, HbAS, HbSS and malaria infection rates (HbAA $X^2_{0.05,10=}$ 7.786, HbAS $X^2_{0.05,\ 10}$= 8.421, HbSS $X^2_{0.05,\ 10}$= 4.1, P-value $_{0.05,\ 10}$ =18.307, Hence $P>0.05$).

Conclusion: Findings in this work are suggestive that Oghara town may be hyper endemic for malaria and this information may be useful to the University Health department as well as the relevant Delta State Health agencies for the necessary antimalarial therapy and prophylactic measures. Findings also indicate a high prevalence of haemoglobin genotype variants particularly heterozygous HbAS. This information can help in the formulation of genetic counseling policies for prospective couples in order to assist them make informed decisions before marriage. This will ultimately reduce the sickling gene pool amongst the study population and beyond. Results of this study also imply that there is no protection against malaria by any of the implicated haemoglobin genotypes.

Keywords: Malaria; parasitaemia; association; haemoglobin genotypes; university students; Nigeria.

1. INTRODUCTION

The enhanced immune activity in both HbC and HbS carriers supports the hypothesis that the protection against malaria of these adaptive genotypes might be at least, partially mediated by acquired immunity against malaria [1]

Epidemiological and clinical studies have indicated that malaria susceptibility and severity are influenced by haemoglobin genotype with haemoglobin (Hb) AS individuals having a selective advantage in malarial environments [2]. Thus, the high frequency of HbAS in human population has been attributed to the decreased malarial morbidity and mortality experienced by HbAS heterozygotes [3,4]. Nevertheless, the mechanism of this resistance remains the subject of considerable debate. While it probably involves innate factors such as the reduced ability of *Plasmodium falciparum* parasites to grow and multiply in HbAS erythrocytes, recent observations suggest that it might also involve the accelerated acquisition of malaria specific immunity.

The mechanism by which HbAS protects against malaria has been the subject of speculation for more than 50 years [5]. While to some extent, it probably relates to the physical characteristics of HbAS erythrocytes, a number of studies suggest that HbAS may also enhance the acquisition of natural immunity [6]. It has been observed that sickle trait provides a survival advantage over people with normal haemoglobin in regions where malaria is endemic [7]. It has also been reported that children who are carriers of haemoglobin (S) have ninety percent protection against severe malaria [8]. Salimonu [9] also reported that patients who are heterozygous for the sickle gene (HbAS) are usually more resistant to *Plasmodium falciparum* infection than those who are homozygous (HbSS, HbAA). It has however been suggested that the protective role is against severe disease and not against infection rate [10].

The allele that causes sickle cell anaemia imparts resistance to malaria infection. However, individuals with HbSS gene are not protected from malaria [11,12]. Malaria parasites infect the red blood cells of those with two normal alleles leading to the bursting of the infected cells, but the red blood cells of individuals with one sickle allele are relatively resistant to malaria and do not normally get sickle cell anaemia [13]. The

absence of malaria infection in haemoglobin SS may be because it interferes with the growth and multiplication of malaria parasites [14]. Homozygous (HbSS) red cells produce membrane associated hemin which oxidizes membrane lipid proteins and probably produce little of such products [15].

Malaria has been shown to be consistently higher in individuals with HbAA genotype compared to those with HbAS [3]. The greater susceptibility of HbAA individuals to *P. falciparum* malaria and the enhanced severity of an attack in this group may be due to low red cell membrane resistance to the invading parasites and a non-hypoxic environment within the red cell which enhances its development [3,16]. It has been reported that sickle cell trait produces higher levels of the superoxide (0_2) and hydrogen peroxide (H_2O_2) which are toxic to a number of pathogens including malaria parasites [17]. Haemoglobin AA individuals are very susceptible to malaria because the red cells are conducive to the growth and development of the parasite [18].

Akinboye et al. [19] in their study, reported that males were more infected with malaria parasites than females and the mean parasite load in males was significantly higher than that in female subjects ($P< 0.05$) and also that HbAA patients were more infected than HbAS patients. In a similar study, Akhigbe et al. [20] reported that subjects with sickle cell disease (HbSS) showed the highest prevalence of both malaria parasitaemia and severe malaria than subjects having sickle cell trait (HbAS, HbAC).

Another related study carried out by Nkuo-Akenji et al. [10] stated that malaria prevalence was lower (57.5%) in HbAS individuals compared with HbAA (74.6%) and HbSS (60.0%). Segeja*et al*. [21] conducted a cross sectional survey to determine the prevalence of HbS in a community based in Tanzania to verify its association with protection against malaria. Malaria parasite prevalence among 415 blood samples was 17.2% in the highlands and 39.6% in the lowlands.

Aidoo et al. [22] reported that HbAS provides significant protection against all-cause mortality, severe malarial anaemia and high density parasitaemia. In a similar study, Otajevwo [23] reported that HbAA children were infected at a rate of 71.4%, HbAS children at 10.0% and HbSS at 2.2% with malaria while Tidi et al. [14] reported malaria infection rates of 38.9%, 6.2% and 0.0% for HbAA, HbAS and HbSS respectively. It is clear from the foregoing therefore that the association of any haemoglobin genotype variant with malaria infection has been somewhat contentious. This work was an attempt therefore, to widen the scope of knowledge in this regard and was aimed at studying the association of immune protection of haemoglobin genotypes with malaria parasitaemia among students of a private University based in Western Delta, Nigeria. The objectives are to determine:

(a) Malaria parasitaemia association with haemoglobin genotypes with respect to age and sex.
(b) The influence of haemoglobin genotypes on malaria infection rates.

2. MATERIALS AND METHODS

2.1 Ethical Clearance

Informed consent was given by students recruited for the study. A consent form (in form of a questionnaire) was given to students (who were all adults) to fill and sign. Participating students also gave their verbal informed consent by presenting themselves for blood sample collection. The University where study was carried out has not instituted an ethical committee. The University was established less than a decade ago. There was no standing ethical committee instituted at the time research was carried out and hence ethical clearance was not officially obtained but consent form was completed by students who also gave their verbal informed consent.

2.2 Sampling

Two millilitres (2ml) of venous blood was collected from 360 randomly selected Western Delta University, Nigeria students made of 150(41.7%) males and 210(58.3%) females from various departments of the University. Blood samples were collected with the aid of 5ml sterile needles and syringes by venipuncture (after tyinga tourniquet around the upper arm and sterilizing it with 70% ethanol to increase blood pressure in the veins) and dispensed into ethylene diamine tetra acetic acid (EDTA) anticoagulated blood containers. Blood containers were rotated by standard method to properly mix blood and anticoagulant and collected samples were labeled appropriately.

Both symptomatic and asymptomatic subjects were used for the study. Symptomatic subjects were those whose malaria parasite tests were positive and showed clinical signs/symptoms of illness. Asymptomatic subjects were those who were apparently healthy although positive for malaria parasite test. For clarity, symptomatic subjects were grouped as having severe malaria parasitaemia with high level of parasite densities while asymptomatic subjects were grouped as having non-severe malaria parasitaemiawith low level of parasite densities.

2.3 Malaria Parasite Detection Tests

The malaria *Plasmodium falciparum* rapid Test Device (whole blood) Micropoint package insert kit by Cook et al. [24] was used to test for presence of specific antigens (proteins) produced by falciparum malaria parasites [25,26]. Antigen targeted is *P. falciparum* specific histidine rich protein-2 (ie PF HRP-2) and aldolase [25,27].

2.4 Rapid (Micropoint, USA) Test Procedure

The test device, specimens, buffer and/ or controls were allowed to equilibrate to 15-30℃ (room temperature) prior to testing. The test device cassette was removed from the foil pouch and used immediately. Using the sample loop (or rubber pipette) provided, 5µl (0.05ml) of whole blood was dropped into sample well (A). Two drops (60µl or 0.06ml) of assay buffer were put in well (B) and the set up was left to stand for 20 minutes after which the result was read. A positive result was one in which a pink or purple coloured control (C) band appeared in addition to a distinct pink coloured band in the test (P.F) region. In a negative result, only one pink or purple band appeared on the test (P.f) region. The quality control region was to confirm if sufficient specimen volume was used and if the correct procedural technique was applied.The specificity and expected values of this rapid test method are well defined by the manufacturers of the kit.

2.5 Giemsa Staining

This manual staining was carried out as quality control of the rapid test method. Thick blood films were made on grease free microscope slides (after appropriate labeling) and allowed to air dry on laboratory working bench. Slides were arranged on a staining rack and flooded with 10% (V/V) Giemsa stain solution for 15minutes [28]. The plus system was used for the determination of parasite density [29]. Only blood samples that showed positive results with the rapid test device and Giemsa staining method were recorded and used for haemoglobin genotyping.

2.6 Determination of Haemoglobin Genotypes

The haemoglobin genotypes were determined by a modified cellulose acetate membrane electrophoresis (CAME) method as described by Awah and Uzoegwu [30]. Haemoglobin lysates were prepared and were spotted in triplicates at a distance of 0.5cm of one end of well dried cellulose acetate strips. Reference HbAS haemolysate was spotted as control. The spots were allowed to dry and the strips were placed in an electrophoresis tank to form a bridge between the anode and cathode of the tank in such a way that haemoglobin bands migrate towards the anode. Both compartments of the tank contained the same volume or quantity of buffer (TRIS-EDTA-Borate buffer, pH 8.9). A constant voltage of 220V was applied by use of a functional stabilizer to achieve excellent separation within 5-10 minutes. After separation, the haemoglobin bands were visualized against the control. HbA migrated fastest followed by HbS and HbC respectively [31].

2.7 Statistical Analysis of Data

Chi square (X^2) analysis using test of independence of two characters or associations using a 4 X 3 contingency table at 95% confidence interval was used. The software used was the statistical package for social sciences (SPSS) version 17.0. Confidence interval at 95% (0.05) was calculated using mean $\pm$ $t_{0.05(6)}$ SE where 6 refers to degree of freedom calculated using (r-1) (c-1).

3. RESULTS

In Table 1**,** the sex, department and age group distribution of students recruited for the study are shown. Students of fifteen (15) departments who gave their informed consent and volunteered their blood samples are shown. Seventy two (20.0%), 66(18.3%), 54(15.0%), 33(9.2%), 30(8.3%), 30(8.3%), 18(5.0%), 18(5.0%), 12(3.3%), 6(1.7%), 6(1.7%), 6(1.7%), 3(0.8%), 3(0.8%) and 3(0.8%) volunteering students of Microbiology/Biotechnology, Accounting, Geology, Economics, Biochemistry, Political

Science, Mass Communication, Business Administration, Computer Science, Chemistry, Physics, Library Science, Philosophy, Hotel/Tourism and Environmental Science departments respectively engaged in this study are shown in that descending order. Out of the 360 sampled students, 150(41.7%) and 210(58.3%) were males and females respectively. Table 1 also shows the age brackets of students used for the study. In decreasing order, 198(55.0%), 111(30.8%), 39(10.8%), 6(1.7%), 3(0.8%) and 3(0.8%) number of students occurred in 21-25 (average age 23 years), 15-20(average age 19years), 26-30(average age 28 years), 31-35(average age 33years), 36-40(average age 40 years) and 41-45(average age 44years) respectively. Again, out of the total recruited in each age group, 90(45.5%), 33(29.7%), 15(38.5%), 6(100.0%), 3(100.0%) and 3(100.0%) represented male students in 21-25, 15-20, 26-30, 31-35, 36-40, and 41-45 age brackets respectively in that descending order while 108(54.5%), 78(70.3%), 24(61.5%),0(0.0%), 0(0.0%) and 0(0.0%) represented female students in the same age groups respectively.

Table 2 shows the sex (gender) distribution of malaria parasitaemia among students engaged in the study. Out of the 360 students recruited, 150(41.7%) and 210(58.3%) were males and females respectively. Out of the same total sample size, 255(70.8%) students were infected (positive) with *P. falciparum parasites* in their peripheral blood circulation of which 114(44.7%) and 141(55.3%) were infected male and female students respectively. Table 2 also shows that 105(29.2%) students were negative for malaria parasites in their blood system of which 36(34.3%) and 69(65.7%) were uninfected male and female students respectively. Malaria parasitaemia was significantly associated with female gender and therefore with sex (X^2 p-value= 3.841, calculated X^2 value= 3.958, P< 0.05).

In Table 3, the data on age and sex association of haemoglobin genotypes on malaria infection rates are shown. Out of 360 students sampled, 240(66.7%), 99(27.5%) and 21(5.8%) were of AA, AS and SS haemoglobin genotypes respectively. Of the 240 AA subjects, 89(37.1%) and 151(62.9%) were males and females respectively. Sixty five (73.0%) AA male students were infected with trophozoites (ring forms) and schizonts of *Plasmodium falciparum* and 99(65.6%) female students were infected with the same malaria parasites. Twenty four (27.0%) and 48(34.4%) AA male and female students respectively were not infected with malaria parasites. Chi square statistical analysis done showed a degree of freedom of 10 from (r-1) (c-1) contingency table, $X^2_{0.05,10}$ = P value = 18.307, calculated X^2= 7.641 for male students and hence, P>0.05. As for the female students, calculated X^2= 4.048 and hence P>0.05. This suggested that there was no significant association between haemoglobin genotype AA and malaria infection rates in both male and female students.

Of the 99(27.5%) AS subjects, 54(54.6%) and 45(45.4%) were male and female students respectively of which 39(72.2%) and 21(46.7%) males and females respectively were infected with ring and other forms of *Plasmodium falciparum*. Fifteen (27.8%) and 24(53.3%) males and females respectively were not infected with the parasites. The results of chi square analysis showed P- value= 18.307, calculated X^2=7.273 and hence P>0.05 for the male AS students. The calculated X^2 for the female students is 7.273 and therefore P>0.05 (Table 3).

Twenty one (5.8%) students had SS genotype and of this, 10(47.6%) and 11(52.4%) were males and females respectively. In this group, 8(80.0%) and 7(63.6%) male and female students respectively were infected with malaria parasites of *P. falciparum.* Only 2(20.0%) and 4(36.4%) males and females respectively tested negative to the parasites. Chi square analysis results showed calculated X^2=3.037 and therefore, P>0.05. In terms of age groups, 55.0%, 30.8%, 10.8%, 1.7%, 0.9%, and 0.9% of students belonged to 21-25, 15-20, 26-30, 31-35, 36-40 and 41-45 age brackets respectively. Age group 21-25 had the highest population of AA, AS and SS students while 41-45 had the least. Also, the highest infection rate occurred in 15-20 and 21-25 age groups while the least infection rate occurred in the 41-45 age group.

In the 15-20 age group, 15(13.5%) male students were in the AA genotype group and more than half (8.1%) of them were infected. In the same age group, there were 18(16.2%) AS male students all of whom (16.2%) were infected with malaria parasites. More than half (1.8%) of the 3(2.7%) SS genotype students in this same age group were infected. In the female AA, AS and SS students in this group, the infection rate was much higher. Out of 54.1% AA recorded, 40.5% were infected, out of 10.8% AS, 8.1% were infected and 3.6% were infected out of 5.4% SS students.

Table 1. Sex and age distribution of Western Delta University recruited for the study

Department	**Sex**	**15-20 yrs average=19 N=111(30.8%)**	**21-25 yrs average=23yrs N=198(55.0%)**	**26-30 yrs average=28 N=39(10.8%)**	**31-35 yrs average=33 N=06(1.7%)**	**36-40 yrs average=40 N=03(0.8%)**	**41-45 yrs average=44 N=03(0.8%)**
Geology	M=36	06	27	00	03	00	00
N=54(15.0%)	F=18	12	03	03	00	00	00
Microbiology	M=21	12	09	00	00	00	00
N=72(20.0%)	F=51	09	33	09	00	00	00
Accounting	M=24	09	12	03	00	00	00
N=66(18.3%)	F=42	15	24	03	00	00	00
Mass comm.	M=03	00	00	00	00	03	00
N=18(5.0%)	F=15	00	06	00	00	00	00
Computer.Sci	M=06	00	03	03	00	00	00
N=12(3.3%)	F=06	03	03	00	00	00	00
Bus.Admin	M=06	00	03	03	00	00	00
N=18(5.0%)	F=12	00	12	00	00	00	00
Biochemistry	M=03	00	03	00	00	00	00
N =30(8.3%)	F=27	21	06	00	00	00	00
Economics	M=18	03	15	00	00	00	00
N=33(9.2%)	F=15	03	09	03	00	00	00
Pol. Science	M=21	03	12	03	00	00	03
N=30(8.3%)	F=09	06	03	00	00	00	00
Env. Science	M=03	00	03	00	00	00	00
N=03(0.8%)	F=00	00	00	00	00	00	00
Chemistry	M=03	00	03	00	00	00	00
N=06(1.7%)	F=03	00	03	00	00	00	00
Physics	M=03	00	00	03	00	00	00
N=06(1.7%)	F=03	00	00	03	00	00	00
Hotel&tourism	M=00	00	00	00	00	00	00
N=03(0.8%)	F=03	00	03	00	00	00	00
Philosophy	M=03	00	00	00	03	00	00
N=03(0.8%)	F=00	00	00	00	00	00	00
Library sci.	M=00	00	00	00	00	00	00
N=06(1.7%)	F=06	00	03	03	00	00	00
Total	M=150(41.7%)	33(29.7%)	90(45.5%)	15(38.5%)	06(100.0%)	03(100.0%)	03(100.0%)
	F=210(58.3%)	78(70.3%)	108(54.5%)	24(61.5%)	0(0.0%)	0(0.0%)	0(0.0%)
	360	**111(30.8%)**	**198 (55.0%)**	**39(10.8%)**	**06(1.7%)**	**03(0.8%)**	**03(0.8%)**

Table 2. Sex distribution of malaria parasitaemia among students recruited for the study

Sex	Positive malaria parasite test (%)	Negative malaria parasite test (%)	Total (%)
Males	114(44.7)	36(34.3)	150(41.7)
Females	141(55.3)	69(65.7)	210(58.3)
Total	**255(70.8)**	**105(29.2)**	**360(100.0%)**

X^2df (1) $_{0.05(6)}$ = 3.841 (P-value); Calculated X^2 =3.958; P < 0.05

Similarly in the 21-25 age bracket, 24.2% of the male AA (28.8%) students were infected. Less than half (6.1%) of the male AS students (13.6%) in this age bracket were infected while all the male SS students were infected. In the females, higher infection rates were recorded. Out of 36.9% AA students, 23.2% were infected, out of 2.5% SS students, 1.5% were infected except for AS female students for which 4.6% infection rate out of 13.6% was recorded. In the 26-30 age group, apart from 7.7% male AS individuals that recorded 7.7% infection rate, less than half (5.1%) AA male students (28.2%) were infected with malaria parasites. There was only one male SS student who was negative in this age group. The female students recorded higher infection rates in this group except for SS genotype for which no single female student was recorded. In the 31-35 age group, all the students were males and 50% each belonged to AA and AS genotypes with none belonging to SS group. All the males in AA and AS types were infected. There were no females in this age group. Only 3 male students were in the 36-40 age bracket and all were AA type and were all infected. Three male students were of the 41-45 group and all were AS and infected with malaria parasites (Table 3). Chi square statistical analyses done on data showed that for male AA/MP association, calculated X^2 = 7.641, critical (P value) $X^2_{0.05}$, df 10 = 18.307 and hence P>0.05. For female AA/MP association, calculated X^2= 4.048 and therefore, P>0.05. Calculated X^2 for AS/MP association as it relates to male and female students were 7.273 and 7.273 respectively and hence P>0.05. Lastly, SS/MP association showed that calculated X^2 for both male and female students were 2.428 and 3.037 respectively and therefore, P>0.05.

The data on the influence of Hb-genotype on malaria infection rates are shown in (Table 4). Out of 240(66.7%) AA subjects, 164(68.3%) were infected and out of 99(27.5%) AS samples processed, 60(60.6%) were infected with malaria parasites. Twenty one (5.8%) SS genotype students had an infection rate of 71.4% in that only 15 of them were infected. There was therefore AA infection rate of 68.3%, AS infection rate of 60.6% and SS infection rate of 71.4%. In terms of age groups, 57(76.0%) AA students out of 75(67.6%) AA students in the 15-20 age group were infected. Twenty seven (90.0%) AS students out of 30(27.0%) were infected while 4(66.7%) SS genotype students out of 6(5.4%) students in the same age group were infected with malaria parasites. All these data translate to AA, AS and SS infection rates of 76.0%, 90.0% and 66.7% respectively in the 15-20 age group.

In the 21-25 group, 94(72.3%) AA students out of 130(65.7) were infected, 21(38.9%) AS students out of 54(27.3%) were infected and 10(71.4%) SS genotype students out of 14(7.1%) were infected. This means AA, AS and SS genotype in this age group recorded malaria infection rates of 72.3%, 38.9% and 71.4% respectively for this age group. Genotype AA in the 26-30 group recorded 29(74.4%) out of which 14(48.3%) were infected. In the same age group, 6(66.7%) AS students out of 9(23.1%) were infected and no SS student was infected. Therefore, infection rates in this age bracket for AA, AS and SS haemoglobin genotypes were 48.3%, 66.7% and 0.0% respectively. Only 6 students were recorded for 31-35 age group. All the 3(100.0%) AA students in this group were infected and all the 3(100.0%) AS students in this group were infected. There was no SS genotype student recorded in this group. Hence infection rates of 100.0%, 100.0% and 0.0% were recorded for AA, AS and SS respectively. There was a similar trend in the 36-40 age group in which all 3(100.0%) AA students were infected. All 3(100.0%) AS students in the 41-45 group were infected. In the 36-40 group therefore, infection rates of 100.0%, 0.0% and 0.0% were recorded for AA, AS and SS respectively and in the 41-45 group, 0.0%, 100.0% and 0.0% infection rates were recorded for AA, AS and SS respectively (Table 4).

Table 3. Age and sex association of haemoglobin genotypes with malaria parasitaemia

Age groups (yrs)	SEX	AA (%)	% mp positive micropoint & giemsa stain tests	% MP negative Micropoint & giemsa stain tests	AS (%)	% MP positive micropoint & giemsa stain tests	% MP negative micropoint & giemsa stain tests	SS (%)	% MP positive micropoint & giemsa stain tests	% MP negative micropoint & giemsa stain tests
15-20	M	15(13.5)	9(8.1)	6(5.4)	18(16.2)	18(16.2)	0(0.0)	3(2.7)	2(1.8)	1(0.9)
n=111	F	60(54.1)	45(40.5)	15(13.5)	12(10.8)	9(8.1)	3(2.7)	6(5.4)	4(3.6)	2(1.8)
(30.8%)										
21-25	M	57(28.8)	48(24.2)	9(4.6)	27(13.6)	12(6.1)	15(7.6)	6(3.0)	6(3.0)	0(0.0)
n=198	F	73(36.9)	46(23.2)	27(13.6)	27(13.6)	9(4.6)	18(9.1)	5(2.5)	3(1.5)	2(1.0)
(55.0%)										
26-30	M	11(28.2)	2(5.1)	9(23.1)	3(7.7)	3(7.7)	0(0.0)	1(2.6)	0(0.0)	1(0.9)
n=39	F	18(46.2)	12(30.8)	6(15.4)	6(15.4)	3(7.7)	3(7.7)	0(0.0)	0(0.0)	0(0.0)
(10.8%)										
31-35	M	3(50.0)	3(50.0)	0(0.0)	3(50.0)	3(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0
n=6	F	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
(1.7%)										
36- 40	M	3(100.0)	3(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0
n=3	F	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
(0.85%)										
41- 45	M	0(0.0)	0(0.0)	0(0.0)	3(100.0)	3(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0
n=3	F	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
(0.9%)										
TOTAL	M	89(37.1)	65(73.0)	24(27.0)	54(54.6)	39(72.2)	15(27.8)	10(47.6)	8(80.0)	2(20.0)
	F	151(62.9)	99(65.6)	48(34.4)	45(45.4)	21(46.7)	24(53.3)	11(52.4)	7(63.6)	4(36.4)
		240(66.7)			**99(27.5)**			**21(5.8)**		

Table 4. Influence of haemoglobin genotypes on malaria infection rates among students sampled

Age groups (Yrs)	**AA (%)**	**% MP positive micropoint & giemsa stain)**	**% MP negative micropoint & giemsa stain)**	**AS (%)**	**% MP positive micropoint & giemsa stain)**	**% MP negative micropoint & giemsa stain)**	**SS (%)**	**% MP positive micropoint & giemsa stain)**	**% MP negative micropoint & giemsa stain)**
15-20 n=111	75(67.6)	57(76.0)	18(24.0)	30(27.0)	27(90.0)	3(10.0)	6(5.4)	4(66.7)	2(33.3)
21-25 n=198	130(65.7)	94(72.3)	36(27.7)	54(27.3)	21(38.9)	33(61.1)	14(7.1)	10(71.4)	4(28.6)
26-30 n=39	29(74.4)	14(48.3)	15(51.7)	9(23.1)	6(66.7)	3(33.3)	01(2.6)	0(0.0)	1(100.0)
31-35 n=6	3(50.0)	3(100.0)	0(0.0)	3(50.0)	3(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
36-40 n=3	3(100.0)	3(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
41-45 n=3	0(0.0)	0(0.0)	0(0.0)	3(100.0)	3(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	**Infection Rates**	**164(68.3)**	**72(31.7)**		**60(60.6)**	**39(39.4)**		**15(71.4)**	**6(28.6)**
HB Types	**240(66.7)**			**99(27.5)**			**21(5.8)**		
*Critical (P) value @ df, 10 = 18.307 Calculated X^2 = **7.786** P>0.05*				*Critical (P) value @ df, 10 = 18.307 Calculated X^2 = **8.4207** P>0.05*			*Critical (P) value @ df, 10 = 18.307 Calculated X^2 = **4.012** P>0.05*		*P>0.05*

4. DISCUSSION

A total of 360 students from fifteen departments of the University were recruited for the study. Of this sample size, 198(55.0%) were of the 21-25 age category and 23 years average age. This, somewhat suggested that the average age of majority of the students engaged in this study was 23 years. This was followed by an average age of 19years as represented by 111(30.8%) students who occurred in the 15-20 age bracket. This finding implied that the sampled students were mature enough to make informed decision in terms of giving their informed consent to be enlisted for the research (Table 1).

The findings in this work further revealed that 255(70.8%) of the 360 students sampled were infected with *Plasmodium falciparum* parasites of various forms and densities. This relatively high parasitaemia rate suggests that Oghara town may be hyper endemic for malaria. This parasitaemia rates appear to be low when compared to 93.4% obtained in Odoakpu, Onitsha south by Ilozumba and Uzozie [32] and 76.8% obtained in Okada, Edo State by Otajevwo [23] as well as 79.3% and 77.4% parasitaemia rates obtained in Warri and Owerri by previous authors [33,34]. Parasitaemia rate in this study appears to be high however, when compared to prevalence rates of 66.9%, 63.1%, 58.3%, 43.2%, 10.0% and 6.0% obtained respectively for subjects in Ogbomosho by Akhigbe et al. [20], outpatients in Wudil, Kano by Abdullahi [35], children in Awka by Mbanugo and Ejins [36], coastal dwellers in Lagos by Nebe et al. [37], blood donors in Ibadan by Edington et al. [38] and blood donors in Maiduguri by Ahmed et al. [39]. These results may suggest existence of regional differences in malaria parasitaemia in Nigeria with the Western, Midwestern and Eastern areas (as represented by Ogbomosho, Warri, Onitsha and Owerri) ranking highest in prevalence rating and the northern area (represented by Maiduguri) occupying the lowest position while Lagos and Ibadan took a middle position. Further studies in this area of research should be undertaken by other authors in others parts of Nigeria before a more definite statement on the apparent trend could be made. Statistical analysis showed that at 95% confidence interval, the female students were significantly more infected than the males, X^2(P-value) = 3.841, calculated X^2 = 3.958, (P<0.05) (Table 2). The reason why the female students were more prone to malaria attack could not be fathomed immediately but it may be due to their peculiar dressing of which most parts of their bodies (such as forearms and hind limbs) are often exposed to mosquito bites compared to the males. This finding was in agreement with the report of some earlier authors [20], but inconsistent with reports of some previous studies [40,32,34].

In this study also, male and female AA genotype infection rates were 73.0% and 65.6% respectively. Genotype AS male and female malaria infection rates were 72.2% and 46.7% respectively while 80.0% and 63.6% were infection rates of male and female SS genotype respectively (Table 3). More AA females appeared to have been infected with malaria parasites than the male AA students. This was not the case with AS and SS male and female students as the reverse was the case. There is no documented evidence that supports or explains any gender/ genotype connection or association with malaria infection.

Combining both sexes, malaria infection rates of AA, AS and SS genotypes in this study were 68.3%, 60.6% and 71.4% respectively. This clearly shows malaria infection rates of SS and AS students were the highest and least respectively. Some previous studies reported malaria infection rates HbAA (67.0%), HbAS (32.0%) and SS (1.0%) [41]. When compared to the report of Awah et al. [41], the findings in this study were small except genotype SS infection rate. Other authors reported higher infection rate of 74.6% for HbAA and lower rates of 57.5% and 60.0% for HbAS and HbSS respectively [10]. Much lower infection rates of 38.9%, 6.2% and 0.0% have been reported for HbAA, HbAS and HbSS respectively by Tidi et al. [14]. It is evident from these reports that HbAA always had the highest malaria infection rate. According to Awah*et al.* [41], high malaria parasitaemia (MP+++) was significantly more common in subjects with genotype AA than those with HbAS. Haemoglobin AA individuals are very susceptible to malaria attack because the red cells are conducive to the growth and development of the parasite [18]. Malaria parasites infect the red blood cells of those with two normal alleles leading to the bursting of the infected cells [13]. Eteng [3] reported that malaria is higher in individuals of HbAA genotype compared to those of HbAS. This greater susceptibility to *P. falciparum* malaria and the enhanced severity of an attack in this group may be due to low red cell membrane resistance to the invading parasite and a non-hypoxic

environment within the red cell which enhances its development [3,16]. Statistical analysis done on HbAA data and malaria infection rates however, showed that there was no significant association between male and female HbAA types and malaria infection ($P>0.05$). There was no significant association between HbAA data and malaria infection ($P>0.05$).

Also based on results obtained in this study and reports of previous and similar studies, it is also clear that malaria infection rates were lowest for HbSS followed by HbAS. This is consistent with the reports of some earlier workers which stated that the allele that causes sickle cell anaemia imparts resistance to malaria infection [11,12]. According to the reports of previous authors, haemoglobin S is known to interfere with the growth and reproduction of malaria parasite [42,43]. Salimonu [9] also reported that patients who are heterozygous for the sickle trait gene (HbAS) are usually more resistant to *Plasmodium falciparum* infection than those who are homozygous (HbSS, HbAA).

In this study, malaria infection rate in HbAS was second to lowest compared to HbSS. This may be due to the production of higher levels of superoxide and hydrogen peroxide (H_2O_2) which are toxic to a number of pathogens including malaria parasites [17]. It has also been reported that the absence of malaria infection in haemoglobin SS may be because it interferes with the growth and multiplication of malaria parasites [14]. Findings in this work are also supported by a report that stated that homozygous haemoglobin S red cells produces membrane associated hemin which oxidizes membrane lipid proteins and probably produce little of such products [44]. Derek [45] stated in a related report that the high frequency of haemoglobin S in malaria endemic areas has been attributed to the selection advantage if offers to carriers of the trait against malaria. It has however been suggested that the protective role of HbAS is against severe disease and not against infection rate [10]. According to Cabrera et al. [46], HbAS probably protects against malaria infection due to increased parasite clearance and induction of antibodies. These two traits are under strong selection pressure by the disease [47]. Also, a similar report by Williams et al. [16] stated that HbAS is associated with reduced parasite densities during intercurrent *Plasmodium falciparum* infections. Awah and Uzoegwu [30] concluded that inheriting both genetic disorders reduces malaria anaemia, parasitaemia and severe malaria symptoms.

Chi square statistical analyses of male HbAS and malaria infection rates and female HbAS and malaria infection rates showed that there was no significant association or relationship in both ($P>0.05$). The situation between male HbSS and malaria infection rates and female HbSS and malaria infection rates was not different as there was no signification association also ($P>0.05$) (Tables 3 and 4).

The highest frequencies of HbAA, HbAS and HbSS were recorded among students of 21-25 age group followed by 15-20 and 26-30 age brackets. This did not necessarily imply that any of the haemoglobin types will occur highest or lowest within a specific age group but was basically because of the peculiarity of the age group(s) that dominated the University population under study and indeed, most other Universities.

4.1 Limitation

A limitation in this study was that the test device is for in-vitro diagnostic test use only. Neither the qualitative value nor the rate increase in Pf antigen concentration can be determined by its qualitative test. The test Device will only indicate the presence of *Plasmodium falciparum* antigen and hence, the specimen should not be used as the sole criteria for the diagnosis of malaria infection. All results must be interpreted together with other clinical information available to the physician. A negative result does not at any time preclude the possibility of malaria infection. The inability of authors to get controls of other haemoglobin genotype variants such as “SC” etc limited the scope of this effort.

5. CONCLUSION

Average age of majority of the students engaged in the study was 19-23 years and 70.8% of sampled subjects were infected with various asexual forms and densities of *Plasmodium falciparum* of which the female students were significantly more infected than the male students. Findings in this regard are suggestive that Oghara town may be hyper endemic for malaria and this information may be useful to the relevant Delta State Health agencies.

More HbAA female students were infected than HbAA male students while more HbAS and HbSS male students were infected than HbAS

and HbSS female students. Malaria infection rates were highest in HbAA, HbAS and HbSS in decreasing order. There was no significant association between male and female HbAA, HbAS, HbSS and malaria infection rates. Hence results of this study imply that there is no protection against malaria by any of the implicated haemoglobin types. The focus of this work was not aimed at explaining the mechanism of protection that HbAS arguably confers against malaria infection. The overall goal was to suggest the need for provision of baseline information on haemoglobin types in areas with different transmission frequencies. Such knowledge may be useful in designing and implementing different malaria interventions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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