Original Article

Prevalence of malaria and human blood factors among patients in Ethiope East, Delta State, Nigeria

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ABSTRACT

Background: Malaria has been and is still a major protozoan disease affecting the human population. Erythrocyte polymorphisms (mainly in blood groups and genotypes) influence the susceptibility to severe malaria. Aim: This study is aimed at assessing the prevalence of malaria in relation to human blood factor and to identify the susceptibility of malaria to these factors. Methods: The prevalence of malaria with blood groups and genotypes were determined in 206 subjects attending the three major medical centres in Eku-Abraka metropolis. Results: It was found that blood group O was present in 60.68% of the population, with 17.98%, 16.99% and 4.37% in B, A and AB respectively, while genotype AA was highest with 75.73% prevalence. Blood group O+ was associated with 85.71% infection, as the most susceptible of the blood groups. It was shown that dominant homozygotes, HbAA, were more susceptible than the sickle cell trait, HbAS, while sickle cell disease, HbSS, were most vulnerable to the plasmodial parasite. A combination of blood group O+ and AS genotype showed the least susceptibility to malaria with significant sample size. Conclusion: This work provides insights into the relationship between malaria, blood groups and genotypes.

Key words: Malaria, prevalence, plasmodium, blood groups, genotypes, erythrocyte polymorphism

INTRODUCTION

Malaria is the most highly prevalent tropical disease with high morbidity and mortality and high economic and social impact.1,2 The disease remains a major public health problem in Nigeria where it is endemic especially in rural populations especially after rains and floods where stagnant water, overcrowding and improper sanitation predisposes to malaria, as is the case elsewhere in Africa.3,4 The disease accounts for 40% of public health expenditure, 30-50% of in-patient admissions and up to 50% of out-patient visits in areas with high malaria transmission.5,6
Human malaria is commonly caused by five *Plasmodium* species: *Plasmodium vivax*, *P. malariae*, *P. falciparum*, *P. ovale* and *P. knowlesi* each with their geographical location and varied incubation periods (IP), from infection to manifestation of symptoms with *P. falciparum* causing 80% of infections and 90% of deaths worldwide.\(^7, 8\)

The extent to which man becomes victim of malaria depends in the first place on how his own habits affect his accessibility to vector.\(^9\) For instance, location and type of housing with reference to vector breed habit, night travels in malarious areas, out-door sleeping, migration and cooperation in control and eradication operations, all are factors that may determine presence or absence of infections.\(^9\) Also, the feeding habit of the vectors could be twilight or night feeders, some species are day feeders and could contribute to the spread of the infection.\(^10\)

When an infectious *Anopheles gambiae* (mosquito) bites, it introduces about 3000 sporozoites into the person at each feeding.\(^8, 11\) The sporozoites travel to the liver whereas series of development take place.\(^8, 9\) Several tropical mosquito species are capable of transmitting the human plasmodia, and as a result, the disease is endemic to many tropical areas. In Africa, where 90% of the world’s malaria cases exist, *A. gambiae* is the main vector for the *Plasmodia* especially *P. falciparum*.\(^12\) This *Anopheles* species is highly anthropophilic (prefer to feed on humans) and survives longer than required *Plasmodium* incubation period.\(^10\)

Host susceptibility to malaria infection and disease is regulated by hereditary and acquired factors such as human blood groups (ABO) and genotypes (AA, AS, and SS).\(^13\) Blood group antigens are hereditary determined and plays a vital role in transfusion safety, understanding genetics, inheritance pattern and disease susceptibility.\(^14, 15\) In Nigeria, malaria results in 25% infant and 30% childhood mortality.\(^16\) More than 90% of the total population is at risk of malaria and at least 50% of the population suffers from at least one episode of malaria each year.\(^17\) Research coordinated by the World Health Organisation (WHO) has found that sleeping under nets treated with insecticide can greatly reduce deaths from malaria, especially among children.\(^18\) By 2025, an estimated 700 million people will live in urban communities in Africa.\(^19\) With such rapid expansion, identification of the risk factors for urban malaria requires urgent attention.\(^5, 20\) Haemoglobin is the oxygen-carrying pigment of the red blood Cells (RBCs) and is a chromoprotein that contains four heme groups, which are the pigment-containing part and globin, the protein part.\(^21\)

This current study reports the aspects of ABO Blood group system, HbA and HbS genotypes and checking the prevalence of malaria in Eku-Abraka metropolis.

**METHODOLOGY**

**Study area and subject selection**

This study was carried out between the month of October and November, 2012 in Eku and Abraka metropolis situated between latitude 5° 54’ and longitude 5° 40’. Eku and Abraka are two major towns in Ethiope East Local Government Area of Delta State, Nigeria. The inhabitants are mainly farmers and traders with high concentrations of primary, secondary and one tertiary institution. Three (3) laboratories of major hospitals in the area (Eku Baptist Hospital; Government Hospital Abraka and Delta State University Health Service Department Laboratories) were used as collection/sampling sites. Blood samples were collected from patients/subjects that came for malaria tests (outpatients) or in patients whose doctors requested for malaria tests. Hospital numbers or IDs and personal data were collected from each patient for easy access and identification.

**Blood sample collection**

After obtaining verbally informed consent, capillary or venous blood samples were collected from a total of 206 patient; (92 males and 114 females) were selected from patients attending the Out-Patients Department of the Eku Baptist Hospital, Government Hospital Abraka and Delta State University Health Center (DELSU), Abraka using a heparinised capillary tube or 2ml volume sterile syringe and needle.

**Laboratory analysis**

Blood samples were collected and analyzed within 2 hours of collection. Thick and thin blood films were prepared according to the technique described by Hanscheid\(^22\) and Cheesbrough.\(^23\) A drop of each blood sample was placed in the center of a grease-free clean glass slide.\(^24\) The reverse side of the slide was cleaned with cotton wool, air-dried and stained with Field’s stain.\(^24\) The

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slide was held with the dried thick film side facing downward and dipped in 3% Giemsa solution for 45 min; washed off gently in clean water and then dipped in Field's stain B (methyl azure) for 5s and washed again in clean water. A back of the slide was cleaned with cotton wool and kept in the draining rack to air-dry for eventual examination using standard methods under the microscope. Determination of blood group

The Coombs Direct Agglutination Method was used with commercially purchased ABD antisera (Anti-A, Anti-B and Anti-D Antisera). A drop of whole un-coagulated blood was mixed thoroughly with a drop of respective antiserum and the mixture rocked gently for one minute. Determination of genotype

A drop of un-coagulated whole blood was diluted with equal volume of distilled water to lyse the blood cells (forming the lysate), releasing the haemoglobin pigment. Cellular acetate strips/paper were soaked in the working buffer for some time, then brought out and dried with filter paper. Haemoglobin lysate was spotted in duplicates at about 1cm from one end of dried cellulose acetate strip. Haemolysate of reference of AS individuals was also spotted on the same acetate strip as controls. The two compartments of the electrophoretic tank, containing same working buffer, where connected to power/electric source; one to the anode (+) and the other to the cathode (-). The cellulose acetate strip with the haemolysate spots was placed on the runs of the tank to form a bridge between the two compartments. The strip was held in place at the two ends by the buffer-dampened filter paper. A constant voltage of 150V (2mA current) was maintained through the power source. Within five to ten (5-10) minutes migration occurs and haemoglobin spots were seen as pink spots on the strip. This method was adopted from Uzoegwu and Onwurah. Ethical clearance

Ethical permit for the study was obtained from the Ethical Committee of the institution. Ethical standards were strictly adhered to and informed consent was obtained from each of the subject examined. Data analysis

Prevalence of Plasmodium was calculated as the proportion of positive samples. The student t-test was used in determining the statistical difference using Microsoft word 2003. The significance was taken at $P<0.05$. RESULTS

A total of 206 subjects (92 (44.66%) males and 114 (55.34%) females) were involved in this study and their ages, sexes, blood groups and genotypes were used for analyses. Majority of the subjects were students of Delta State University, residing both in and outside the university campuses. Other subjects were either staff of the university, traders, farmers or residents around the sampling sites. From questioning, it was found that majority of the subjects were ignorant of their blood groups and genotypes. Some had no idea of what they meant, even among the students. Overt febrile symptoms were seen in majority of the subjects.

Table 1 shows that age group 21-30 had the highest attendance (76/206) and as well the highest infection in the population (85.53%). Age group 61-70 had the least attendance of 6 subjects and 5 of them (83.33%) were infected. The table also shows that the prevalence of malaria in the population is very high, with 173 (83.98%) infected subjects. As shown in table 2, blood group AB, had the least percentage occurrence of 4.37% with no subject found to be rhesus negative (AB). Blood group A and B were shown to be of almost same prevalence of 16.99% and 17.96% respectively, although blood group A had a higher prevalence of rhesus positive (Rh+) subjects. Blood group O manifested the highest prevalence of 60.68%. 21 subjects (10.19%) were rhesus negative (Rh), while 185 (89.81%) were rhesus positive (Rh+). Data from table 3 shows that males were with a higher prevalence (89.13%) and hence were invariably more susceptible to malaria infection. Females showed a lesser susceptibility with a prevalence of 79.82%. In each blood group, females also showed a relatively lesser susceptibility across all blood groups. One would observe from table 4 that blood group B+ had least infection with 66.67% infection. Blood groups O+, A+ and O- had higher infections of 85.71%, 84.86% and
84.61% respectively. Blood groups B+ and AB+ had prevalence of 80.63% and 77.78% respectively. All subjects of A- blood group were infected. Total prevalence of malaria was at a high prevalence of 83.98%.

As shown in table 5, subjects with genotype AA amounted to a total of 156; 67 males (42.95%) and 89 females (57.05%), which represents 75.73% of the total sample population. Genotypes AS and SS were found in 19.90% and 4.37% of the total population with males having relatively higher prevalence in both genotypes.

Table 6 shows that in AA and AS genotypes females were less vulnerable to plasmodial infections, with prevalence of 80.90% and 70.00% respectively as compared to 89.55% and 85.71% in males. SS genotype showed equal prevalence as all subjects were infected in both males and females.

From Table 7, it can be observed that all subjects of genotype SS (abnormal haemoglobin) were infected with plasmodial parasite. AS genotype shows least susceptibility with 78.05% infection, while that of AA was 86.84%.

It can be deduced from Table 8 that the subjects with the most susceptible combination of blood group and genotype are those of A+:AS, A+:SS, A-:AA, B+:SS, O+:SS, O-:AS, and AB+:AS, all with 100% infection. O+:AS combination had the second least prevalence of 70.83% infection. Following closely are AB+:AA and B+:AS combinations, both having same prevalence of 71.43%.

DISCUSSION

In tropical Africa especially Nigeria, malaria epidemics are common in mostly rural, less privileged population without effective alert systems. Although levels of transmission in urban areas may be lower than in contiguous rural areas, high population densities and possible lower immunity (due to lack of repeated infections with multiple strains of malaria parasites) may result in more disease impact in urban settings.

The prevalence of malaria in the present study population was 83.98%. This is high compared to the overall prevalence of 59.9% reported in a study by Ojo and Mafiana among children under15 years in Abeokuta, also in Southwestern Nigeria and 51.5% reported by Epidi et al among blood donors in Abakaliki, southeastern Nigeria. Findings from this study are quite higher than previous estimates from passive surveillance of suspected malaria case-patients. Anumudu et al reported 17% prevalence in Eastern Nigeria while Umeaneato and Ekejindu reported 46% prevalence in Nwewi, Anambra state. Atif et al reported an incidence of 10.5% malaria infection among 1000 patients in Hyderabad, Pakistan. It appears that the 83.98% observed in this study is the highest malaria prevalence recorded in recent times.

Studies have also shown seasonal variations in the rate of infections and differences in the types of malaria parasite depending upon the climatic condition. Plasmodium malariae causes Quartan malaria (every 72 hours) with Incubation Period (IP) of 18-40 days. P. vivax and P. ovale cause benign tertian malaria (every 48 hours) with IP of 12-17 days and 16 –18 days respectively. P. falciparum causes subtertian or malignant malaria (every 24-48 hours or continuous) with IP of 9-14 days. P. falciparum causes 80% of infections and 90% of deaths worldwide. Its merozoites hide in dead liver cells and release cloaking chemicals so that the body does not clean up those dead cells like it should. P. falciparum displays over 60 variations of adhesive surface proteins on surface of infected RBCs, which stick to walls of blood vessels and prevents travel to spleen (where they would have been destroyed).

Malaria parasites do not develop well in ovalocytes and it has been suggested that ovalocytosis, which is quite common in some malarious areas, such as New Guinea, may reduce the prevalence of malaria. Some investigators have suggested that Glucose 6-Phosphate Dehydrogenase (G6PD) deficiency as well as a number of other haemoglobinopathies (including the thalassemias and haemoglobin E), also protect against malaria infection, though the evidence for these associations are not so compelling. Pre-infection has also been shown to confer some level of immunity to malaria (Acquired immunity). This comes from studies in malarious areas where both prevalence and severity of malaria infections decrease with age.
Table 1: Prevalence of malaria among subjects by age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number Examined</th>
<th>Number Infected</th>
<th>% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>28</td>
<td>23</td>
<td>82.14</td>
</tr>
<tr>
<td>11 – 20</td>
<td>22</td>
<td>18</td>
<td>81.82</td>
</tr>
<tr>
<td>21 – 30</td>
<td>76</td>
<td>65</td>
<td>85.53</td>
</tr>
<tr>
<td>31 – 40</td>
<td>27</td>
<td>23</td>
<td>85.19</td>
</tr>
<tr>
<td>41 – 50</td>
<td>31</td>
<td>26</td>
<td>83.87</td>
</tr>
<tr>
<td>51 – 60</td>
<td>16</td>
<td>13</td>
<td>81.25</td>
</tr>
<tr>
<td>61 – 70</td>
<td>6</td>
<td>5</td>
<td>83.33</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>173</td>
<td>83.98</td>
</tr>
</tbody>
</table>

Table 2: Distribution of blood group among subjects

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>A+</th>
<th>A-</th>
<th>B+</th>
<th>B-</th>
<th>O+</th>
<th>O-</th>
<th>AB+</th>
<th>AB-</th>
<th>Total</th>
<th>Number examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+</td>
<td>33</td>
<td>02</td>
<td>31</td>
<td>06</td>
<td>112</td>
<td>13</td>
<td>09</td>
<td>00</td>
<td>206</td>
<td></td>
</tr>
<tr>
<td>Percentage of population</td>
<td>16.02</td>
<td>0.97</td>
<td>15.05</td>
<td>2.91</td>
<td>54.37</td>
<td>6.31</td>
<td>4.37</td>
<td>0.00</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Total of blood type (+&amp;-)</td>
<td>35</td>
<td>37</td>
<td>125</td>
<td>09</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Overall Percentage of population</td>
<td>16.99</td>
<td>17.96</td>
<td>60.86</td>
<td>4.37</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Prevalence of malaria among subjects by blood groups and sex

<table>
<thead>
<tr>
<th>Blood groups</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Examined</td>
<td>Infected (%)</td>
</tr>
<tr>
<td>A+</td>
<td>16</td>
<td>14 (87.50)</td>
</tr>
<tr>
<td>B+</td>
<td>15</td>
<td>13 (86.67)</td>
</tr>
<tr>
<td>O+</td>
<td>48</td>
<td>43 (89.58)</td>
</tr>
<tr>
<td>AB+</td>
<td>03</td>
<td>03 (100.00)</td>
</tr>
<tr>
<td>A-</td>
<td>02</td>
<td>02 (100.00)</td>
</tr>
<tr>
<td>B-</td>
<td>03</td>
<td>02 (66.67)</td>
</tr>
<tr>
<td>O-</td>
<td>05</td>
<td>05 (100.00)</td>
</tr>
<tr>
<td>AB-</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Total (%)</td>
<td>92 (44.66)</td>
<td>82 (89.13)</td>
</tr>
</tbody>
</table>
Table 4: Malaria Infection among subjects by blood groups

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>A+</th>
<th>A-</th>
<th>B+</th>
<th>B-</th>
<th>O+</th>
<th>O-</th>
<th>AB+</th>
<th>AB-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Examined</td>
<td>33</td>
<td>02</td>
<td>31</td>
<td>06</td>
<td>112</td>
<td>13</td>
<td>09</td>
<td>00</td>
<td>206</td>
</tr>
<tr>
<td>Number Infected</td>
<td>28</td>
<td>02</td>
<td>25</td>
<td>04</td>
<td>96</td>
<td>11</td>
<td>07</td>
<td>00</td>
<td>173</td>
</tr>
<tr>
<td>Percentage (%) Infected</td>
<td>84.85</td>
<td>100</td>
<td>80.63</td>
<td>66.67</td>
<td>85.71</td>
<td>84.81</td>
<td>77.78</td>
<td>00</td>
<td>83.98</td>
</tr>
</tbody>
</table>

Table 5: Distribution of Subjects with respect to genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Examined</th>
<th>Males</th>
<th>Females</th>
<th>Percentage (%) Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>156</td>
<td>67 (42.95)</td>
<td>89 (57.05)</td>
<td>75.73</td>
</tr>
<tr>
<td>AS</td>
<td>41</td>
<td>21 (51.22)</td>
<td>20 (48.78)</td>
<td>19.90</td>
</tr>
<tr>
<td>SS</td>
<td>09</td>
<td>05 (55.56)</td>
<td>04 (44.44)</td>
<td>04.37</td>
</tr>
</tbody>
</table>

Table 6: Prevalence of malaria in genotypes by sex

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AA</th>
<th>AS</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Examined</td>
<td>67</td>
<td>89</td>
<td>21</td>
</tr>
<tr>
<td>Infected</td>
<td>60</td>
<td>72</td>
<td>18</td>
</tr>
<tr>
<td>Percentage Infected (%)</td>
<td>89.55</td>
<td>80.90</td>
<td>85.71</td>
</tr>
</tbody>
</table>
Immunity (or, more accurately, tolerance) to malaria parasitaemia does occur naturally, but only in response to repeated infection with multiple strains of malaria, especially among adults in areas of moderate or intense transmission conditions.\[39\] Host susceptibility to malaria infection and disease is regulated by hereditary and acquired factors.\[40,41\] The virtual absence of *P. vivax* infections in many areas of Africa is explained by the fact that most blacks do not express the Duffy protein on their RBCs.\[40\] They are carriers of the Duffy null-phenotype \(\text{Fy}(a^b^-)\), found in 68% of black people but rare in white populations, therefore most blacks are immune to *P. vivax* infection.\[42\] The Duffy glycoprotein is a receptor chemical that is secreted by blood cells during inflammation and also happens to be a receptor of *P. vivax*; without the Duffy antigen, the parasite cannot invade the RBCs.\[43\] The major institution in the study area is the Delta State University, hence the high occurrence of subjects within the age group of 21-30 in the study area. The University environment cannot be classified as healthy, especially during the wet season. The population of the inhabitants of the university...
The high prevalence of infection in blood group O shows that they are most susceptible to infection. This corresponds to the result gotten by Rowe et al.\cite{52} in Mali, where blood group O had a higher prevalence of uncomplicated malaria. This study considered only mild or non-severe cases of malaria infection, hence the observed prevalence.

The population having a high percentage AA genotype shows natural selection against sickle cell anemia, characteristic of the SS genotype. However, the occurrence AS and SS individuals amounting to 19.90% and 4.37% of the population respectively, shows that a balancing natural selection force against malaria is still active in the population, as AS individuals are less susceptible to malaria. High infection in SS subjects is not surprising as plasmodial infections of Sickle Cell Anemia persons trigger off a concentration-dependent polymerization of HbS in RBC.\cite{53} Following the HbS polymerization, the RBCs containing them will assume sickle shape, become fragile and abnormal and are consequently destroyed by the immune cells.\cite{53,54} Subsequently, haemolytic anemia sets in to provoke sickle cell crisis.\cite{26} On the other hand, plasmodial infection of AS RBCs is not as serious. Only HbS is polymerised on infection leaving HbA intact.\cite{26} This event could also stimulate the immune cells to destroy the RBC containing the Hbs and the plasmodial parasites.\cite{26} The parasitised HbA-RBC survive.\cite{26} This fact makes dominant homozygotes more susceptible to malaria than sickle heterozygotes (AS).\cite{26} Confirmation of resistance factor of blood group O and genotype AS is made clear in a combination of both (O+:AS), showing the least infection, when significant sample sizes are considered.

CONCLUSION

The high prevalence malaria in the population undermining blood groups and genotypes is as a result of the onset of the wet season, which favours the activities of mosquito vectors of malaria. From the results, it shows that a combination of blood group O and AS genotype is the least susceptible to malaria infection. However a longer period of sampling and considerations
of severe cases of malaria are needed to properly ascertain the rate of survival of the patients of the various blood groups and genotypes.

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Conflict of Interest: None declared