Association between *Plasmodium* infection, Haemoglobin genotypes and Blood groups among Under-five nomadic Fulani of Northeastern Nigeria

TIDI, S.K.¹, Amos, J. T.¹, and Firyanda, E.²

¹Department of Biological Sciences Federal University Wukari, Nigeria
²Department of Haematology, Federal Medical Centre, Yola, Nigeria.

Email for Correspondence: stephentiddi@yahoo.com, Tel:+2348036269745

Abstract: Malaria is perhaps the most dreaded disease in the tropical regions of the world to which a lot of resources are committed in order to understand and control its effect on development of African communities. This study was to determine the association between *Plasmodium* infection, Haemoglobin genotypes and Blood groups of Northern Nigerian nomadic Fulani under-five children. It will be interesting to if certain haemoglobin genotypes and blood groups reduces vulnerability to *Plasmodium* infection as the most severe public health problems in developing countries. Blood samples were examined for for *Plasmodium* infection, haemoglobin genotypes and blood groups. Results show that participants of haemoglobin genotypes AA were significantly ($X^2$, \(p<0.05\)) infected than other haemoglobin genotypes. *Plasmodium falciparum* was more commonly associated with blood group A (49.1%) than any other blood group ($X^2$, \(p<0.05\)). Study appear to show significant (\(p<0.05\)) association between *P. falciparum*, haemoglobin genotypes and blood groups. Hence haemoglobin genotypes and blood groups are predisposing factor for *Plasmodium* infection in under-five nomadic Fulani children.

Keywords: Plasmodium, Haemoglobin genotypes, ABO Blood groups, Nomadic Fulani, Northeastern Nigeria.

INTRODUCTION

Malaria is one of the most severe public health problems and a leading cause of death in many developing countries especially Africa. According to latest World Malaria Report there were about 219 million cases of malaria in 104 endemic countries (WHO 2012). Individuals with less immunity are at most risk from the disease. These include young children, pregnant mothers, travellers and people from endemic areas who are not regularly exposed to infection (Gaëtan et al; 2013 and Erica et al; 2012).

Malaria is caused by sporozoan parasites of the genus *Plasmodium*. Four main species infect man: *Plasmodium falciparum, plasmodium malariae, Plasmodium ovale* and *Plasmodium vivax*. The four main species are transmitted to man through the bite of infected female Anopheline mosquitoes (Oyerinde, 1998). Malaria parasites are known to respond differently to their environment in the human host such as the structure of haemoglobin. Malaria parasite does not thrive well in sickle cell individuals (Hills, 1996). This natural protection has made the haemoglobin S gene resilient in malaria-infested areas, particularly Africa. The protection against malaria is bestowed only on people who have sickle cell trait and have inherited just a single gene because haemoglobin S is known to interfere with the growth and reproduction of malaria parasite (Goldfarb et al; 2009 and Aidoo et al; 2009). The allele that causes sickle cell anaemia imparts resistance to malaria infection. However, individual with HbSS gene are not protected from malaria (Bougouma et al; 2012 and Kreuels et al; 2010). 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 (Kwiatkowski, 2000). The sickle cell trait infected with malaria parasite under low oxygen tension sickled more readily than uninfected cells. Since sickle cells are removed from the circulation and destroyed in the reticuloendothelial system, infected sickle trait red cells reduces the parasite burden in people with sickle cell trait (Modiano et al; 2001). Malaria parasite could also be changed or killed directly in sickle cell
trait at low oxygen tension. The sickling process that occurs at low oxygen tension is responsible in harming the malaria parasite. During the process of sickling oxygen redicals are formed in sickle trait; thereby retarding the growth and even killing the malaria parasite. Sickle cell trait produce higher levels of the superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), which is toxic to a number of pathogens including malaria parasites (Agarwal et al; 2000). The risk of malaria is lower in subjects homozygous for haemoglobin C. Heterozygotes haemoglobin C have fewer episodes of malaria than homozygous haemoglobin A. Haemoglobin AA are very susceptible to malaria because the red cells are conducive to the growth and development of the parasite (Okwa, 2004).

ABO blood group system is one important factor associated with infections and diseases. There is relationship between ABO and malaria, and it is possible that the infection is responsible for the distribution of ABO Blood groups in humans (Fairhurst et al; 2003). Individual blood groups are selective to malaria infection and have resulted in the persistent increase in the prevalence of group O individuals, especially after migration. Group O genes have been further enriched among humans, inhabiting malaria regions outside Africa through the introduction of independent group O mutations (Kwiatkowski, 2005). The ratio of group O to A is higher in geographic regions where malaria is currently endemic (Christine and Walter 2007). High prevalence of group O and low prevalence of group A is found throughout sub-Saharan Africa where malaria parasite persists. The distribution of group A and O, generally matches malaria’s tropical distribution. In contrast, group A is a predominant blood group in the colder regions of the world. The survival from malaria is associated with blood group A (Uneke et al; 2006). The direct role of group A and B antigens in cytoadherence rosette formation with P. falciparum erythrocytes membrane protein (PfEMP-1) than in blood group O may explain why group A or B individual has prone to malaria infection (Degarege et al; 2012). It is documented that malaria responses differ between ethnic groups. Studies in Mali had shown that nomadic Fulani are likely to have some form of immunity against malaria parasite when compared with their sedentary neighbors (Agarwal et al; 2000). Unfortunately studies in Nigeria have not revealed such observations (Akogun et al; 2012a and Akogun et al; 2012b). The current report is part of a series of studies to understand the social, cultural and biological response of the nomadic Fulani group in Northeastern Nigeria to malaria. We believe such understanding is important for planning a comprehensive and sustainable intervention against the disease within this unique population.

MATERIALS AND METHODS

The study was conducted in Adamawa State, Nigeria (7° and 11°N, 11° and 14°E) which has been well described in previous studies as an important zone for nomadic population activity Akogun et al; 2012a and Akogun et al; 2012b). The study was carried out as part of a large socio-epidemiological study of nomads in the Northeastern Nigeria and involved 23 camp communities spread across the Benue-trough pastoral block. Participants informed consent was obtained before blood samples were collected.

The method for malaria parasite determination, count and Plasmodium specie identification was followed as described by Cheesbrough, (1998).

Determination of Haemoglobin Genotypes

The method for haemoglobin electrophoresis described by John and Lewis, (1986), was followed. Fifty micro-liters of washed cells were added into khan tubes containing 50 micro-liter of 0.1 % white saponin and were mixed thoroughly (haemolysate). The haemolysate was centrifuged to remove any debris. The supernatant was used for the test. Cellulose acetate papers were soaked and blotted. Controls used include: HbA, Hbf, Hbs and Hbc. One hundred ml of the Tris-EDTA and boric acid buffer was added into each of the outer section of the electrophoresis chamber. One micro-liter of each haemolysate sample (tests and controls) was transferred into the well plate. Using an applicator, 0.5 micro-liter of the haemolysate (samples and controls) was applied onto the cellulose acetate paper leaving about 0.5 cm gap for each sample. The cellulose paper was placed on a cathode bridge of the electrophoresis chamber containing Tris-EDTA and boric acid buffer. Two hundred voltages were applied for 15 minutes, and the results recorded.

Determination of ABO Blood Groups

The method for ABO blood grouping test described by Cheesbrough (1998) was followed. Twenty percent of cells suspension was prepared from the previous 2ml washed blood cells, by adding 0.2 ml of the washed cells to 0.8 ml of physiological saline and mixed gently. Controls used was known group A and B. One volume of anti-A and B was dispensed onto a marked white tile (cells grouping). One volume of the Volunteers’ antiserum was dispensed onto the
Table 1. *Plasmodium* infection based on Hb-genotypes and Blood groups.

<table>
<thead>
<tr>
<th>Hb-genotype</th>
<th>No. Examined</th>
<th>% infected</th>
<th>% Non infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>599</td>
<td>40.6</td>
<td>59.4</td>
</tr>
<tr>
<td>AS</td>
<td>65</td>
<td>6.2</td>
<td>93.8</td>
</tr>
<tr>
<td>SS</td>
<td>8</td>
<td>0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

\[X^2 = 28.951\]

\[df = 2\]

\[p < 0.05\]

<table>
<thead>
<tr>
<th>Blood group</th>
<th>No. Examined</th>
<th>% infected</th>
<th>% Non infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>55</td>
<td>49.1</td>
<td>50.9</td>
</tr>
<tr>
<td>B</td>
<td>142</td>
<td>44.4</td>
<td>55.6</td>
</tr>
<tr>
<td>AB</td>
<td>17</td>
<td>41.2</td>
<td>58.8</td>
</tr>
<tr>
<td>O</td>
<td>458</td>
<td>32.8</td>
<td>67.2</td>
</tr>
</tbody>
</table>

\[X^2 = 10.441\]

\[df = 2\]

\[p < 0.05\]

\[p < 0.05\]


Table 2. *Plasmodium* species based on Hb-genotype and Blood group.

<table>
<thead>
<tr>
<th>Hb-genotype</th>
<th>No. Examined</th>
<th>% Plasmodium negative</th>
<th>% <em>P. falciparum</em></th>
<th>% <em>P. malariae</em></th>
<th>% <em>P. ovale</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>599</td>
<td>59.4</td>
<td>38.9</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>AS</td>
<td>65</td>
<td>93.8</td>
<td>6.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SS</td>
<td>8</td>
<td>100.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\[X^2 = 34.610\]

\[df = 6\]

\[p = 0.000\]

\[p < 0.05\]

<table>
<thead>
<tr>
<th>Blood group</th>
<th>No. Examined</th>
<th>% Plasmodium negative</th>
<th>% <em>P. falciparum</em></th>
<th>% <em>P. malariae</em></th>
<th>% <em>P. ovale</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>55</td>
<td>50.9</td>
<td>47.3</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>142</td>
<td>55.6</td>
<td>40.8</td>
<td>2.8</td>
<td>0.7</td>
</tr>
<tr>
<td>AB</td>
<td>17</td>
<td>58.8</td>
<td>41.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O</td>
<td>458</td>
<td>67.2</td>
<td>32.8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\[X^2 = 17.246\]

\[df = 9\]

\[p < 0.05\]

\[p = 0.045\]

\[p < 0.05\]

same marked white tile in a correspondingly way (serum grouping). One volume of 20% cells was added onto the A and B anti-sera. One volume of standard 20% washed A cells, B cells and O cells was added onto the volunteers antiserum. Using a clean stirrer, it was mixed, spread and tilted back and forth for signs of agglutination.

Data analysis

Data were entered into Epidata version 3.1 transferred to Statistical Analysis System (SAS) version 8.0 for analysis. Pearson chi-square was used to determine the level of association between parameters and P<0.05 was considered significant.

RESULTS

Result in Table 1 shows that 36.8% of the participants were infected with *Plasmodium*. Haemoglobin genotype AA participants were 40.6% infected. Only 6.2% of the haemoglobin AS participants was infected. There was no *Plasmodium* detected among haemoglobin SS participants. However, there was significant difference (\[X^2 = 28.951, df = 2, p < 0.05\]) between haemoglobin genotypes and *Plasmodium* infection. Blood group A were more (49.1%) infected than blood group B (44.4%) and blood group O (33.6%) participants.

Result in Table 2 shows that 35.3% of the participants were infected with *Plasmodium falciparum* specie, while 1.3% with *P. malariae* and 0.1% were infected with *P. ovale*. Haemoglobin AA had *P. falciparum* infection of 38.9%, while haemoglobin AS had 6.2%. This was significantly different (\[X^2 = 34.610, df = 6, p < 0.05\]). Blood group A participants were more (47.3%) infected with *P. falciparum* than blood group B (40.8%), AB (41.2%) and O (32.8%) participants.

Similarly, blood group B were more (2.8%) infected with *P. malariae* than blood group A (1.8%), AB (0%) and O (0.9%) Participants. However, there was statistical significant difference (\[X^2 = 17.246, df = 9, p < 0.05\]) between *Plasmodium species* and ABO blood groups.
DISCUSSION

This study was designed to determine the association of ABO blood group and haemoglobin variants with Plasmodium infection. The study showed that Plasmodium infection in haemoglobin genotype AS was low probably because red blood cells infected with malaria parasite deforms as a result of reduction in oxygen tension within the erythrocytes as it carries out its metabolism (Pascal et al; 2004). The result further showed that haemoglobin genotype SS were not infected with malaria. The absence of malaria infection in haemoglobin SS may be because it interferes with the growth and multiplication of malaria parasites. Homozygous haemoglobin-S red cells produces membrane associated hemin which oxidizes membrane lipid proteins and probably produce little of such products (Pascal et al; 2004). Plasmodium infection was more common among haemoglobin AA individuals compared to haemoglobin AS due to factors earlier mentioned in this study, this may support the hypothesis that haemoglobin AS individuals suffer less malaria infection than those of haemoglobin AA (Moluneaix and Cricmara, 1980)

Blood group A was more significantly infected than other blood groups, followed by blood group B and least infected was blood group O (Table 1). The result held the same view with the reports of Christine and Walter, (2007), which revealed that many polymorphic human genes are implicated in the determination of infectivity or resistance to Plasmodium infection to some extent which may include blood group A and O respectively. In this study participants of blood group O were more populated followed closely by blood group B, blood group A and the least was blood group AB. This is similar to the report of Uneke, et. al. (2006), that the ratio of O to other blood groups is higher in geographic regions where malaria is endemic. The high prevalence of group O coupled with low prevalence of group A is found throughout Sub-Sahara Africa, where P. falciparum persist. Thus survival from malaria may be associated with group O and mortality with group A. Other possible reason of high blood group O compared to other blood groups could be because of the direct role of group A and B antigens in cytoadherence rosette formation with P. falciparum erythrocytes membrane protein (PiEMP-1) than in blood group O (Degarege et al; 2006). In addition the malaria cases were more as likely to be blood group A as O, and to be blood group B as O. Probably because of the underlying factors cited in this study which provide the strongest statistical evidence of an association between ABO and malaria infection.

P. falciparum was the highest (Table 2) species found among the infected blood groups and haemoglobin genotypes as compared to others. This is in accordance with the findings of Moluneaix and Cricmara 1980, D’alleandro et al; 1995 and Salako 1997. The high rate of P. falciparum could be due to the availability of mosquitoes’ vectors for the transmission of infection and suitable environmental conditions for the multiplication of P. falciparum. The low rate of P. malariae and P. ovale may be because they tend to be selective in the type of blood cells they infect for example, P. malariae and P. ovale prefers leucocytes and young red blood cells than the old blood cells. The Duffy blood group antigens necessary for the growth and multiplication of P. vivax is negative among the people of West Africa which could be responsible for the low rate of infection in ABO blood groups. Participants of haemoglobin AA were more infected with P. falciparum compared to P. malariae and P. ovale. This suggest higher infection of the population with P. falciparum than other P. species, may be due to factors associated with Hb-genotypes and Plasmodium infection earlier reported in this study. The results of the study further revealed that participants of blood group A were higher infected with P. falciparum than other malaria species. This was in consonance with the findings of Christine and Walter (2005), that blood group A was more infected with P. falciparum than other species of malaria and blood group B was commonly infected with P. malariae and P. ovale. The reason may be due to the availability of specific binding sites of blood group A and B to P. falciparum and P. malariae respectively, which increased the cytoadherence of P. species to blood group antigens as opined by Ronnaly et al; 2012 and Uneke et al; 2006.

The study shows that Plasmodium infection was more among the participants of haemoglobin genotypes AA and blood group A, implying high risk of malaria infection among people belonging to these blood parameters. Apart from focusing on pregnant women and under-five children in the control of malaria, government should also consider individuals of Hb-genotype AA and blood group A in the control of the disease.

Competing interests

The authors declare that they have no competing interests.

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References


