The Interaction between Malaria parasites and Blood Groups in Port Harcourt, Nigeria.

ABSTRACT

The link between blood group Rhesus antigens and different malaria parasites as well as the prevalence of other Plasmodium species were studied among adults in Port Harcourt, Nigeria. Thick blood smears and filter paper blood spots were made from finger prick for microscopy and molecular genotyping of parasite strains. Two hundred and forty six participants aged 16-60 years attending the Braithwaite Memorial Hospital and blood donors presenting at the University of Port Harcourt Teaching Hospital Blood Bank were enrolled into the study. Blood samples were analysed for ABO antigen grouping using commercially prepared potent antisera. Isolated DNA was extracted from dried filter papers and analysed for Plasmodium specie identification using nested PCR method. Results obtained reveal that blood group O was most prevalent while the least was blood group AB. Parasitaemia results by microscopy shows that blood group O Rh positive was the highest with 163 (66.2%) followed by blood group A Rh positive 43 (17.5%), B Rh positive 26 (10.6%), O Rh negative 7 (2.85%), AB Rh positive 5 (2.03%), B Rh negative 1 (0.41%) and A Rh negative 1(0.41%). Nested PCR revealed the presence of low prevalence of *P.ovale* and *P.malariae* species (4.3% each) and a very high prevalence of *P. falciparum* (82.1%) as well as mixed infections of the species. These results show the association of blood groups, rhesus positivity and *Plasmodium* parasites as well as the presence ~~of other species~~ of *ovale* and *malariae* in the study area.

Key Words: malaria parasites, blood group, interaction

1. Introduction

The global burden of malaria infection remains more severe in Africa especially among children and pregnant ~~mothers~~ women. Malaria is a protozoan infection in humans caused by the parasite Plasmodium which until recently was thought to be classified into five species *P. falciparum, P. ovale, P. malariae, P. vivax* and *P. knwolesi.* However current study[1] ~~1~~ ~~(Sutherland et al., 2010)~~ has shown that there are two non-recombining species of *ovale*

(*ovale curtisi* and *ovale wallikeri*) which are non-sympatric in nature [2][2]~~(Oguike et al., 2011)~~. Of all these, *Plasmodium falciparum* is the agent of the most malignant form of malaria, usually presenting with severity mostly in children in sub-Saharan Africa.

The pathogenesis of *Plasmodium* infection entails merozoite invasion of erythrocyte, which implies an implicit interaction between the red cell membrane proteins and the invading plasmodium antigens. Blood group antigens are constituents of these proteins that are embedded in erythrocyte cellular membranes and are encoded by alleles at a single gene locus or at closely linked gene loci of which more than 300 specificities have been reported [3,4]~~(Johnson, 1992[3]; Contreras, 2001)[4]. –~~Additionally, blood group antigens have been reported to serve as genetic markers of several clinical conditions [5-8] ~~(Nwauche et al 2003, Vestibo et al, 1993, Slater, 1993, Brooks et al, 1988)[5-8]~~ while several workers have shown a definite relationship between the ABO and Rh blood group antigens with malaria [9,10]. ~~(Ademowo et al, 1993; Cserti et al, 2007)[9,10]~~. The clearest example of this is the interaction between *Plasmodium vivax* and the Duffy antigen [11,12] ~~(Langhi & Bordin, 2006; Carlos et al, 2007)[11,12].~~

1.1. Study Area

The study was carried out in two major hospitals: the University of Port Harcourt Teaching Hospital (UPTH) and the Braithwaite Memorial Specialist Hospital (BMH) both in Port Harcourt, Nigeria. The study was conducted from September 2010 to September 2011. Malaria is holoendemic in Nigeria. Nigeria has two main seasons, the dry seasons which occur from October to March and the wet or rainy season occurring from April to September with peak of the rains between May and July when malaria transmission is very intense. Port

Harcourt exhibits lengthy and heavy rain seasons with annual rainfall of more than 3,550 millimeters and average temperatures between 25˚C-28˚C.

The main vector of malaria in Nigeria is *Anopheles gambiae* but *A. funestus* and *A. arabiensis* are also commonly encountered. *A. melas* is found in the coastal areas [13] ~~(NPC & NMCP, 2012)[13]~~. The commonest species responsible for ~~to~~ 98% of the confirmed malaria cases in Nigeria in *Plasmodium falciparum.* The other specie that has been reported in Nigeria is the *P. malariae*, but it usually occurs as a mixed infection with *P. falciparum*. [13]. ~~(NPC & NMCP, 2012)[13].~~

2. Methods

2.1. Study Participants

Two hundred and forty six participants: 142 males (57.72%) and 104 females (42.28%) aged 16-60 years attending the Braithwaite Memorial Hospital and blood donors presenting at the University of Port Harcourt Teaching Hospital Blood Bank were enrolled into the study. Apart from the blood donors, many of the participants were general outpatients who attended the hospital for mild clinical conditions and were subsequently screened for malaria. The first participant was recruited based on meeting the eligibility criteria (positivity to malaria parasite by microscopy, willingness to participate in the study) and presenting on the start date of commencing the study. Subsequent participants were randomly recruited on a consecutive basis as they met the eligibility criteria. Consent was obtained from the participants through the signed informed consent forms. Ethics approval was obtained from the Ethics Committee of both hospitals and the London School of Hygiene and Tropical Medicine. The study was conducted from September 2010 to September 2011.

2.2. Screening for ABO and Rhesus D antigen

Blood donors were sampled and screened for ABO and Rhesus D antigen. The ABO and Rhesus D antigen testing was carried out according to previously described method ~~(Dacie and Lewis, 2001[14])~~ using commercially prepared potent antisera (anti-A, anti-B, anti-D) [14]. Briefly described, 3mls of blood were transferred to sterilin containers and red cells were separated and suspended in saline. One drop of 20%cell suspension was added to two drops of the antisera (cell to serum) in the following tubes-Saline, albumin and anti-human globulin (AHG) and were incubated at 37˚C. Positive samples were identified by agglutination.

Thick blood smears on glass slides and dry blood spots on Whatman filter papers were made from finger prick for microscopy and molecular genotyping of parasite strains. Participants were recruited if the blood samples collected were found *Plasmodium*-positive by microscopy. Genotyping of the DNA extracted from the filter paper blood spots for specie identification was carried out in the London School of Hygiene and Tropical Medicine, London for identification of *Plasmodium* species.

2.3. Isolation of DNA

Parasite DNA was extracted from dried filter papers using Chelex method as previously reported [15]. ~~(Dlamini et al, 2010)[15].~~ Briefly described, blood spots punched out from dried filter papers were soaked in a deep well plate in 0.5% saponin 1 x PBS solution and incubated overnight at 37˚C. Debris and saponin were removed after centrifugation and the filter papers were washed in PBS solution. They were subsequently suspended in 6% Chelex®100 and incubated in a water bath for 25 minutes at 100˚C in 6% Chelex®100 and centrifuged thereafter to remove resin. Approximately 100µl of supernatant containing the DNA was removed and stored in -20˚C freezer.

2.4. Plasmodium species identification by nested PCR

Amplification of the small sub-unit of ribosomal gene (SSU rRNA) of the *Plasmodium species* was carried out on extracted DNA using nested PCR to identify the different species present [16]. ~~(Snounou et al., 1993)[16].~~ PCR reactions was carried out under the following conditions: 95°C for 5min/ [58°C for 2min-72°C for 2min-94^{0}C for 1min] x25 cycles/58°C for 2min-72°C for 5min using primers (rPLU5+rPLU6) as listed below.

rPLU6	5'	TTAAAATTGTTGCAGTTAAAACG	3'genus F2
rPLU5 new	5'	CYTGTTGTTGCCTTAAACTTC	3'genus R

The amplified products were nested with primer mix consisting of 5 µM Primer mix (Pf/Pm/Pv/Po) under the cycling conditions– 95°C for 5mins, (58°C for 2mins; 72°C for 2mins; 94°C for 1min) x 30cycles; 58°C for 2mins, 72°C for 5mins, ~~4°C forever~~ for species differentiation using Snounou protocol [16] ~~[16]~~. The amplified products were run on agarose gel and size against Hyperladder IV to identify the different species. PCR conditions and primer sequences used are listed below.

rFAL1 5'-TTAAACTGGTTTGGGAAAACCAAATATATT-3'
rFAL2 5'-ACACAATAGACTCAATCATGACTACCCGTC-3'
rMAL1 5'-ATAACATAGTTGTACGTTAAGAATAACCGC-3'
rMAL2 5'-AAAATTCCCATGCATAAAAAATTATACAAA-3'
rVIV1 5'-CGCTTCTAGCTTAATCCACATAACTGATAC-3'
rVIV2 5'-ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA-3'
Pad Po 5' CTGTTCTTTGCATTCCTTATGC 3'

<u>Cycling conditions</u> – 95°C for 5mins, (58°C for 2mins; 72°C for 2mins; 94°C for 1min) x30cycles; 58°C for 2mins, 72°C for 5mins, 4°C forever. The band size obtained for the

various species differ and each was identified according to the band size. The band sizes obtained are here below listed.

Table 1: *Plasmodium* species with the expected band sizes

P. falciparum primers(rfal1+rfal2)	205 bp product
P. malariae (rmal1 + rmal2)	144bp
P. vivax (rviv1+rviv2)	120bp
P. ovale (pad Po)	800bp

3. Results

Observation from the baseline distribution of blood groups shows that the largest blood group in the set of study samples was blood group O (Fig.1). Results of the blood group screening against microscopy for *Plasmodium falciparum* shows that blood group O Rh positive was the highest with 170 (69.1%) followed by blood group A 44 (17.9%), B 27 (11%), AB 5 (2 %) [Fig. 2]. Close observation of the results also show that the samples from Rhesus positive were more parasitaemic than the Rhesus negative samples Table 2.

From the analysis of the PCR data obtained from the specie identification, we found a similar pattern of distribution of the parasitemia among the blood groups with Blood group O having the highest number of parasitaemia positive. However, we observed that a very high percentage (71.5%) of the samples were aparasitaemic suggesting a very poor microscopic diagnosis. Further observation from the obtained PCR positive results, showed that the samples with Rhesus positivity had higher parasitaemia (Table 2). Analysis of the specie identification from the PCR results, revealed that the most prevalent specie is *P. falciparum (82.9%),* followed by *P. malariae (5.7%), P. ovale* (5.7%) and then mixed infections of *falciparum* and *ovale (1.4%), falciparum and malariae* (4.3%). Closer observation showed

that the few ovale and malariae parasites seen were predominantly in the blood group O Rhesus positive samples while 25% (1/4) of each of these parasites were from the blood group B Rhesus positive samples. The mixed infections were all from the blood group O Rhesus positive samples. There was no *P. vivax* seen in all the samples analysed (Table 2).

Table 2. Distribution of Plasmodium species and ABO-Rh antigens using PCR

Bld Gp	A+	A-	B+	B-	AB+	AB-	O+	O-	Total
Pf	11		3				40	4	58
Pm			1				3		4
Po			1				3		4
Pf+Pm							3		3
Pf+Po							1		1
Total	11		5				50	4	70

Fig. 1 distribution of blood group among the study subjects.

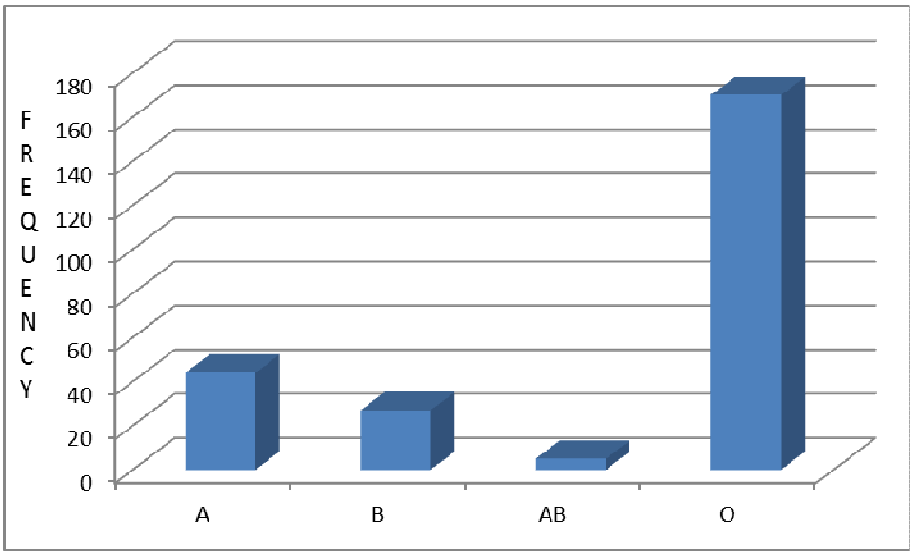
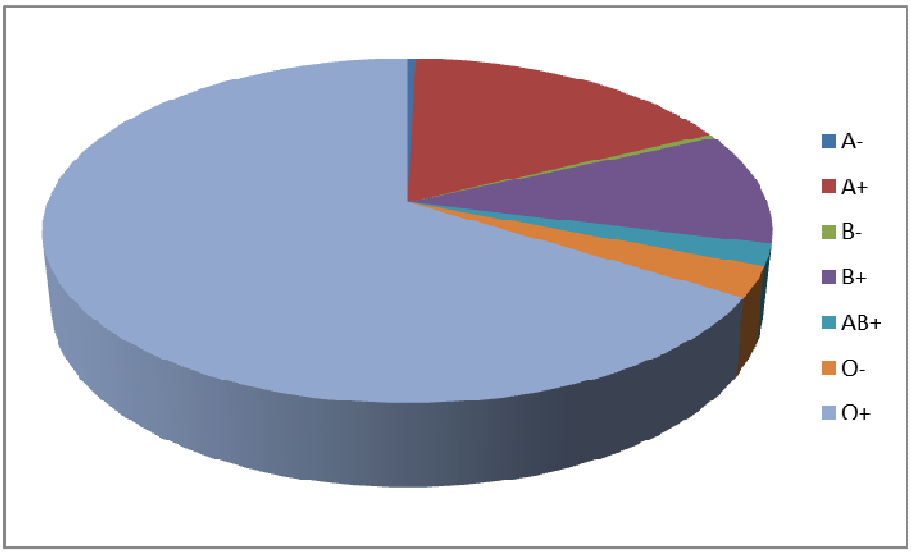


Fig. 2 Pie chart showing *P. falciparum* parasitaemia by microscopy and blood group distribution



4. Discussion

A total of 246 adults presenting blood donors were recruited into the study. Results obtained showed that the most prevalent blood group was Blood group O while the least was blood group A. This is in consonance with earlier studies [17-19]. ~~(Jeremiah, 2006, Nwauche et al, 2012, Otajevwo and Igoniwari, 2014)[17-19]~~ and the much earlier study on nationwide distribution of ABO blood group as reported by Worlledge et al. ~~1974[20]~~.[20]

Analysing the results of the Rhesus positivity, the observation was that *Plasmodium* parasite selects for the Rhesus D antigen hence the samples which were Rhesus positive had higher prevalence of parasitaemia regardless of the ABO blood grouping. The combined analysis of the blood grouping and Rhesus antigen, against microscopy revealed that blood group O Rh positive had the highest parasitaemia positive while the least was seen in the blood group A Rhesus negative. This observation is in consonance with previous reports of ~~Singh et al, 2015[21]~~ of higher prevalence of malaria among Rhesus positive persons [21].

Further findings of the study reveal that the most prevalent specie in the study sample is *Plasmodium falciparum*. This is consistent with various studies ~~([22,23])~~ that have reported the high prevalence of *P. falciparum* in Nigeria [22,23]. National Malaria control ~~(NMC, 2012)[13]~~ reports that *P. falciparum* is the most virulent of the Plasmodium species and accounts for about 98% of the malaria cases in the country [13]. In malaria endemic areas of tropical Africa, almost all malaria attacks are attributed to *P. falciparum*, possibly due either to underdiagnosis of *P. ovale* and *P. malariae* clinical attacks, to partial cross-immunity between malaria species, or to rapidly acquired species-specific protective immunity against *P. ovale* and *P. malariae* [24]. ~~(Roucher et al, 2014)[24].~~ The presence of these species *seen* though with a very low prevalence is in consonance with the report from Malaria Indicator Survey [13] ~~(2010)[13]~~ and it buttresses the importance of further studies on the prevalence of other species of *Plasmodium* in Nigeria.

In a 20 year study in Senegal, Roucher et al, ~~2014[24]~~ established that *Plasmodium ovale* and *malariae* were a common cause of morbidity in the villagers [24]. This underlines the importance of surveillance to these parasites which have been overshadowed by the abundant presence of *falciparum* malaria and understanding these interactions could have an important influence on the deployment of interventions such as malaria vaccines [25]. ~~(Mueller et al, 2007)[25].~~

Plasmodium ovale was the last of the malaria parasites of humans to be described and it is primarily distributed throughout sub-Saharan Africa [26]. ~~(Collins and Jeffrey, 2005)[26]~~. The presence of *P. ovale* was first reported in Nigeria in 1930 [27]. ~~(James et l, 1930)[27]~~. It is important that the diagnosis of *ovale* malaria be made appropriately to enhance proper and accurate management since *Plasmodium ovale* is a relapsing malaria parasite with a latent liver stage that often persists for many months. There have been reports of relapses occurring

as long as 45 months after infection [26, 28]. ~~(Collins and Jeffrey 20015, Marty et al, 1987)~~[26,28]~~.~~ On the other hand, *Plasmodium malariae* has been reported to carry a high rate of mortality both in children and adults ~~(Mueller et al, 2007~~[25]~~)~~ and have been attributed to be responsible for chronic nephrotic syndrome in a US traveler 14 years after leaving Nigeria. [25,29]. ~~(Hedelius et al, 2011)~~[29]~~.~~

A major limitation to the study is the inexperience of the microscopists such that a greater percentage of the study participants were actually aparsitaemic. This resulted in lower number that would be used for statistical analysis. Despite this limitation, we were able to present a picture of the interaction between ABO Rh blood group and Plasmodium species in the study environment. Further studies with a larger sample size is therefore very imperative

In conclusion, this study is first to document the interaction between different *Plasmodium* species and ABO antigen blood groups in Nigeria. The study showed that there is higher prevalence of malaria parasitaemia among blood group O Rhesus positive persons regardless of the *Plasmodium* specie thereby underscoring the relationship between blood group O antigen, Rhesus positivity and parasitaemia. This association maybe beneficial in designing targeted treatment for malaria infection. Apart from the ABO blood group, there has been a long established interaction between *Plasmodium vivax* and the Duffy antigen. Therefore the association and interaction between blood group types can also serve as possible diagnostic tool. The study also highlighted the presence of mixed infections in Port Harcourt and its relationship with blood group O Rhesus positive. There is need for further investigations of the interaction between blood groups and various malaria parasites since this may be beneficial in malaria vaccine development.

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