AN INVESTIGATION INTO HETEROZYGOUS HAEMOGLOBIN GENOTYPE ASSOCIATION WITH MALARIA PARASITAEMIA IN A COMMUNITY SCHOOL BASED IN BENIN CITY, NIGERIA.

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One hundred and thirty six subjects made up of 67(49.3%) males and 69(50.7%) females of the Covenant Christian Academy Uteh, Benin City, Nigeria were used for the study. Subjects were grouped into 33 (24.1%), 41(29.9%), 13 (9.5%) and 8(5.8%) kindergarten, primary school, junior secondary school, senior secondary school and teachers respectively. Blood samples (2ml volume) which were collected into ethylene diamine tetra-acetic acid anti-coagulated containers were screened for malaria parasitaemia by the malaria Plasmodium falciparum Rapid test Device (Global diagnostics, Malaysia). Haemoglobin genotype test was done by the cellulose acetate paper electrophoresis method. Out of 21(63.6%) HBAA kindergarten children, 15 (71.4%) were infected with malaria parasite. Twenty three (82.1%), 26(78.8%), 6(66.7%) and 5(100.0%) primary school, junior secondary school, senior secondary school and teachers respectively were infected with malaria out of 28(63.3%), 33(78.6%), 9(69.2%) and 5(62.5%) sampled subjects respectively. In the heterozygous HBAS group, 1(8.3%), 3(27.3%), 3(33.3%), 1(50.0%) and 2(66.7%) of kindergarten, primary school, junior secondary, senior secondary and teachers respectively were infected out of 12(36.4%), 11(26.8%), 9(21.4%), 2(4.8%) and 3(37.5%) sampled subjects respectively. There was no clear cut HbSS association with malaria infection ostensibly due to not too large a sample size used in study. Whereas malaria infection was significantly associated with HBAA (P<0.05), the association with HbAS was insignificant (P>0.05). Also, there was no sex discrimination (advantage) of HbAS protection over malaria infection. Findings may suggest the need for provision of baseline information on HbAS in areas with different transmission frequencies which may be useful in designing and implementing different malaria interventions.

Key words: Malaria , Parasitaemia, Haemoglobin, Genotype, Nigeria.

Introduction

Despite substantial evidence of protection against clinical malaria given by the haemoglobin variant – HbC and HbS, the precise mechanism(s) are still under debate (Verra et al., 2007). 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 (Verra et al., 2007).

Malaria is endemic in tropical Africa and some individuals including pregnant women, children and sickle-cell disease (SCD) patients have an increased susceptibility to its infection (Bouyou-Akotet et al., 2003; Okogun and Amadi, 2005; Flemming, 1989). In addition, it is a factor that has maintained the prevalence of SCD at a constant level in the tropics over the years with the sickle-cell trait acting as a genetic modifier against malaria infection. Malaria is believed to be a major cause of morbidity in SCD patients; it is a precipitating factor for the frequent vasoocclusive crises experienced by these patients: and may thus be responsible for hospital...
admission. It is customary, therefore, to prescribe malaria chemoprophylaxis for almost every SCD patient who is in crisis regardless of whether they are symptomatic or not (Kotila, 2005).

Malaria resistance by the sickle cell trait (genotype HbAS) has served as the prime example of genetic selection for over half a century. 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.

Sickle-cell trait (genotype HbAS) confers a high degree of resistance to severe and complicated malaria (Aidoo et al., 2002) yet the precise mechanism remains unknown. To some extent it almost certainly relates to the peculiar physical or biochemical properties of HbAS red blood cells: invasion, growth, and development of Plasmodium falciparum parasites are all reduced in such cells under physiological conditions in vitro (Freidman, 1978), and parasite-infected HbAS red blood cells also tend to sickle (Freidman, 1978, Roth et al., 1978), a process that may result in their premature destruction by the spleen (Freidman, 1978, Shear et al., 1993). Nevertheless, while such factors appear to be important, recent observations suggest that the mechanism might also involve an immune component. For example, in a study conducted in Gambia, it was found that the immune recognition of P. falciparum-infected red blood cells was enhanced in HbAS children (Marsh et al., 1989), and up-regulation of malaria-specific cell-mediated immune responses has also been observed in HbAS children (Marsh et al., 1989), and up-regulation of malaria-specific cell-mediated immune responses has also been observed in HbAS individuals in Sudan (Abu- Zeid et al., 1992, Bayoami et al., 1990). While potentially important, such observations could represent epi-phenomena, rather than proximate effects of the HbAS red cell phenotype. Establishing whether or not immune processes are involved may prove useful in learning about malaria protection more generally.

The mechanism by which HbAS protects against malaria has been the subject of speculation for more than 50 years. 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 (Marsh et al., 1989; Guggenmoos et al., 1981). However, establishing this relationship 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.

These Hb genetic disorders have been associated with protection against malaria morbidity (Moormann et al., 2003). Studies have shown that children with sickle cell trait (HbAS) are protected from both mild and severe malaria (Aidoo et al., 2002). HbAS is also associated with reduced parasite densities during intercurrent Plasmodium falciparum infections (Williams et al., 2005) and enhanced acquired immunity, which suggests that HbAS probably protects against malaria infection due to increased parasite clearance and introduction of antibodies (Cabrera et al., 2005). These two traits are therefore under strong selection pressure by the disease (Francis and Pete, 2006). Segeja et al. (2008) 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.

The high frequency of the sickle-cell haemoglobin (HBS) gene in malaria endemic regions is believed to be due to a heterozygote (HbAS) advantage against fatal malaria. Aidoo et al. (2002) showed, based on data generated from their study that HbAS provides significant protection against all-cause mortality, severe malarial anaemia, and high density parasitaemia. Compared to HbAA, HbAS was significantly associated with a reduction in all-cause mortality only during the period from 2 to 16 months of age (risk ratio 0.45 (95% CI 0.24-0.84), p=0.0001, figure). However, when compared with HbAA, there was no HbAS-associated reduction in mortality during the first 2 months or >16 months of age (1.2 [0.7-2.1]; p=0.5). At ages 2-16 months, HbSS was not associated with any survival advantage when compared with HbAA.

Intriguingly, an enhanced immune recognition of variant surface antigens (VSA) was initially observed in HbAS individuals by Marsh et al. (1989) and further demonstrated in a study showing that the presence of HbAS genotype was associated with enhanced recognition of two randomly selected clinical isolates amongst Gabonese children (Cabrera et al., 2005). A cohort study in Kenya lends further support to the hypothesis of an accelerated acquisition of immunity against mild clinical malaria in HbAS children less than 10 years old (Williams et al., 2005).

A research to relate malaria infection with heterozygous HbAS genotype in Benin City, Nigeria, is not known to have been carried out. This project therefore, is aimed at investigating the heterozygous haemoglobin genotype association with immune protection against malaria parasitaemia in a community school based in Benin City, Nigeria, with the following objectives:
a. Determine the age and sex association of haemoglobin genotypes with malaria parasitaemia.
b. Determine the influence of age on association of haemoglobin genotype and malaria parasitaemia.

Materials and methods

Ethical Approval:

The researcher obtained ethical clearance from the management of the Covenant Christian Academy, Benin City, Nigeria which had earlier gotten consent from parents and teachers of subjects as part of admission requirements. All subjects used for study were confirmed to be asymptomatic for malaria and had not been on any antimalarial medication for at least, seven days before samples were taken.

Two millilitres of venous blood was collected by vein puncture with sterile 5ml needles and syringes from a total of 137 subjects made up of 67(49.3%) males and 69(50.7%) females of covenant Christian academy, Uteh Benin City. Subjects were grouped 33(24.1%), 41(29.9%), 42(30.7%), 13(9.5%) and 3(5.8%) kindergarten, primary school, junior secondary school, senior secondary school and teachers respectively. Blood samples which were collected by informed consent of the school authority and parents of wards were and dispensed into appropriately labelled sequestrinized (ethylene diamine tetra acetic acid) anti-coagulated containers and mixed.

Malaria parasite detection test (Test Kit content/storage)

The Malaria Plasmodium falciparum rapid Test Device (whole blood) package insert kit by Maconell (2001) and Cooke et al., (1999) was used to test for presence of malaria parasite in the specimens. The content of test kit included: test devices, test tubes, package insert, disposable specimen droppers, transfer droppers and buffer. The test kit was stored at room temperature on laboratory bench according to manufacturers’ instruction. Test Kit was used within expiration date stated on the sealed pouch.

Rapid test procedure

The test device, specimens, buffer and/or controls were allowed to equilibrate to 15-30°C (room temperature) prior to testing. For processing of each sample, the test device cassette was removed from the foil pouch and used immediately. Using the transfer dropper (provided), and held vertically one dropper of whole blood sample approximately 20µl (0.02ml) was transferred to the test tube. Added to this, were three full drops of buffer (approximately 120 µl. or 0.12ml) after which the mixture was allowed to stand for 1 minute. The test device (cassette) was placed on a clean and level surface. The test tube was inverted five times to mix the specimen and buffer completely. The entire content (of about 140 µl) was then transferred to the specimen well in the cassette with the transfer dropper and the set up was left for 15-20 minutes.

Interpretation of results

A test was positive if two distinct coloured lines appeared. One line in the control region (c) and another line should be in the test region (T). The intensity of the colour in the test line region (T) may vary depending on the concentration of Plasmodium falciparum present in the specimen. A test was termed negative if one coloured line only appeared in the control region (C) with no apparent coloured line appearing in the test region (T).

Quality control

Internal procedural controls were included in the test. A coloured line appearing in the control region (C) was used as an internal positive procedural control. The quality control was to confirm if sufficient specimen volume was used and if the correct procedural technique was applied.

Sensitivity of test device

The Malaria P.F Rapid Test Device (whole blood) has been tested within or thick blood smears on clinical samples. The results show that the sensitivity of the test Device (whole blood) is > 99% (greater than 99%) relative to blood smears (Cooke et al., 1999).

Specificity

The Test Device uses an antibody that is highly specific for malaria Plasmodium falciparum antigen in the blood. The Test Device has a specificity of > 99% relative to blood smears (Cooke et al., 1999).

Expected values

The Test Device has been compared with traditional thick or thin blood smears microscopic analysis. The correlation between the two systems is >99% (Cooke et al., 1999).

Haemoglobin Genotype test

The haemoglobin genotype test was carried out by haemoglobin cellulose acetate paper electrophoresis method originally described by Ochei and Kolhatkar (2008) but modified present by authors.

Preparation of haemolystate

The anti-coagulated whole blood samples were centrifuged at 2500rpm for five minutes. The plasma was decanted and the red-blood cells sediments were washed
with large volumes of saline three times. After the third washing, the red blood cells were lysed by adding equal volume of distilled water. Mixing was done by inversion. The mixture was then centrifuged to remove the cell debris. The resulting haemolysate was then transferred to a clean tube.

**Electrophoresis**

The chamber of the electrophoresis tank was filled with Teb Tris Buffer, pH 8.6 (India). The Whatman No 1 papers soaked and positioned in the chamber. The cellulose acetate paper was pre-soaked in the buffer for 5 minutes and excess buffer was removed by partial drying in between absorbent papers. Applicator sticks were used to spot the haemolysate of each sample (about 0.02ml or a drop) on marked lines of origin on the acetate paper. Controls known as AS and SS were also spotted. The spotted acetate paper was then placed firmly across in between soaked Whatman No 1 paper in the chamber of the tank. The electrophoresis was then run at 220 volts for 5 minutes. The relative mobility of the controls samples were compared and related to the test samples visually and results recorded.

**Statistical Analysis**

The Student paired T-test contained in SPSS software was used to calculate significant and non-significant association between haemoglobin genotype and malaria parasitaemia at 95% and 99% confidence intervals.

**Results**

The haemoglobin genotype and malaria parasite results of 33 (24.1%), 41 (29.9%), 42 (30.7%), 13 (9.5%) and 8 (5.8%) kindergarten, primary school, junior secondary school, senior secondary school and teachers respectively in terms of their age and sex relationships are presented in Table 1.

Nine (69.2%) male kindergarten children were infected with malaria parasite out of 13 (39.4%) of the kids in the HbAA group. Out of 8 (24.2%) females having HbAA in this group, 6 (75.0%) were infected. Less than 20% of the heterozygous HbAS male children were infected out of 8 (24.2%). Of the 4 (12.1%) female HbAS subjects, none was infected with malaria parasite. There were no sickle cell anaemia (HbSS) children found in this group. Nine (27.3%) male kindergarten children were infected with malaria parasites out of 13 (39.4%) of the kids in the HbAA group. Out of 8 females having HbAA in this group, 6 (18.2%) were infected.

In the primary school (5-11 years) category, 7 (87.5%) males were infected out of 8 (19.5%) HbAA female pupils were infected. Among the HbAS heterozygous group, of the 4 (9.8%) males, only 1 (25.0%) was infected while 2 (28.6%) females out of 7 (17.1%) had malaria parasitaemia. All the 2 (4.8%) male HbSS children were infected. There were no female sickle cell anaemia children in this group (Table 1).

The junior Secondary School (10-16 years) group recorded 15 (79.0%) and 11 (78.8%) male and female students being infected with *Plasmodium falciparum* parasites out of 19 (45.2%) and 14 (33.32%) male and female HbAA students respectively. As far HbSS, whereas 1 (25.0%) male out of 4 (9.8%) was infected, 2 (28.6%) females out of 7 (17.150 were parasitized. The only 1 (2.45) female HBSS student was not infected (Table 1).

In the Senior Secondary group (14-17 years), male student out of 6 (46.2%) HbAA students were infected while 2 (66.7%) females out of 3 (23.1%) HbAA female students were infected. The only 1 (7.7%) female HbSS students did not record malaria parasite in her blood. There was no male or female record of HbSS (Table 1).

There was no HbAA record for the male teachers. All the 5 (62.5%) teachers having HbAA were infected with *Plasmodium falciparum* parasites. Two (5.0%) male teachers had HbAS haemoglobin genotype out of which 1 (50.0%) was infected. The only 1 (12.5%) female with HbAS was infected with the parasite (Table 1).

On the whole, out of 46 (47.950) HbAA male subjects in this study, 35 (76.1%) had *Plasmodium falciparum* parasitaemia while out of 50 (52.1%) HbAA females, 34 (68.0%) were infected. Whereas out of 19 (51.4%) male HbAS subjects, 3 (15.8%) were infected, 6 (33.35) female HbAS were infected out of 18 (48.7%) record. All the 2 (66.7%) male HbSS subjects were infected while the only 1 (33.3%) female was not at all.
Table 1: Age and Sex Association of Haemoglobin Genotypes with Malaria parasitaemia.

<table>
<thead>
<tr>
<th>CLASS GROUPING (YRS)</th>
<th>SEX</th>
<th>AA (%)</th>
<th>MP (%)</th>
<th>AS (%)</th>
<th>MP (%)</th>
<th>SS (%)</th>
<th>MP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kindergarten</td>
<td>M</td>
<td>13(39.4)</td>
<td>9(27.3)</td>
<td>8(24.2)</td>
<td>1(3.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
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<tr>
<td></td>
<td>F</td>
<td>8(24.2)</td>
<td>6(18.2)</td>
<td>4(12.1)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
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<td></td>
<td>n=33</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Primary</td>
<td>M</td>
<td>8(19.5)</td>
<td>7(17.1)</td>
<td>4(9.8)</td>
<td>1(2.4)</td>
<td>2(4.8)</td>
<td>2(4.8)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>20(48.8)</td>
<td>16(39.0)</td>
<td>7(17.1)</td>
<td>2(4.8)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
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<td></td>
<td>n=41</td>
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</tr>
<tr>
<td>Junior S 1-3</td>
<td>M</td>
<td>19(45.2)</td>
<td>15(35.7)</td>
<td>4(9.5)</td>
<td>1(2.4)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
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<tr>
<td></td>
<td>F</td>
<td>14(33.3)</td>
<td>11(26.2)</td>
<td>5(11.9)</td>
<td>2(4.8)</td>
<td>1(2.4)</td>
<td>0(0.0)</td>
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<td></td>
<td>n=42</td>
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</tr>
<tr>
<td>Senior S 1-2</td>
<td>M</td>
<td>6(46.2)</td>
<td>4(30.8)</td>
<td>1(7.7)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3(23.1)</td>
<td>2(6.1)</td>
<td>1(7.7)</td>
<td>1(7.7)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
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<td></td>
<td>n=13</td>
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<tr>
<td>TEACHERS</td>
<td>M</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>2(25.0)</td>
<td>1(12.5)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
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<tr>
<td></td>
<td>F</td>
<td>5(62.5)</td>
<td>5(62.5)</td>
<td>1(12.5)</td>
<td>1(12.5)</td>
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<tr>
<td>TOTAL</td>
<td>M</td>
<td>46(47.9%)</td>
<td>35(76.1%)</td>
<td>19(51.4%)</td>
<td>3(15.8%)</td>
<td>2(66.7%)</td>
<td>2(66.7%)</td>
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<tr>
<td></td>
<td>F</td>
<td>50(52.1%)</td>
<td>34(68.0%)</td>
<td>18(48.7%)</td>
<td>6(33.3%)</td>
<td>1(33.3%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td></td>
<td>n=96(71.1%)</td>
<td>n=37(27.4%)</td>
<td>n=3(2.2%)</td>
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</tbody>
</table>

Presented in Table 2 are data to show the influence of age on association of haemoglobin genotype and malaria parasitaemia.

In the kindergarten group, 15(71.4%) children out of 21(63.6%) HbAA were infected. Only 1(8.3%) was infected out of 12(36.4%) HbAS subjects while there was no HbSS cases recorded.

Twenty three(82.15) out of 28(68.3%) HbAA pupils were infected. Only 3(27.3%) out of 11(26.8%) HbAS children were infected while all the 2(4.9%) HbSS children were infected.

In the JS group, 26(78.8%) out of 33(78.6%) were infected in the HbAA genotype group. AS for the HbAS class, only 3(33.3%) out of 9(21.4%) were infected while the only 1(2.4%) HbSS had no malaria infection.

Out of 9(69.25) and 2(4.8%) HbAA and HbAS respectively senior secondary students, 6(66.7%) and 1(50.0%) respectively had malaria infection. There was no record of HbSS students in this group.

All the 5(62.5%) HbAA teachers were infected with malaria parasite while 2(66.7%) out of 3(37.5%) HbAS teachers were infected. There was no record of HbSS in this group.
Statistically, the paired t-test analysis of HbAA association with malaria parasite showed a mean ± standard error of 19.2 ± 1.24 HbAA as against 15.0 ± 1.24 that had malaria parasitaemia calculated t=3.39, book t (p value)=2.776, hence P<0.05. This suggests that malaria parasite association with HbAA in this study is significant. This again means that HbAA individuals are significantly susceptible to malaria parasite attack.

The mean ± standard errors of HbAS as against malaria parasite were 7.4±1.97 HbAS and 2.2+1.97 malaria parasite. Calculated t value=2.75, book t value=2.776, hence P>0.05. This suggests the HbAS association with malaria parasite in this study is not significant. This also implies that the heterozygous genotype offers some protection on the carrier against the parasite.

The association of the parasite with HbSS appears not to be significant in terms of 0.6 ± 0.2 HbSS as compared with 0.4 ± 0.2 with t=2, book t value=2.776, and hence P > 0.05.

Table 2: Influence of Age on Association of Haemoglobin Genotype and Malaria parasitaemia.

<table>
<thead>
<tr>
<th>CLASS GROUP</th>
<th>AA (%)</th>
<th>MP (%)</th>
<th>AS (%)</th>
<th>MP (%)</th>
<th>SS (%)</th>
<th>MP (%)</th>
</tr>
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<tbody>
<tr>
<td>KG 1-3</td>
<td>21(63.6)</td>
<td>15(71.4)</td>
<td>12(36.4)</td>
<td>1(8.3)</td>
<td>0(0.0)</td>
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<tr>
<td>2-6 YEARS</td>
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<tr>
<td>Primary 1-5</td>
<td>28(68.3)</td>
<td>23(82.1)</td>
<td>11(26.8)</td>
<td>3(27.3)</td>
<td>2(4.9)</td>
<td>2(100.0)</td>
</tr>
<tr>
<td>5-11 YEARS</td>
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<td>n=41</td>
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<tr>
<td>Junior S 1-3</td>
<td>33(78.6)</td>
<td>26(78.8)</td>
<td>9(21.4)</td>
<td>3(33.3)</td>
<td>1(2.4)</td>
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<td>10-16 YEARS</td>
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<tr>
<td>Senior S 1-2</td>
<td>9(69.2)</td>
<td>6(66.7)</td>
<td>2(4.8)</td>
<td>1(50.0)</td>
<td>0(0.0)</td>
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<td>14-17 YEARS</td>
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<td>n=13</td>
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<tr>
<td>TEACHERS 25-45 YEARS</td>
<td>5(62.5)</td>
<td>5(100.0)</td>
<td>3(37.5)</td>
<td>2(66.7)</td>
<td>0(0.0)</td>
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</table>

| TOTAL               | 96(71.1%) | 75(78.1%) | 37(27.4%) | 11(29.7%) | 3(2.22%) | 2(77.6%) |
| MEAN±S.E            | 19.2±1.24 | 15±1.24 | 7.4±1.97 | 2.2±1.97 | 0.6±0.2 | 0.4±0.2 |
| P-VALUE             | P < 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |

Discussion

Despite substantial evidence of protection against clinical malaria given by the haemoglobin S (HbS), the precise mechanisms are still under debate (Verra et al., 2007). The enhanced immune reactivity in 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 (Verra et al., 2007). The HbS genetic disorder has been associated with protection against malaria morbidity (Moorman et al., 2003). Studies have shown
that children with sickle cell trait (HbAS) are protected from both mild and severe malaria (Aidoo et al., 2002).

Findings in this study did not clearly show sex discrimination in HbAA association with malaria parasitaemia apart from the teachers group of which there was no male HbAA subjects recorded with malaria parasite. Out of the 13(39.4%) HbAA kindergarten children sampled, 9(27.3%) were infected. Similarly, out of 8(19.5%) primary school pupils, 19(45.2%) junior secondary school students and 6(46.2%) senior secondary subjects sampled, 7(17.1%), 15(35.7%), 4(30.8%) were infected respectively.

The trend in the female subjects was not too different. Out of 8(24.2%) kindergarten children, 20(48.8%) primary school pupils, 14(33.3%) junior secondary school students, 3(23.1%) senior secondary school students and 5(62.5%) teachers sampled, 6(18.2%), 16(39.0%), 11(26.2%), 2(6.1%) and 5(62.5%) were infected respectively (Table 1). This clearly shows a high susceptibility of HbAA individuals to malaria parasitaemia. The paired t-test analysis done on data showed that the association is a significant one (P<0.05). Findings above also did not clearly show any age discrimination of HbAA as against malaria infection as both males and females were infected almost at the same rates and frequencies. This finding is not similar to those of Schulman et al. (1999) and Maricelle et al. (2003) who reported that malaria parasitaemia is commoner in the younger age groups than in the older ones. The association of HbSS with malaria infection in this study was not clear cut. This was due to a small sample size although statistically, the association is not significant (P>0.05) Table 2. Aidoo et al. (2002) reported that HbSS was not associated with any survival advantage when compared with HbAA.

There was marked reduction in terms of numbers of HbAS subjects that were infected. Based on findings, it appears age is a factor in the association of heterozygous HbAS with malaria parasite. In the 2-6 years age bracket, out of 12(36.4%) sampled, 1(8.3%) was infected. Only 3(27.3%)-Primary, 3(33.3%)-Junior Secondary, 1(50.0%)-Secondary and 2(66.75%)-teachers were infected out of 12(36.4%), 11(26.8%)-0, 9(21.4%), 2(4.8%) and 3(37.5%) sampled respectively. A paired t-test analysis on data showed that there was no significant association between both variables (P>0.05). This confirms that HbAS somewhat offers protection against malaria attack whether mild or severe. Our finding is supported by Aidoo et al. (2002) who showed that HbAS provides significant protection against all-cause mortality, severe malarial anaemia, and high density parasitamae. The report of Segeja et al. (2008) also supports findings in this study. According to Cabrera et al. (2005), HbAS probably protects against malaria infection due to increased parasite clearance and induction of antibodies. These two traits (HbA and HbS) are under strong selection pressure by the disease (Francis and Pete, 2006). Also, a similar report by Williams et al. (2005) stated that HbAS is associated with reduced parasite densities during intercurrent Plasmodium falciparum infections. According to Awah and Uzoegwu (2006), less severe clinical malaria attack. They concluded that inheriting both genetic disorders reduces malaria anaemia, parasitaemia and severe malarial symptoms.

Again, there was no sex discrimination in HbAS association with malaria infection. Whereas out of a total of 19(51.4%) sampled males, only 3(15.8%) were infected out of 18(48.7%) that were sampled. The association of HbAS with malaria in terms of both sexes was not significant to be different. The percentage infected subjects as against percentage sampled was small at the 2-6 years, kindergarten group (8.3%). This infection rate increased to 27.3% (in the 5-11 years primary group), 33.3% (10-16 years junior secondary), 50.0% (14-17 years, teachers). This trend appears to imply that HbAS confirms Williams’ protection up to certain. According to Williams et al., (2005), HbAS protection against mild malaria increased with age from 20% in the first two years of life to a maximum of 56% by the age of ten(10years) and then decreased to 30% in people older than ten years.

Finding in this study different slightly from the report of Aidoo et al. (2002) which stated that HbAS protection against malaria covered the period from 2 to 16 months only. They also reported that the lack of an apparent protection against mortality among children with the HbAS gene in the first 2 months of life could be due to maternally transferred protective immunity or the presence, in the first few months of life due to high levels of fetal haemoglobin which probably supports the growth of Plasmodium falciparum. Although this age bracket does not accommodate the 2-6 year kindergarten group, there might be a tendency for this protection to extend even to the first 24 months of life.

**Conclusion**

The high frequency of the gene for sickle cell haemoglobin (HbS) in malaria endemic regions despite the high mortality rate among homozygote’s is thought to be due to a selective advantage conferred by HbAS against malaria mortality.

Although it has been suggested that HbAS would provide a protective advantage early in life before the acquisition of clinical immunity to malaria, definitive data to support this assumption especially in high malaria transmission areas are lacking.

This study has not attempted to explain the mechanism of protection that HbAS confers against
malaria infection. Data generated, May however, help to give backing to previous similar researchers. The overall goal is to suggest the need for provision of baseline information on HbAS in areas with different transmission frequencies. Such knowledge may be useful in designing and implementing different malaria interventions.

Findings shows that there is no sex discrimination in HBAA association with malaria parasitaemia in the study area (i.e. both male and female HBAA individuals stand equal chance of being infected). There is also no age discrimination in HBAA association with malaria infection (i.e. HBAA school children from kindergarten to senior secondary school as well as adults or teachers) stand equal chance of being infected. There is no clear or cut advantage of HBSS over malaria parasitaemia. More studies on this homozygous recessive genotype should be done involving large sample size to verify this.

Lastly, whereas there is no sex discrimination (advantage) of HBAS protection over malaria infection, there is age advantage while children in the 2-6 years age bracket may possess the highest protection, protection reduced drastically from 7 years and above. More elaborate research is recommended to confirm this finding.

REFERENCES


