Frequencies of some human genetic markers and their association with *Plasmodium falciparum* malaria in the Niger Delta, Nigeria

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**Abstract**

*Background & objectives:* There is paucity of information on the association between *Plasmodium falciparum* malaria and some human genetic markers in the Niger Delta region of Nigeria. Hence, a study was undertaken in children to assess the current level of subclinical malaria due to *P. falciparum*.

*Methods:* Blood groups ABO and Rhesus factor, haemoglobin electrophoretic pattern, G-6-PD deficiency status and malaria were determined among 240 apparently healthy children in a cross-sectional descriptive study using standard procedures.

*Results:* The prevalence of *P. falciparum* malaria in this region was high (27.5%). Blood group O (51.3%) dominated the study population, followed by B (23.8%), A (21.3%), and AB (3.8%). Rhesus D positive accounted for 91.3% while Rh D negative was 8.7%. Sickle-cell trait (HbAS) prevalence was 12.5% while HbAA accounted for 87.5%. In all, 5.42% of the children were G-6-PD deficient while 94.58% had normal G-6-PD status. Chi-square analysis revealed that only blood group O and Rh D negative had a significant association with *P. falciparum* malaria (\(\chi^2 = 4.3636, \ p < 0.05\) and \(\chi^2 = 5.760, \ p < 0.02\) respectively). No significant association was found to exist between *P. falciparum* malaria and other genetic markers.

*Conclusion:* This study has provided the current prevalence rates of some genetic markers in a malaria endemic region of Niger Delta, Nigeria. Of all the genetic markers tested, only Blood group O and Rh D negative had significant and positive associations with *P. falciparum* infection.

**Key words** ABO blood group; G-6-PD deficiency; HbAA; HbAS; Niger Delta; Nigeria; *Plasmodium falciparum* malaria; Rh factor

**Introduction**

Despite all efforts to roll back malaria in Africa, the malaria parasite *Plasmodium falciparum* still remains the leading cause of child mortality which annually kills >1 million children in Africa alone. This death toll is only one aspect of the global burden of malaria\(^1\). In Nigeria, malaria still constitutes a serious health problem. It is responsible for 60% outpatient visits to health facilities, 30% childhood deaths, 25% of deaths in children under one year and 11% of maternal deaths (4500 die yearly)\(^2\). In Nigeria, a child will be sick of malaria between 2 and 4 times in one year and 70% of pregnant women suffer from malaria contributing to maternal anaemia, low birth weight, still birth, abortion and other pregnancy related complications. The financial loss due to malaria annually is estimated to be about 132 billion naira in the form of treatment cost, prevention, loss of man-hours etc\(^2\). In regions of high malaria transmission, every member of the community might be chronically infected and as such there could be a high
prevalence of sub-clinical malaria. *Plasmodium falciparum* has been called “the strongest known force for evolutionary selection in the recent history of human genome.” Thus, it has been hypothesized that *P. falciparum* has shaped the distribution of ABO blood groups in man, i.e. the current worldwide distribution of ABO groups is consistent with an effect from *P. falciparum* and that certain blood groups protect humans from the lethal effect of *P. falciparum* infection. Malaria has also been hypothesized as the evolutionary driving force behind sickle-cell disease, glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and other erythrocyte defects like Duffy null phenotype. It has been reported that HbS homozygotes suffer from sickle-cell disease but heterozygotes (HbAS) have a 10-fold reduced risk of severe *P. falciparum* malaria.

Deficient G-6-PD enzyme activity has also been shown to correlate with protection against severe malaria in Nigerian children. A study of more than 2000 Gambian and Kenyan children found that the common African form of G-6-PD deficiency (G-6-PDA−) is associated with about 50% reduced risk of severe malaria in female heterozygotes and in male hemizygotes. Reduced parasite replication in G-6-PD deficient erythrocytes is thought to be the mechanism of protection but the parasite appears to counter this by manufacturing G-6-PD itself.

The main objective of this study was to assess the current level of subclinical malaria due to *P. falciparum* in the Niger Delta region of Nigeria, the prevalence of some genetic markers among children and evaluate the extent of association between *P. falciparum* and these genetic markers.

### Material & Methods

**Study area:** This study was conducted between March 2005 and April 2006 in Rumueme, Port Harcourt, Nigeria. The geographical location is latitude 447° 21’ and longitude 659° 55’. The study area lies in a typical deltaic wetlands (Niger Delta) and perennial malaria transmission is present. The study population consisted of 240 children aged 1–8 yr of both sexes recruited from households and schools in a cross-sectional descriptive study. All the children had auxiliary body temperature of <37.5°C. None had symptoms suggestive of malaria or any other systemic illness. After obtaining informed consents from their parents, the children were brought to the research center where 2 ml of blood sample was collected into ethylenediamine tetracetic acid (EDTA) containing tubes. This study received ethical approval from the Rivers State University of Science and Technology, Port Harcourt, Nigeria. *Plasmodium falciparum* diagnosis was done using microscopic examination of Giemsa stained thin and thick blood smears under oil immersion lens at x100 magnification. PCR techniques could not be employed for the diagnosis due to some technical constraints and higher cost.

Haemoglobin electrophoretic pattern of the participants were determined using haemoglobin electrophoresis by cellulose acetate membrane at pH 8.9 as described by Brown. Haemolysates from blood sample of known haemoglobin (i.e. AA, AS, AC) were included as controls. A qualitative determination of G-6-PD in red cells of participants was carried out using the G-6-PD deficiency screen reagent set (Pointe Scientific, USA. Lot 513704). Red cell phenotyping was carried out with standard tube techniques as described by Brecher. ABO blood typing was done using anti-A, anti-B and anti-AB (Biotec, Ipswich, UK) agglutination method. Rhesus D typing was done using anti-D serum (Biotec, Ipswich, UK) agglutination method and the negative results were confirmed using the indirect agglutination test (IAT) procedure.

**Statistical analysis:** Data were analyzed using SPSS (version 12) for windows (SPSS Inc, Chicago). Statistical analysis included frequency distribution and chi-square test for measure of association and *p* <0.05 were considered statistically significant.

### Results

The prevalence of *P. falciparum* malaria in the study
The distribution of ABO and Rh blood groups are as follows: O (51.3%), B (23.8%), A (21.3%), AB (3.8%), Rh D positive (91.3%) and Rh D negative (8.7%). A significant association was found to exist only between blood group O, Rh D negative and *P. falciparum* malaria. This association is a positive association that 33 in group O subjects were expected to be parasitized, but more (45) were observed to be parasitized, and out of five in Rhesus D negative group expected, nine were parasitized (Table 2).

Similarly, no association was found to exist between haemoglobin electrophoretic phenotypes and *P. falciparum* malaria, 12.5% were HbAS while 87.5% were HbAA. Of the 12.5%, HbAS, 6 (2.5%) were infected with malaria. G-6-PD deficient subjects accounted for 5.42% out of which 4 (1.6%) were parasitized. No association was also found to exist between G-6-PD status and *P. falciparum* parasitaemia (Table 2).

**Discussion**

In the last 20 years, there has been increasing evidence that blood groups have a function and play a biological role in disease association especially malaria. Much of these reports relate to severe malaria with little or no reference to subclinical or asymptomatic infection. Children were chosen as subjects because they belong to the most vulnerable group to *P. falciparum* infection. In this study, group O subjects dominated the study population, followed by B, A, and AB which is in consistent with previous reports that group O is the dominant blood group among Nigerians13,14.

Group O individual lack the terminal glycosyl transferases necessary to produce A or B antigen and carry the disaccharide H antigen (Fucα1–2 Galβ1). The A and B trisaccharides are thought to act as receptors for rosetting on uninfected erythrocytes and direct binding between the parasite rosetting ligand PfEMP1 and the A antigen has been demonstrated. *P. falciparum* rosettes are able to form in group O
cells but these rosettes tend to be smaller and more easily disrupted than rosettes formed in group A, B or AB erythrocytes. Thus, through this mechanism of rosetting and cytoadherence, blood group O is able to protect against severe *P. falciparum* malaria.

There is a high prevalence of subclinical malaria (27.5%) in this region even among children. A significant association was seen to exist between blood group O and *P. falciparum* malaria which is in consonance with few cross-sectional studies involving asymptomatic children and few longitudinal and case-control studies involving apparently healthy children. However, this study is at variance with almost all the studies in Nigeria where absence of significant association was observed between *P. falciparum* and ABO blood group. Evidence from the literature has established that parasitized erythrocytes form rosettes more readily with RBCs of either A, B or AB blood groups than with those belonging to blood group O. It is also well established that this parasite-triggered RBC rosette formation is associated with the severity of clinical disease. This may explain why severe malaria, lower haemoglobin levels, jaundice or central nervous system disorders were relatively more frequent in individuals of non-O blood groups as was observed in Zimbabwe. However, this study observed that more than expected, number of subjects were significantly infected with *P. falciparum* malaria. The implication is that the association between blood group O and malaria is a positive association.

Concerning the Rh system, an association with Rhesus D negative was established with malaria in this study. Earlier report showed that E phenotype individuals exhibited a higher number of malaria episodes than ee phenotype. No literature was encountered that supported the association of Rhesus D negative blood group with *P. falciparum* infection. Since long, it has been known that sickle-cell trait (HbAS) affords protection against *P. falciparum* infection. In our data, no significant association between AS phenotype and malaria was established.

An important form of defense against oxidative stress within the erythrocyte is the production of the electron donor nicotinamide dinucleotide phosphate by the enzyme G-6-PD. This enzyme is encoded by G-6-PD on chromosome X of which many different variants are known, and those that markedly compromise enzyme activity result in hemolytic anaemia. In Africa, the dominant strain that is associated with protection against severe *P. falciparum* is G-6-PD A–8. In a recent report, the X-linked G-6-PD deficiency has been found to protect homozygous males but not heterozygous females against malaria. In this study, we did not genotype to isolate the strain prevalent here in Port Harcourt. This study only screened the children for G-6-PD deficiency of which 5.42% were deficient. The sex differences were not studied either, however, we found no association between G-6-PD status and *P. falciparum* infection in this study. It is noteworthy that in our setting, PCR techniques have not been introduced as a routine procedure for malaria detection probably due to costs, hence microscopic examination still remains the method of choice in a developing country such as ours. The implication is that the prevalence rate may be lower in cases of subclinical malaria. Sensitive procedures like the PCR if used could pick some of the parasites that would have been missed by microscopy.

This study has provided the current prevalence rates of some genetic markers in a malaria endemic region.
of Niger Delta, Nigeria. Of all the genetic markers tested, only blood group O and Rhesus D negative had significant associations with *Plasmodium falciparum* infection but sadly enough, this association was not found to be favourable and needs to be confirmed.

References


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