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Research Article



## Examining the association between malaria infection and the occurrence of ABO/Rhesus and hemoglobin variants in patients at a tertiary hospital in Port Harcourt, Nigeria

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### Abstract

The spread of the ABO-Rh blood group and the haemoglobin genotype differs widely between racial and ethnic groupings. Patterns could fluctuate over time within the same community in various regions of the globe, which might indicate that a specific blood type gave resistance to an infectious illness. Therefore, it is crucial to have accurate and current information on how these blood types are distributed across human demographics. A total of 451 individuals were chosen at random, ranging in age from 1 to 15 years. The sample size was selected utilising Cochran sample size calculation as well as a rate of plasmodium in youngsters in Port Harcourt of 60.6%. Using a normal phlebotomy method, 2 ml of venous blood was extracted with little immobility within sterile circumstances from the dorsum of the hand or ante-cubital vein, as the occasion may indeed be. Employing thick and thin Giemsa-stained blood smears, malaria density was evaluated microscopically. The genotype of haemoglobin was determined using cellulose acetate membrane electrophoresis in Tris-EDTA borate buffer (PH 8.9). Atlas Medical ABO reagent was used to identify ABO blood type qualitatively. Data management and statistical analyses were carried out using the Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA), with p values less than .05 deemed statistically significant. Four hundred fifty-one (451) people took part in this study. The amount of research participants (394) tested rhesus positive (87.4%). Amongst ABO variations, blood type B+ 8 (32%), A+ 7 (28%), and O+ 6 (24%), were shown to be higher common with complex plasmodium. Blood type O+ 198 (46.48) was by far the most common of simple ABO/Rhesus + blood groups, followed by B+ 97(22.77%) and A+ 72 (22.77%). (16.90). Similarly, among ABO/Rhesus negative blood groups with uncomplicated falciparum malaria, blood group O- predominated with 41 (9.62%). A+, A-, B+, B-, AB+, O+, and O- blood types of class for uncomplicated plasmodium species load revealed a mean of 18114.03/l, 52760.10/l, 20021.47/l, 3428.33/l, 11084.33/l, 12460.34/l, and 4445.59/l in the identical sequence, with O- exhibiting the least susceptibility to malaria. In addition, for uncomplicated malaria parasite density, the haemoglobin genotype status indicated a mean of 113073.44/l, 111435.65/l, and 107250.3/11 for AA, AS, and SS in the same order, with AA indicating the highest sensitivity to plasmodium. The prevalence of haemoglobin genotypes AA, AS, and SS in the study participants was 72.5%, 20.6%, and 6.9%, respectively. Men made up 54.5% (246/451), although females made up 45.5% (205/451). The haemoglobin genotype AA predominated, with men (178) outnumbering girls (149), followed by AS (56 vs 37) and SS (12 against 31), correspondingly. The most frequent ABO blood type was O (54.3%), second by B (24.6%), A (19.7%), and AB (1.3%). The bulk of Rh+ blood cells (87.4%) were found in the Rh bloodstream. The bulk of Rh bloodstream (87.4%) were Rh+, whereas 12.6% comprised Rh-. O+ (45.2%), B+ (23.3%), A+ (17.5%), O- (9.1%), A- (2.2%), AB+ (1.3%), B- (1.3%), and AB (0%) were always the mixed ABO and Rh blood types. Men's blood groups B+ (60), B- (4), AB+ (4), and O+ (112) were significantly higher than female blood groups B+ (45), B- (2), AB+ (2), and O+ (94), whereas female blood groups A- (7) and O- (23) were higher than male blood types A- (2) and O- (23) (16).

**Keywords:** Malaria, Infection, Haemoglobin Genotype, ABO, Rhesus, Blood Group, Frequency.

## Introduction

Surviving plasmodium appears to be the most powerful selection force influencing blood group composition. Red cells without or with altered forms of blood group-active molecules are often observed in malaria-endemic areas across the world, most notably the Duffy, Fy(a-b-) phenotype and the MNS, S-s- phenotype in African and the Gerbich, Ge- and SAO phenotypes in South East Asia (David, 2010). The architecture of the various blood group systems and their antigens have been widely examined, and a plethora of knowledge is now accessible, particularly with the introduction of molecular genetic methods (Lögberg et al., 2011). The prevalence of ABO and Rh antigens vary by community, despite the fact that the antigens implicated are constant in life (Lova et al., 2007). ABO and Rh antigen frequency varies in different population even though the antigens involved are stable throughout the life (Lova et al., 2007). Several studies in different regions across the globe have revealed variations in the incidence of abnormal haemoglobin variants, ABO and Rh blood groups.

Regardless of the fact that now the antigens involved are fixed in life, the frequency of ABO and Rh antigens varies by population (Lova et al., 2007). Even though the antigens responsible are constant across life, the prevalence of ABO and Rh antigens vary by population (Lova et al., 2007). Several investigations conducted in various parts of the world have found differences in the prevalence of aberrant hemoglobin variants, ABO and Rh blood categories.

The ABO-Rh blood type is distributed differently in different races and ethnic groups across the world (Akigbe et al., 2009). Aside from regional and ethnic/racial variances, the ABO-Rh blood type frequencies in a particular population can alter over time (Patel et al., 2008). As a result, having credible and up-to-date statistics on the distribution of these blood types in diverse populations is critical.

Aside from their importance in blood transfusion practise, the ABO and Rh-D blood group antigens are now used by lawyers in paternity suits, useful in population genetic studies, researching population migration patterns, assessing the likelihood of haemolytic disease in newborns, and resolving certain medico-legal issues, particularly disputes in paternity/maternity and for forensic purposes (Reid and Lomas-Francis, 2004).

The ABO blood group antigens seem to have played crucial during our development since the proportions of individual ABO blood types vary between groups, implying that a certain blood group offered a selective benefit such as resistance to an infectious illness (Reid and Lomas-Francis, 2004). Notwithstanding their evident clinical significance, the metabolic activities of ABO blood type antigens are unknown. Individuals with the prevalent blood type O do not exhibit the

A or B antigens and are healthier (O'Donnell and Laffan, 2001).

Several studies have found links between some ABO traits and higher illness vulnerability. Persons with blood type O, for example, have lower quantities of the protein von Willebrand factor (vWF), which aids in blood coagulation (O'Donnell and Laffan, 2001). It is generally known that low concentrations of FVIII and vWF produce excessive bleeding; consequently, larger levels may increase the likelihood of clotting, raising the danger of both arterial (ischemic and heart disease) and venous (thromboembolic illness). Non-group O people are at a higher likelihood of both arterial and venous diseases (O'Donnell and Laffan, 2001).

The antigen of the ABO blood type are expressed by a single gene locus, the ABO locus, that has triple alternative (allelic) variants, A, B, and O. A kid inherits one of the three alleles from each parent, giving birth to six genotypes (AA, AO, BB, BO, AB, and OO) and four blood types (phenotypes) (Daniels, 2002). Plasmodium falciparum is the most common parasite in tropical Africa, owing to a rare incidence of a negative Duffy blood type, that has preference for *P. vivax* and *P. ovale* (Brown, 2021). Human immunodeficiency virus 2 (HIV-2) infections have also been linked to blood type AB (Abdulazeez et al., 2008).

The highly polymorphic genes that encode the rhesus blood group antigens are the source of the antigens' complexity. RHD and RHCE are two genes that are closely related. Numerous genomic rearrangements between them have resulted in hybrid Rh genes that encode a diverse variety of Rh antigens (Daniels, 2002). The lack of the Rh complex changes the structure of the RBC, increases its osmotic fragility, and shortens its lifetime, resulting in a moderate haemolytic anaemia (Daniels, 2002). Because they may generate antibodies against many Rh antigens, these individuals are at risk of dangerous transfusion responses (Westhoff, 2004). There have been associations identified between rhesus and COVID-19. Abdallah et al. (2020) observed that Rh-positive but not Rhesus-negative blood group are more vulnerable to Sars-Cov-2 infection.

Hemoglobin is a metal oxygen-transport protein found in red blood cells. It is made up of two alpha chains plus two beta chains, each of which are wrapped around a heme molecule (Sankaran and Orkin, 2012). Each polypeptide chain is linked to an iron-containing porphyrin pigment termed haem, which binds oxygen reversibly to each haem group's ferrous (Fe<sup>2+</sup>). Haemoglobin variations modify the structure and metabolic characteristics of haemoglobin, with physiological implications ranging from minor to severe modifications in the chain, notably the globin chains (Kohne, 2011). They are hereditary traits that are determined by various combinations of these chains. Hb AA, HbAS, HbAC, HbSC, HbSD, HbSE, and HbSS are among them (Kohne, 2011).

In regions where falciparum malaria is endemic, sickle cell trait carriers are thought to have lower parasite densities and to be better protected against dying from severe malaria. This is most likely because HbS and HbA differ in that HbS has valine in place of glutamic acid at position 6 in the  $\alpha$ -chains, whereas HbC has lysine in place of glutamic acid at position 6 in the  $\beta$ -chains, which makes it different from Hb (Piel et al., 2010).

There are many aberrant haemoglobin genotypes, but only a few number of them are common and highly harmful to public health in different parts of the world (Abdulazeez et al., 2008). According to the World Health Organization, Nigeria has the greatest incidence of sickle cell disease. Additionally, according to the World Health Organization, 150,000 newborns in Nigeria typically have sickle cell disorder (Bakare et al., 2004). The aberrant haemoglobin genotypes AS, AC, SC, and SS are the most often found among Nigerians. There are six main haemoglobin genotypes found in Nigeria: HbAA, HbSS, HbAS, HbSC, HbAC, and HbCC (Erabor et al., 2010).

The prevalence of the remaining variations is as follows: 55-75% HbAA, 20-30% HbAS, and 3-4% HbSS. The haemoglobin C model is said to be mostly restricted to the Yorubas residing in the south-western region of the nation with a predominance of 6% (Akinyanju, 1989; Erabor et al., 2010; Jeremiah, 2006; Bakare et al., 2004).

According to reports, persons with a variety of moderate to severe hemolytic anaemia due to defective haemoglobin genotypes are more likely to suffer from morbidity and mortality as well as renal medullary cancer and hyposthenuria (Akigbe et al., 2009; Patel et al., 2008). Both the minimal incidence of COVID-19 in malaria-endemic areas and the beneficial impact of the sickle cell trait on the *Plasmodium* parasite have already been demonstrated (Elliot et al., 2008). (Hussein, 2020).

The interplay of *Plasmodium falciparum* with other blood type antigens, such as Rhesus systems, are still unknown. Hence, the geographically specific preference forces might affect the aggregate frequencies of blood group and haemoglobin genotype, with *P. falciparum* playing a significant but non-exclusive role. It is additionally important to understand the frequency of haemoglobin genotypes and blood groups since they have been linked to a variety of clinical diseases. In order to assess the relationship between falciparum malaria and the incidence of ABO/Rhesus blood group and haemoglobin genotypes.

## Materials and Method

### Study Design

The study, which was conducted on 451 paediatric patients with probable malaria infection recorded at the University of Port Harcourt Teaching Hospital in Rivers State, Nigeria,

during the months of March and August 2020, was cross-sectional in nature. After receiving administrative clearance and ethical permission for the study, parent or guardian were given informed consent/assent forms that detailed the study's goals, risks, and benefits as well as the amount of blood that would be drawn from each kid. The children's voluntary engagement in the research was emphasised, and it was made clear that their reluctance to participate would not have any bearing on the standard of care they would get.

All participants that agreed to participate in the study have been engaged. The children's enrollment in the research might at any moment be terminated by the parent or the legal guardian. Each kid had a blood sample taken in order to determine their haemoglobin genotype, ABO blood group, and plasmodium parasite density. Using the World Health Organization's guidelines and related criteria, the clinical manifestations of malaria were utilized to split the participants onto 2 categories. The simple malaria group, which has moderate to light symptoms, is distinguished by something like a feverish that is unrelated towards other infections and a lack of signs of *p. falciparum* malaria. One with a severe high parasitic infection (100,000 parasites/L) was classified as having complex severe malaria (cerebral malaria). The malaria infected children were recruited from the Paediatrics Department in the University of Port Harcourt Teaching Hospital after approval from institutional Ethical Clearance Committee.

### Study Area

Port Harcourt, the capital of Nigeria's Rivers State, was the site of the research. Geographically, Port Harcourt is located at 4°49'27"N 7°2'1"E. Port Harcourt has tropical wet weather with short dry weather and long, intense erratic rainfall. In the city, only December and January actually fall under the category of the dry season. Port Harcourt experiences less of the harmattan, which has an impact on several towns in West Africa due to its meteorological characteristics. With a rainfall total of 367 mm, September is the wettest month in Port Harcourt. With or without an average annual rainfall of 20 mm, December is often the driest month of the year. The city experiences very stable temperatures throughout the year with little seasonal change. Average temperatures are typically between 25°C-28°C in the city. University of Port Harcourt Teaching Hospital, Rivers State, Nigeria; was used as the center for the study. University of Port Harcourt Teaching Hospital, Choba Port Harcourt was established in 1980 with 500 bed spaces.

### Study Population

A total of four hundred and fifty-one (451) paediatrics with age range of 1<-15 years were randomly selected. Children registered in the paediatric ward of University of Port Harcourt Teaching Hospital, UPTH with suspected *P. falciparum* parasitaemia presenting with complicated and uncomplicated febrile illness were recruited for this study.

## Eligibility Criteria

### Inclusion criteria

With the parents' or guardians' informed agreement, youngsters of both genders experiencing feverish who have been admitted to the University of Port Harcourt Teaching Hospital and had a confirmed malaria test were used in the study.

### Exclusion criteria

Children with temperature including both gender that weren't recorded inside the unit of University of Port Harcourt Teaching Hospital were ruled out of the study.

### Sample Size

The number of patients registered in the paediatrics clinic at the University of Port Harcourt Teaching Hospital in Rivers State was taken into consideration while choosing the participants using a purposeful sampling and randomization process.

The Cochran's sample size method was used to compute the sample size, and the results are given below. The ideal sample size was derived using the prevalence of malaria in children in Port Harcourt, which was calculated as 60.6% (Yaguo-Ide and Awopeju, 2018; Cochran, 1977).

$$N = \frac{Z^2 pq}{d^2}$$

Where N = The desired sample size  
 Z = The Standard Normal deviate usually set at 1.96 corresponding to the 95% Confidence level  
 p = The prevalence of target population (60.6%)  
 q = 1 - p  
 d = degree of accuracy desired set at 0.05  
 Therefore,  $N = \frac{(1.96)^2 \times 0.606 \times (1-0.606)}{(0.05)^2}$   
 N = 366.894

By adding 10% of non-respondent, the sample size was 404.

### Sample Collection and Handling

All recruited patients were given study numbers which was used for all data collection /laboratory processes. Blood sample (2ml) was withdrawn with minimum stasis under aseptic conditions from the dorsum of the hand or ante-cubital vein as the case may be (Epidi et al., 2008). This was done by fastening a soft tourniquet to help locate and define peripheral veins to achieve successful and safe venipuncture not more than 2 minutes to enable the index finger feel a suitable vein. The punctured site was then cleaned with 70% methylated spirit (methanol) before collection of samples with 21G dry sterile hypodermic needle and syringe into EDTA. The sample

bottles were then assigned a study code with a non-water soluble ink with date, sex and time of collection and logged on to a paper log after dispensing the blood sample into the sample bottles. The sample was rocked gently to mix and kept at room temperature and malaria parasites slide were made within 1 hour of collection to prevent loss of morphological characters of the *Plasmodium falciparum*.

### Laboratory Methods of Analysis for Specific Objectives

#### Malaria Diagnosis

Light microscopy of thick and thin Giemsa stained blood smears method was used for diagnosing malaria as described by WHO, (2010). These stains contain eosin which is an acidic anionic dye and methylene blue (azure) which are basic cationic dyes. When diluted in buffered water at pH of 7.2, ionization occurs. Eosin component stains the parasite nucleus red, while the methylene blue components stain the cytoplasm blue. Pre-cleaned frosted ends slides were labeled according to the code on the bottles. Using a micro pipette, 5µl of blood was collected from each bottle and placed on the labeled slide to prepare a thick film, and 2µl to prepare a thin film. The films were then air dried on a slide rack on a flat surface and protected from debris. Methanol was used to fix the thin blood film.

Given the stable overlay of RBC, this permitted morphological identification of the parasite to the species level, which was crucial in precise interpretation of the developmental phases that may suggest the potential of an increasingly serious clinical context and the concentration of parasitized RBCs. The precise thickness of blood film was created, through which newspaper was nearly invisible. It was left to dry for 30 mins and not methanol-fixed. This permitted the red blood cells to be haemolyzed, resulting in the only identifiable components were leukocytes and any trophozoites present.

It increased the sensitivity of the blood film to identify low levels of parasitaemia. The lysis of a red blood cell (RBC) can change the plasmodia appearance, making species classification difficult, as does scanning for parasites in a stained slide. As a result, thick smears were employed to identify infection and quantify parasite concentrations in WBCs. All films were stained for 30 minutes with the slow (3% Giemsa stain working solution) pH 7.0 procedure. In a Coplin jar, 3ml of stock Giemsa was mixed with 7ml of buffered water to make a 3% working solution. After that, the films were temporarily cleaned by dipping the slide in and out of a Coplin jar of buffered water (two dips). Please keep in mind that prolonged washing can cause discoloration.

Afterward, air aired in a vertical posture. The films were subsequently inspected with an X40 objective to look for debris, stain reactivity, thickness, and film area. After that, I switched to an X100 objective (oil immersion) and thoroughly inspected the smear (horizontally or vertically). Before reporting a negative result, at least 200 oil immersion optical

layer at X100 magnification were inspected on both thick and thin smears. The blood film was first checked for parasite presence and species identification before counting using two hand tally counters, one for counting leukocytes and the other for counting parasites. The asexual parasites were counted against a predetermined number of WBC (200) using two hand tally counters, one for counting leukocytes and another for counting parasites. If 10 asexual parasite stages were detected following 200 WBCs, the overall number of asexual parasites was documented; if parasites remained detected but outnumbered Ten asexual parasite phases following 200 WBCs, the counting was expanded to 500 leukocytes. The parasite density count of parasites/l of blood was calculated by dividing the number of parasites by the actual leukocytes/l using the method below.

$$\frac{\text{Parasite count X Absolute WBC Value}}{\text{Number of leucocytes (WBC)counted (200)}} = \text{Parasite}/\mu\text{l}$$

Parasitaemia was categorized as negative (0 Parasite/ $\mu\text{l}$  blood), moderate (1,000-4,999 Parasite/ $\mu\text{l}$  blood), high (5,000-99,999 Parasite/ $\mu\text{l}$  blood), and hyperparasitaemia ( $\geq 100,000$ ).

### Estimation of ABO Blood group

The ABO blood group was determined using the haemagglutination technique (Muramatsu et al., 2014) using Atlas Medical ABO reagents. The idea is based on agglutination, in which red cells containing the antigen agglutinate in the presence of the appropriate antibody, suggesting that the result is positive. When no reaction emerges, the test is declared negative. The chemicals were kept at room temperature (18-25°C). The wax pen was employed to split the slide into proper numbers of parts. Using the vial dropper, a drop of 40110l of each reagent was applied to its corresponding split on the slide. Each drop of reagents received 25l of the precipitated cells. The reagent and cells were combined using a cleaned swirling stick across a diameter measuring approximately 20-40 mm. The slides were then incubated at room temperature (18-25oC) for 30 seconds without being stirred. The slides was held and gently shaken for 3 minutes before being examined macroscopically for agglutination, and responses were instantly read. If reaction occurred, the outcome were interpreted as positive; otherwise, it was read as negative.

### Determination of Haemoglobin electrophoretic pattern

The genotype of the haemoglobin was identified using cellulose acetate membrane electrophoresis (CAME), as reported by Awah and Uzoegwu (2006). Haemoglobin is a negatively charged protein at alkaline pH and will move towards the anode in an electromagnetic current.

Structural variations with different surface charges will dissociate from HbA, whereas those with no charge variation will not. EDTA-anticoagulated whole blood was used to make haemolysate. To prepare the electrophoretic tank, 300ml of Tris EDTA Borate (TEB) buffer was added to the tank (pH 8.9). Wicks were cut from Grade NO. 3 chromatography paper and set along the tank's 22cm bridges. The cellulose acetate membranes were cut into 40 x 100mm pieces and immersed in TEB buffer for 5 minutes (shiny side down). Five strips were blotted and placed on the electrophoresis tanks but were not allowed to dry out before sample application. To equilibrate the membranes with the buffer, a voltage current of 250 volts was delivered to the membranes for 5 minutes. The current was then switched off, and 8-10l haemolysate (10g/l) was administered to each adhering membranes by inserting a small amount (10l) of each sample solution into a specimen well. After that, the applicator was dipped into the sample wells and applied to the cellulose acetate membrane. The voltage was then set to 250 volts with a steady current of 2 mA for each strip. The electrophoresis was conducted for around 10-15 minutes, or until there was a clear region between both the control sample bands. After turning off the current, the dissociated Hb on the cellulose acetate membrane was monitored and recorded on the working sheet. HbA migrate faster followed by HbS respectively.

### Statistical Analysis

Data for this study was entered into a spreadsheet and statistically analyzed using the statistical tool Statistical Analysis System (SAS) version 9.4. Inferential statistics mean separation using one-way analysis of variance (ANOVA) was used to compare and assess differences between means. Variables like genotype and blood group were given as percentages, while quantitative data was expressed as mean standard deviation and a threshold of significance of P0.05.

### Results and Discussion

Being rhesus negative may be advantageous in parasite endemic regions. According to this study, blood group O rhesus D negative individuals were shown to be less vulnerable to malaria infection. This might be related to the lack of the Rh complex, which changes the structure of the RBC, increases its osmotic fragility, and cuts down its lifetime, culminating in a moderate haemolytic anaemia. The hemoglobin variation SC was not discovered in this investigation, however 5.59% of SS was. This conclusion contradicts the findings of Erabor et al (2010). This might be related to the age disparity among research participants, which included older adults in this study. In this research, 0% of the SC gene was found (Bakare et al. 2006).

**Table 1. Frequency of ABO/Rhesus blood group and Haemoglobin Genotype in the Study Participants**

Characteristic	Malaria Infected Subjects N %	
	N	%
<b>Overall</b>	451	100
<b>ABO/Rhesus Blood Group</b>		
A+	79	17.5
A-	10	2.2
B+	105	23.3
B-	6	1.3
AB+	6	1.3
O+	204	45.2
O-	41	9.1
<b>ABO Blood Group</b>		
A	89	19.7
B	111	24.6
AB	6	1.3
O	245	54.3
<b>Rhesus Blood Group</b>		
Rhesus positive	394	87.4
Rhesus Negative	57	12.6
<b>Haemoglobin Genotype</b>		
AA	327	72.5
AS	93	20.6
SS	31	6.9

Within characteristic, percentages may not add up to 100 due to rounding.

N, total number, %, percentage

[Table 1](#). shows the frequency of ABO/Rhesus blood group and Haemoglobin Genotype in the Study Participants. A total of four hundred and fifty-one (451) participated in this study. Of this blood group O rhesus positive took dominance representing two hundred and four (204) representing 45.2%.

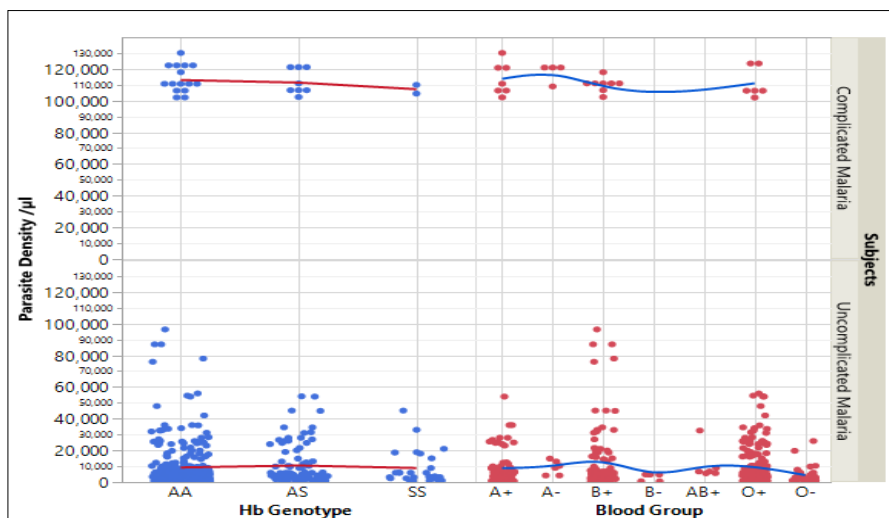
Majority of the study participant were rhesus positive (394) representing 87.4%. Most of the participants 327(72.5%) had haemoglobin electrophoretic pattern of AA.

**Table 2. Characteristics of Paediatric Subjects with Complicated and Uncomplicated Malaria, and Non-Malaria Infected (Control)**

Characteristic	Complicated		Uncomplicated	
	N	%	N	%
<b>Overall</b>	25	3.04	426	51.82
<b>Blood Group</b>				
A <sup>+</sup>	7	28.00	72	16.90
A <sup>-</sup>	4	16.00	6	1.41
B <sup>+</sup>	8	32.00	97	22.77
B <sup>-</sup>	0	0.00	6	1.41
AB <sup>+</sup>	0	0.00	6	1.41
O <sup>+</sup>	6	24.00	198	46.48
O <sup>-</sup>	0	0.00	41	9.62

N, total number, %, percentage

[Table 2](#). This demonstrates the relationship between Malaria Infection and the prevalence of ABO/Rhesus blood type and haemoglobin variations in Study Participants. Among the ABO variations, blood type B+ 8 (32%), A+ 7 (28%), and O+ 6 (24%), were shown to be more common with complex malaria. Blood type O+ 198 (46.48) was the most common of simple ABO/Rhesus positive blood groups, followed by B+ 97(22.77%) and A+ 72 (22.77%). (16.90). Similarly, among ABO/Rhesus negative blood groups with uncomplicated malaria infection, blood group O- predominated with 41 (9.62%).



**Fig. 1 Scatter Plot Showing the Association Between Parasite Density, Genotype and Blood Group of Pediatric Subjects Infected with Complicated and Uncomplicated Malaria**

Fig. 1 shows the association Between Parasite Density, Genotype and Blood Group of Pediatric Subjects Infected with Complicated and Uncomplicated Malaria. ABO/Rh blood groups class for malaria density showed a mean of 18114.03/ $\mu$ l, 52760.10/ $\mu$ l, 20021.47/ $\mu$ l, 3428.33/ $\mu$ l, 11084.33/ $\mu$ l, 12460.34/ $\mu$ l, and 4445.59/ $\mu$ l for A<sup>+</sup>, A<sup>-</sup>, B<sup>+</sup>, B<sup>-</sup>, AB<sup>+</sup>, O<sup>+</sup> and O<sup>-</sup> in the same order with O<sup>-</sup> showing the least susceptibility to malaria. Also, the haemoglobin genotype status for uncomplicated malaria parasite density showed a mean of 113073.44/ $\mu$ l, 111435.65/ $\mu$ l and 107250.3/ $\mu$ l for AA, AS and SS in the same order with AA showing the most susceptibility to malaria.

Table 3. shows frequency of some haemoglobin genotype with gender among paediatrics in the study population. Haemoglobin genotypes AA, AS and SS were seen in the study population and their prevalence were, 72.5%, 20.6% and 6.9% in the same order. Males constituted 54.5% (246/451), while females constituted 45.5% (205/451). Haemoglobin genotype AA took dominance which was higher in males (178) than females (149) followed by AS (56 versus 37) and SS (12 versus 31) respectively.

**Table 3. Frequency of Some Haemoglobin Genotype with Gender Among Paediatrics in the study population.**

Gender/Hb genotype	AA	AS	SS	Total	Percentage
Male	178	56	12	246	54.5
Female	149	37	19	205	45.5
Total	327	93	31	451	
Percentage	72.5	20.6	6.9		

Table 4. shows the distribution of blood antigens in the study population. In the ABO blood group system, O was the most common (54.3%), followed by B (24.6%), A (19.7%), and AB (1.3%). In the Rh blood system, the majority (87.4%) were Rh<sup>+</sup>, while 12.6% were Rh<sup>-</sup>.

**Table 4. Distribution of Blood Antigens in the Study Population**

Gender/blood group	A	B	AB	O	Total	Rh <sup>+</sup>	Rh <sup>-</sup>	Total
Male	36	64	4	128	232	210	23	233
Female	53	47	2	117	219	184	34	218
Total	89	111	6	245	451	394	57	451
Percentage	19.7	24.6	1.3	54.3		87.4	12.6	

**Table 5. Prevalence of combined ABO and RH blood groups in the Study Population**

Gender/blood group	A+	A-	B+	B-	AB+	AB-	O+	O-	Total
Male	34	2	60	4	4	0	112	16	232
Female	45	8	45	2	2	0	92	25	219
Total	79	10	105	6	6	0	204	41	451
Percentage	17.5	2.2	23.3	1.3	1.3	0	45.2	9.1	

Table 5. shows the combined prevalence of ABO and Rhesus blood groups in the study population. The combined ABO and Rh blood groups were in the order of O<sup>+</sup> (45.2%), B<sup>+</sup> (23.3%), A<sup>+</sup> (17.5%), O<sup>-</sup> (9.1%), A<sup>-</sup> (2.2%), AB<sup>+</sup> (1.3%), B<sup>-</sup> (1.3%), and AB<sup>-</sup> (0%). Blood group B<sup>+</sup> (60), B<sup>-</sup> (4), AB<sup>+</sup> (4) and O<sup>+</sup> (112) were higher in male compared to female B<sup>+</sup> (45), B<sup>-</sup> (2), AB<sup>+</sup> (2) and O<sup>+</sup> (94), and blood group A<sup>-</sup> (7) and O<sup>-</sup> (23) were higher in female compared to male A<sup>-</sup> (2) and O<sup>-</sup> (16).

The zero frequency of SC in the current study population could be attributed to the fact that those that harbor the SC gene were not have been recruited into this study by chance.

The prevalence estimates of AA and AS in this research are comparable to those found by Akigbe et al. (2009) and Nwogoh et al (2012). Nevertheless, there really are minor differences between geographical locations. This backs up the idea that inherited hemoglobin variations differ extensively between populations and ethnicities worldwide. The predominance of AA in this study (72.5%) falls within the normal range of 55%-75% previously reported by Erabor et al. (2010); Jeremiah, (2006); Bakare et al. (2004), while the prevalence of AS in this study (20.6%) is within the reference value of 20%-30% quoted for Nigeria and the 20%-40% in

Africa in particular by Erabor et al. (2010), but while tending more towards the cut-off point. The decrease in the incidence of haemoglobinopathies observed in this study could be attributed to increased awareness of sickle cell anaemia, increased response to genotyping prior to marriage and childbearing, increased prenatal diagnosis of genetic haemoglobin disorders, and improved socioeconomic conditions. When compared to HbAS, the prevalence rate of parasite density among various genotypes was substantially greater in HbAA. This observation is consistent with the findings of Elliot et al (2008). This is most likely owing to the difference between HbS and HbA caused by the substitution of valine for glutamic acid at position 6 in the  $\alpha$ -chains of HbS, resulting in its elimination from the system before the full 120 days.

According to the findings of this study, blood antigen O (54.3%) predominates over other ABO blood group antigens. This is consistent with prior findings (Jeremiah, 2006; Lova et al. (2007); Abdulazeez et al (2008). However, when complex and simple malaria individuals were separated, blood type B<sup>+</sup> was shown to have the highest prevalence among severe malaria

victims. This is consistent with the findings of Hameed et al. (2002) and Mohammed et al. (2004), who found that blood type AB had the lowest incidence in this research. This is consistent with prior research by Abdulazeez et al. (2008), Bakare et al. (2004), Erabor et al. (2010), Jeremiah (2006), and Lova et al (2007). The findings of this investigation revealed that the frequency of ABO antigens is increasing.

Nonetheless, this contradicts certain other research, such as that of Jeremiah (2006), who reported the prevalence of ABO blood system in the sequence of O > A > B > AB. The high prevalence of blood group O in this community supports the argument that malaria survival is the most important selection factor influencing blood group expression. The 19.7% prevalence of blood type A in this study differs from the 21.6% observed among healthy infants in Ibadan, Nigeria by Jeremiah (2006). It is also greater than some prior findings from other regions of Nigeria by Bakare, (2004); Enosolease and Bazuaye, (2004). (2008).

This study's 24.6% prevalence of blood type B is greater than the 21.4% observed among healthy newborns in Ibadan by Bakare (2004) and Port Harcourt, Nigeria by Jeremiah (2004). (2006). Blood type AB was the least common (1.3%) in this study, which is consistent with earlier findings by Abdulazeez et al. (2008), Bakare et al. (2004), Erabor et al. (2010), Jeremiah (2006), and Lova et al (2007). However, this percentage is lower than the 2.8% recorded by Bakare (2004) among healthy newborns in Ibadan, Nigeria, and the 2.97% reported by Nwogoh et al. among persons in Benin City, Nigeria (2013). This study discovered that the prevalence of RhD phenotype was 12.6% and the frequency of RhD+ phenotype was 87.4%, which is greater than previously reported.

In this study, no AB<sup>-</sup> was encountered (0%). The reason for this is not clear, probably because both blood group AB and RhD<sup>-</sup> individuals are very rare in Nigeria. Rhesus negative incidence among females in the study population signifies more attention should be given to the female of child bearing age which could reduce haemolytic disease of newborn associated with such age. The findings have clearly demonstrated the existence of difference malaria parasite density among different ABO and Rh blood groups and among different haemoglobin variants in the study population.

## Conclusion

This study discovered a link between Rhesus factor and malaria infection. Malaria infection was less likely in people with blood group O Rhesus negative. This study also found that blood group O is more common than other blood groups, whereas AB is the least common and Rh+ is much more common. Because blood type A is more vulnerable to malaria

parasite infection, priority is given to blood group O, which is less susceptible to malaria infection, in blood transfusion medicine, with specific preference given to blood group O rhesus D negative. The relationship between malaria susceptibility and haemoglobin genotype AA has already been established, and being less vulnerable among sickle cell trait is also supported by this study.

## Ethical Approval

The malaria-infected children were recruited from the Paediatrics Department at the University of Port Harcourt Teaching Hospital after approval from the institutional Ethical Clearance Committee.

## Conflict of Interest

The author hereby declares no conflict of interest.

## Consent for publication

The author declares that the work has consent for publication.

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