J Vector Borne Dis 47, March 2010, pp. 11–16

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

Z.A. Jeremiah[a], T.A. Jeremiah[b] & F.O. Emelike[c]

[a]*Department of Medical Laboratory Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State;* [b]*Pollution Control Department, Ministry of Environment, Rivers State;* [c]*Department of Medical Laboratory Sciences, Ambrose Ali University, Ekpoma, Edo State, Nigeria*

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 (χ^2= 4.3636, p <0.05 and χ^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[3]. *Plasmodium falciparum* has been called "the strongest known force for evolutionary selection in the recent history of human genome"[4]. 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. falciparium* and that certain blood groups protect humans from the lethal effect of *P. falciparum* infection[5]. 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[4]. 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[6,7].

Deficient G-6-PD enzyme activity has also been shown to correlate with protection against severe malaria in Nigerian children[8]. 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[9] but the parasite appears to counter this by manufacturing G-6-PD itself[10].

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[11]. 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[12]. ABO blood grouping 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, Ipcswich, 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

Table 1. Prevalence of asymptomatic malaria among 240 children in Port Harcourt, Nigeria

Age groups (yr)	*P. falciparum* positive (%)	Total (%)	χ^2
1– ≤5	36 (36.36)	99 (41.25)	2.832^{ns}
5 – 9	30 (21.3)	141 (58.75)	1.985^{ns}
Total	66 (27.5)	240 (100)	

ns–Non-significant.

population is presented in Table 1. As the *P. falciparum* prevalence was 27.5%, our null hypothesis therefore, was that for any given genetic marker, the percentage of individuals with *P. falciparum* parasitaemia should be 27.5% and the percentage with no parasite should be 72.5% and if a given genetic marker has an association with asymptomatic parasitaemia, it would cause a significant deviation from the null hypothesis. Based on this, Chi-square values were calculated for any given genetic marker for the parasitized (asymptomatic) and non-parasitized group. Table 2 shows the frequencies of human genetic markers and association with *P. falciparum* malaria. 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).

Table 2. Frequencies of human genetic markers and *P. falciparum* malaria in Niger Delta, Nigeria

Parameter	Parasitized	Non-parasitized	Total	χ^2
Blood groups				
A	9 (3.8)	42 (17.5)	51 (21.3)	1.785^{ns}
B	9 (3.8)	48 (20)	57 (23.8)	1.066^{ns}
O	45 (18.8)	78 (32.5)	123 (51.3)	4.364*
AB	3 (1.3)	6 (2.5)	9 (3.8)	0.150^{ns}
Rh factor				
D positive	57 (23.8)	162 (67.5)	219 (91.3)	0.150^{ns}
D negative	9 (3.8)	12 (5.0)	21 (8.7)	5.760*
Hb Electrophoretic pattern				
AA	60 (25)	150 (62.5)	210 (87.5)	0.083^{ns}
AS	6 (2.5)	24 (10.0)	30 (12.5)	0.843^{ns}
G-6-PD				
Normal	62 (26.3)	165 (68.8)	227 (94.58)	3.735^{ns}
Deficient	4 (1.6)	9 (3.75)	13 (5.42)	0.148^{ns}

*Significant association with malaria (p <0.05); ns – Non-significant; Figures in parentheses are percentages.

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 Nigerians[13,14].

Group O individual lack the terminal glycosyl transferases necessary to produce A or B antigen and carry the disaccharide H antigen (Fucα 1–2 Galβ–1). 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[15].

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[16,17] and few longitudinal and case-control studies involving apparently healthy children[18,19]. 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[20–23]. 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[24,25]. It is also well established that this parasite-triggered RBC rosette formation is associated with the severity of clinical disease[24]. 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[17]. 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[26]. 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[27]. In our data, no significant association between AS phenotype and malaria was established. The difference between the expected and observed counts was not significant. However, a number of mechanisms have been proposed to mediate the protection afforded by HbAS; these include accelerated sickling of parasite infected HbAS erythrocytes, poor parasite invasion and growth rates in HbAS erythrocytes, and enhanced phagocytosis of infected erythrocytes but the relative contribution of any or all of these *in vivo* is not known[28]. Recent reports indicate that the sickle-cell trait is associated with enhanced immunoglobulin G antibody responses to *P. falciparum* variant surface antigens[29]. This may probably apply in severe infection rather than asymptomatic state.

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[4]. 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[30]. 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 *P. falciparum* infection but sadly enough, this association was not found to be favourable and needs to be confirmed.

References

1. Snow RW, Guerra CA, Norr AM, Myint HY, Hay SI. The global distribution of clinical episodes of *P. falciparum* malaria. *Nature* 2005; *434:* 214–7.
2. Federal Ministry of Health, Nigeria. National malaria control programme in Nigeria. *Annual Report* 2005; p.1–27.
3. Trape JF, Rogier C, Konate L, Diagne N, Bouganali H, Canque B, Legros F, Badji A, Ndiaye G, Ndiaye P, Brahimi K, Faye O, Druilhe P, Pereira da Silva L. The Dielmo Project: a longitudinal study of national malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *Am J Trop Med Hyg* 1994; *51:* 123–37.
4. Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria? *Am J Hum Genet* 2005; *77:* 171–92.
5. Cserti CM, Dzik WH. The ABO blood group system and *Plasmodium falciparum* malaria. *Blood* 1997; *110:* 2250–8.
6. Hill AV, Allsopp CE, Kwiatkowski D, Anstey NM, Twamasi P, Rowe PA, Bennett S, Brewster D, McMichael AJ, Greenwood BM. Common West African HLA antigens are associated with protection from severe malaria. *Nature* 1991; *352:* 595–600.
7. Ackerman H, Usen S, Jallow M, Sisay-Joof F, Pinder M, Kwiatkowski DP. A comparison of case-control and family-based associated methods: the example of sickle-cell and malaria. *Ann Hum Genet* 2005; *69:* 559–65.
8. Gillies HM, Fletcher KA, Hendrickse RG, Lindner R, Reddy S, Allan N. Glucose-6-phosphate dehydrogenase deficiency, sickling and malaria in Africa children in south-western Nigeria. *Lancet* 1967; *1:* 138–40.
9. Luzzatto L, Usanga EA, Reddy S. Glucose phosphate dehydrogenase deficient red cells: resistance to infection by malaria parasites. *Science* 1969; *164:* 839–42.
10. Usanga EA, Luzzatto L. Adaptation of *Plasmodium falciparum* to glucose-6-phosphate dehydrogenase deficient host red cells by production of parasite encoded enzyme. *Nature* 1985; *313:* 793–5.
11. Brown BA. *Hematology: principles and procedures*. VI edn. Philadelphia, USA: Lea and Febiger 1993; p. 1–358.
12. Brecher M. *Technical manual.* XIV edn. Bethesda, USA: AABB 2002; p. 669–86.
13. Bakare AA, Azeez MA, Agbolade JO. Gene frequencies of ABO and Rhesus blood groups and haemoglobin variants in Ogbomosho, south-west Nigeria. *African J Biotechnol* 2006; *5:* 224–9.
14. Enosolease ME, Bazuaye GN. Distribution of ABO and Rh-D blood groups in the Benin area of Niger Delta: implication for regional blood transfusion. *Asian J Transfus Sci* 2008; *2:* 3–5.
15. Rowe A1, Handel IG, Thera MA, Deans AM, Luke KE, Kone A, Diallo DA, Raza A, Kai O, Marsh K, Piowe CV, Doumbo OK. Blood group O protects against severe *Plasmodium falcipanum* malaria through the mechanism of reduced rosetting. *Proc Natl Acad Sci* 2007; *104:* 17471–6.
16. Pant CS, Gupta DK, Bhatt RM, Gautam AS, Sharma RC. An epidemiological study of G-6-PD deficiency sickle-cell haemoglobin and ABO blood groups in relation to malaria incidence in Muslim and Christian communities of Kheda, Gujarat, India. *J Commun Dis* 1992; *24:* 199–205.
17. Fischer PR, Boone P. Short report: severe malaria associated with blood group. *Am J Trop Med Hyg* 1998; *58:* 122–3.
18. Migot-Nabias F, Mombo LE, Luty AJ, Duboiz B, Nabias R, Bisseye C, Millet P, Lu CY, Deloron P. Human genetic factors related to susceptibility to mild malaria in Gabon. *Genes Immun* 2000; *1:* 435–41.
19. Beigueloman B, Alves FP, Moura MM, Engracia V, Nunes ACS, Heekmann MIO, Ferreira RGM, Pereira da Silva LH, Camargo EP, Krieger H. The association of genetic markers and malaria infection in the Brazilian Western region. *Mem Inst Oswaldo Cruz* 2003; *98:* 455–60.
20. Martin SK, Miller LH, Hicks CU, David-West A, Ugbode C, Deane M. Frequency of blood group antigens in Nigerian children with falciparum malaria. *Trans R Soc Trop Med Hyg* 1979; *73: 216*–8.
21. Kassim OO, Ejezie GC. ABO blood groups in malaria and *Schistosoma haematobium*. *Acta Trop* 1982; *39:* 179–84.
22. Uneke CK, Ogbu O, Nwojiji V. Potential risk of induced malaria by blood transfusion in south-eastern Nigeria. *MJM* 2006; *9:* 8–13.
23. Akinboye DO, Ogunrinade AF. Malaria and loaisis among blood donors in Ibadan, Nigeria. *Trans R Soc Trop Med Hyg* 1987; *81:* 398–9.
24. Rowe A, Obeiro J, Newbold CL, Marsh K. *Plasmodium falciparum* rosetting is associated with malaria severity in Kenya. *Infect Immun* 1995; *63:* 2323–6.
25. Baragan A, Kremsner PG, Wahlgren M, Carlson J. Blood

group A antigen is a co-receptor in *Plasmodium falciparum* rosetting. *Infect Immun* 2000; *68:* 2971–5.

26. Camargo LMA, Noronha E, Salcedo JMV, Dutra AP, Krieger H, Pereira da ilver LH, Camargo EP. The epidemiology of malaria in Rondonia (Western Amazon region, Brazil): study of a riverine population. *Acta Trop* 1999; *72:* 1–11.
27. Allison AC. The distribution of sickle-cell trait in East Africa and elsewhere and its apparent relationship to the incidence of subtertian malaria. *Trans R Soc Trop Med Hyg* 1954; *48:* 312–8.
28. Abu-Zeid YA, Abdulahi NH, Hviid L. Lymphoproliferative responses to *Plasmodium falciparum* antigens in children with and without the sickle cell trait. *Scand J Immunol* 1991; *34:* 237–42.
29. Cabrera G, Cot M, Migot-Nabias F, Kremsner PG, Deloron P, Luty AJF. The sickle-cell trait is associated with enhanced immunoglobulin G antibody responses to *Plasmodium falciparum* variant surface antigens. *J Infect Dis* 2005; *191:* 1631–8.
30. Guindo A, Fairhuerst RM, Doumbo OK, Wellems TE, Diallo DA. X-linked G6PD deficiency protects hemizygous males but not heterozygous females against severe malaria. *PLOS Med* 2007; *4:* e66.

Corresponding author: Dr Z.A.Jeremiah, P.O. Box 1437, Diobu, Port Harcourt, 500001, Nigeria.
E-mail: zacjerry@yahoo.com; drzajeremiah@yahoo.com

Received: 8 October 2009 *Accepted in revised form:* 2 January 2010