European Institute of Knowledge & Innovation

Annals of Innovation in Medicine (AIM) ISSN: 2977-0335

Research Article

Haemoglobin Variants, ABO/Rh Blood Groups and their Associations with Levels of Malaria Parasitaemia amongst Infected Subjects at Rivers State University, Port Harcourt, Nigeria

Serekara Gideon Christian[1*], Precious Nwaeri Okwu[1], Baridomamene Banaanyone Barivole[1], Dodoru Tuemi Robinson[2]

[1] *Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.*
[2] *Institute for Biomedical Research and Innovations, College of Health Sciences, University of Uyo, Uyo, Nigeria.*
* Correspondence: serekara.christian1@ust.edu.ng; Tel.: (+234-8126576875)

https://doi.org/eiki/10.59652/aim.v2i2.217

Abstract: The aim of the study was to associate haemoglobin variants, ABO/Rh blood groups with levels of malaria parasitaemia amongst infected subjects at Rivers State University, Port Harcourt, Nigeria. ABO/Rh D blood groups were analyzed using monoclonal antisera, and haemoglobin electrophoresis was analyzed using the alkaline cellulose acetate electrophoresis method, while malaria parasites were identified by microscopic examination of stained blood films. Graph Pad Prism version 8.0 was used to statistically analyze odd ratios, confidence intervals, likelihood ratios and relative risks. All 147 subjects (87 females, 60 males) were positive for malaria (*Plasmodium falciparum*). For 3+ *falciparum* malaria, the order of infection for haemoglobin genotype was AA > AS/SS; ABO blood group was B > A > O > AB; Rh blood group was Rh D+ > Rh D-; gender was females > males at $p > 0.05$. At $p > 0.05$, for 2+ *falciparum* malaria: haemoglobin genotype was SS >AA > AS; ABO blood group was B > A > O > AB; Rh blood group was Rh D- > Rh D+; and gender was females > Males. At $p > 0.05$, for 1+ *falciparum* malaria infection: haemoglobin genotype was AS >AA > SS; ABO blood group was AB > O > A > B; Rh blood group was Rh D+ > Rh D-; and gender was males > females. Conclusively, 3+ *Plasmodium falciparum* malaria infection is common amongst individuals with: AA haemoglobin genotype, blood group B, Rh D+, and females; 2+ *P. falciparum* infection is common amongst individuals with: haemoglobin genotype AA, blood group B, Rh D-, and females; while 1+ *P. falciparum* malaria infection is common amongst individuals with: AS haemogobin genotype, blood group AB, Rh D+, and amongst males than females.

Keywords: Malaria Parasite; Haemoglobin Electrophoresis; ABO Blood group; Rh D Blood Group; falciparum malaria.

Received: 23/05/2024
Accepted: 01/06/2024
Published: 02/06/2024

1. Introduction

Blood is a fluid tissue that transports nutrients and oxygen to all parts of the body while also removing waste (carbon dioxide) from the body. It is composed of red blood cells, white blood cells and platelets suspended in plasma, (1); the red blood cell contain haemoglobin that malaria parasites feed on.

Blood group antigens are present on the surfaces red blood cell (RBC) membrane and their specificity can be determined by a number of genes that can be allelic or extremely closely linked on the same chromosome. The blood type describes the pattern of response to testing antisera and can be identified serologically in the laboratory based on antigen-antibody reactions (2).

The International Society of Blood Transfusion (ISBT) has a list of 33 blood group systems that represent more than 300 antigens (3; 4). The majority of them have been sequenced and cloned. ABO continues to be the most important of the 33 systems for transfusion and transplantation since anybody older than 6 months has clinically significant levels of anti-A and/or anti-B antibodies in their serum. While blood group O lacks the A/B antigen but does carry both of its antibodies in serum, blood group A carries an antibody against blood group B and vice versa (2).

The Rh system is the second most significant blood group system after ABO (5). Many years ago, the Rh blood type system was initially described. After giving birth to a stillborn child with erythroblastosis fetalis, a mother who had blood transfusions from her husband experienced a severe transfusion reaction (6). Later on, Landsteiner and Wiener (7), discovered that 85% of human RBC samples were agglutinated by sera from rabbits (and later guinea pigs) immunized with Macaca mulatta RBCs (Macacus Rhesus in the original publication). At first, it was believed that the antibodies from animals and humans had found Rh on the surface of human and Rhesus RBCs. This was not the case, as was quickly realized (8).

Haemoglobin is necessary for the transport of oxygen throughout the body (9;10). It has the ability to combine with oxygen in a reversible manner. Due to the four haem groups' enhanced interaction in an environment with high oxygen tension, the haemoglobin molecule rapidly saturates with oxygen to create oxy-haemoglobin (11). The haemoglobin that is found in red blood cells is what malaria parasites (*Plasmodium falciparum*) feeds on.

Normal haemoglobin has a valine in place of glutamic acid in position six of the beta chain of the globin molecule, which distinguishes sickle cell haemoglobin (HbS) from normal haemoglobin (HbA). Erythrocytes with sickle cell haemoglobin develop sickle-shaped cells instead of the usual round, biconcave disc shape when oxygen levels are decreased. Anaemia is nearly universally experienced by sickle cell homozygotes (HbS/HbS). The HbA/HbS sickle cell heterozygote possesses malaria parasitemia resistance and is only mildly anaemic (12).

The haemoglobin variants present in the blood, which directly or indirectly influence the amount of haemoglobin, are important for predicting the blood's capacity to deliver oxygen to the body's tissues and the likelihood that the person will have sickle cell anaemia (13), and moreso, the amount of haemoglobin available for malaria parasite to feed on.

Malaria is a parasite-borne illness spread by the Anopheles species of mosquitoes and caused by *Plasmodium* parasites. The five *Plasmodium species* which affect humans include *P. falciparum, P. vivax, P. ovale, P. malariae*, and *P. knowlesi*. The two most prevalent species are *P. falciparum* and *P. vivax,* with *P. falciparum* being more prevalent in tropical and subtropical regions, particularly in Africa (and Nigeria, where the study was conducted), and *P. vivax* being more prevalent in Asia, Latin America, and portions of Africa (14; 15; 16).

A study has indicated a correlation between the ABO blood group and the resistance, susceptibility, and severity of malaria infection caused by *P. falciparum*. Blood group "O" is thought to provide protection against complex cases of *falciparum* malaria, while blood group "A" has been proven to be particularly susceptible to the disease (17).

The sickle cell mutation is related to malaria because the malaria parasite causes hypoxia when it infects a red blood cell. When such blood cells sickle in individuals with the AS genotype, the body's immune system macrophage cells remove them, thereby reducing the risk of infection (18).

Sickle cell trait carriers are less resistant to mild occurrences of malaria but more resistant to severe episodes. The acquired immunity that both AA and AS individuals get after multiple exposures to the disease is not the same as the mechanism by which carriers are protected from malaria (19).

2. Materials and Methods

2.1 Study Design

This study was a cross-sectional study carried out among malaria-infected subjects attending the Rivers State University Medical Centre, Port Harcourt.

2.2 Study Area

Rivers State University, formerly called Rivers State University of Science and Technology is a university located in the Diobu area of Nkpolu-Oroworokwu, Port Harcourt, Rivers State in South–South, Nigeria. All participants were recruited from Rivers State University, Port Harcourt. Rivers State University is located on latitude 4.7958^0 N and longitude 7.0246^0 E. The analysis was carried out at Prof. Nimi Briggs Hospital (the Medical Center), Rivers State University. Port Harcourt, the capital of Rivers State is located on latitude 4.75^0 N and longitude 7.00^0 E and lies along Bonny River in the Niger Delta.

2.3 Study Population

Based on convenient sampling, a total of one hundred and forty-seven (147) human subjects consisting of sixty (60) males and eighty-seven (87) females, confirmed positive for *falciparum* malaria, attending the Rivers State University Medical Centre, Port Harcourt, Rivers State were recruited for the study. The study subjects were adults varying between 16-60 years of age.

2.4 Sample Collection

After pre-test counselling and explanations, venous blood collection was drawn from the antecubital fossa of the subjects with the use of vacutainer as described by Ochei and Kolhatkar (11). Three (3.0) ml of venous blood was collected into a glass vacutainer sample bottle that contains 0.5ml of 1.2mg/ml of dipotassium ethylene diamine tetra-acetic acid (EDTA). All blood samples were analyzed within 24 hours of collection.

2.5 Methodology

2.5.1 Determination of ABO and Rh D Blood Group Using Atlas Monoclonal Antisera.

Principle: The presence or absence of A, B on human red blood cells can be determined by testing the red blood cells with the respective antisera, specifically Anti-A, Anti-B, Anti-AB. The procedure is based on the principle of agglutination (20).

Procedure: For ABO blood group, a drop of anti-A, anti-B, and anti-AB (Atlas Medical), each was placed in a clean tube labeled A, B, AB and O. A drop of red cell was added to the part labeled A, B, AB and O and anti-A, anti-B, anti-AB was dropped in the part labeled A, B, AB and O, the mixture was centrifuged for about 30 seconds at 3500rpm and thereafter observed for agglutination. Presence of agglutination indicated a positive result while absence of agglutination indicates a negative result. Same procedure was applied for Rh blood group, a drop of anti-D (Atlas Medical) was placed in a clean glass tube labeled D. A drop of red cell was added to the tube labeled D, the mixture was centrifuged at 3500rpm for 30 seconds and thereafter observe for agglutination. Presence of agglutination indicated a positive result while absence of agglutination indicates a negative result (20).

2.5.2 Determination of Haemoglobin Genotype

Principle: In an alkaline buffer at pH 8.4-8.6 using a cellulose acetate membrane, haemoglobin variants separate at different rates due to differences in their surface electrical charge as determined by their amino acid structure (20).

Procedure for Haemoglobin Genotype: The red blood cells were haemolyzed with the haemolyzing reagent and the electrophoresis tank was prepared by placing equal amount of Tris buffer in the different compartments. The chamber wicks were wetted in the buffer

and placed one across the bridge ensuring there is contact with the buffer. Thereafter, the cellulose acetate paper was soaked into a reserve buffer for 5 minutes. With the aid of an applicator stick, the haemolyzed sample was loaded to the blotting paper (cellulose acetate paper). The cellulose acetate paper was placed across the bridge and power was turned on for electrophoresis to take place at 350V for 25 minutes. After 25 minutes of electrophoresis, the cellulose acetate paper was transferred to the staining reagent to fix for 5 minutes. Excess stain was removed by washing for 5 minutes in acetic acid and 10 minutes in the remaining de-staining reagent. The membrane was labeled and results were interpreted (20).

Interpretation of Results: The results of a haemoglobin electrophoresis test are interpreted by comparing the pattern of bands on the gel or paper strip to a known pattern. The known pattern will show the different types of haemoglobin that were present in the blood sample.

2.5.3 Identification of Malaria Parasite

Principle of Giemsa Stain: Giemsa solution is composed of eosin and methylene blue (azure). The eosin component stains the parasite nucleus red, while the methylene blue component stains the cytoplasm blue (20).

Procedure for Thick Film Preparation: Two drops of blood were placed on a clean glass slide and the blood was mixed with the head of a pipette in circular motion over an area about two centimeter in diameter. The blood film was allowed to air-dry at room temperature (20).

Procedure for Staining of Thick Film: 1 in 30 dilution of Giemsa's stain was made by mixing 1ml of the stain in 29ml of buffered water, the film was dried for some hours. The film was covered with diluted Giemsa's stain for 30 minutes, and washed in buffered water at pH of 7.2. The film was dried in a vertical position, and later examined microscopically using oil immersion objective (100x) (20).

Interpretation of Results: 1–10/100 HPF = 1+**;** 11–20/100 HPF = 2+**;** 1–10/10 HPF = 3+**;** 11–20/10 HPF = 4+

2.6 Statistical Analysis

Data collected were statistically analyzed for percentage distribution, odd ratio, relative risk, confidence interval and likelihood ratio using Graph Pad Prism version 8.0. Data are represented in Tables.

3. Results

This section can be divided into multiple sub-sections to ensure that the results are presented in the best possible format. We strongly recommend using tables, and figures, in this part of the article.

3.1: Demographic Details of Studied Population

A total of 147 *falciparum* malaria infected subjects participated in the study. Males were sixty (60) while females were eighty-seven (87).

3.2: Frequency and Percentage Distribution of Malaria based on Levels of Parasitemia

The frequency and percentage distribution of *falciparum* malaria based on levels of parasitemia were analyzed and recorded. Details are shown in Table 1.

Table 1. Frequency and Percentage Distribution of *falciparum* Malaria based on Levels of Parasitemia

Parameters	Frequency (f)	Percentage (%)
1+ *falciparum* malaria	98	66.7%
2+ *falciparum* malaria	45	30.6%
3+ *falciparum* malaria	4	2.7%

3.3: Frequency Occurrence and Percentage Distribution of Malaria Infection in the Studied Population based on gender

The frequency and percentage distributions of malaria infection based on gender were analyzed and recorded. Details are shown in Table 2.

Table 2. Frequency Occurrence and Percentage Distribution of Malaria Infection in the Studied Population based on gender

Parameters	Total Population (N)	Frequency	Percentage (%)
1+ *falciparum* malaria	98	M = 42	M = 28.6
		F = 56	F = 38.1
2+ *falciparum* malaria	45	M = 17	M = 11.6
		F = 28	F = 19.0
3+ *falciparum* malaria	4	M = 1	M = 0.7
		F = 3	F = 2.0

3.4: Comparison of Odds Ratios, Relative Risks and Likelihood Ratios of 3+ Plasmodium falciparum Malaria based on Genotype, Blood Groups and Gender Differences

Based on their odds the likelihood of having 3+ infection with Plasmodium falciparum malaria for the studied variables at $p > 0.05$, were in the order of: Genotype – AA > AS/SS with odds of infinity and 0.00 respectively; ABO blood group – B > A > O > AB with odds of 2.16 > 0.79 > 0.24 > 0.00 respectively; Rh blood group – Rh D+ > Rh D- with odds of infinity > 0.00 respectively; and Gender – Female > Male with odds of 2.05 > 0.48 respectively. Details are shown in Table 3.

Table 3. Odds Ratios, Relative Risks and Likelihood Ratios of being infected with 3+ *Plasmodium falciparum* Malaria based on Genotype, Blood Group and Gender Differences

Variables	Odds Ratio	Relative Risk	Likelihood Ratio	p-value
Genotype AA	Infinity	Infinity	1.26	0.5837 ^{NS}
A				

	CI 0.279 to Infinity	CI 0.242 to Infinity		
Genotype AS	0.00 CI 0.279 to Infinity	0.00 CI 0.242 to Infinity	0.00	>0.9999^{NS}
Genotype SS	0.00 CI 0.000 to 88.79	0.00 CI 0.000 to 36.75	0.00	>0.9999^{NS}
Blood Group A	0.79 CI 0.119 to 5.311	0.00 CI 0.000 to 36.75	0.00	>0.9999^{NS}
Blood Group B	2.16 CI 0.159 to 14.93	2.11 CI 0.305 to 13.81	1.87	0.4467^{NS}
Blood Group AB	0.00 CI 0.000 to 337.5	0.00 CI 0.000 to 49.43	0.43	>0.9999^{NS}
Blood Group O	0.24 CI 0.018 to 1.66	0.25 CI 0.036 to 1.70	0.00	0.3142^{NS}
Blood Group Rh D+	Infinity CI 0.022 to Infinity	infinity CI 0.048 to Infinity	1.03	>0.9999^{NS}
Blood Group Rh D-	0.00 CI 0.000 to 1.355	0.00 CI 0.000 to 1.193	0.00	0.2208^{NS}
Females	0.00 CI 0.299 to 27.10	0.00 CI 0.297 to 13.98	1,26	0.6487^{NS}
Males	0.00 CI 0.036 to 3.339	0.00 CI 0.071 to 3.358	0.61	0.6487^{NS}

3.5: Comparison of Odds Ratios, Relative Risks and Likelihood Ratios of 2+ Plasmodium falciparum Malaria based on Genotype, Blood Groups and Gender Differences

Based on their odds, the likelihood of having 2+ infection with Plasmodium falciparum malaria for the studied variables at $p > 0.05$, were in the order of: Genotype – SS >AA > AS with odds of 1.64 > 1.21 > 0.76 respectively; ABO blood group – B > A > O > AB with odds of 1.38 > 1.16 > 0.75 > 0.00 respectively; Rh blood group – Rh D- > Rh D+ with odds of 1.34 > 0.74 respectively; and Gender – Female > Male with odds of 1.24 > 0.80 respectively. Details are shown in Table 4.

Table 4. Odds Ratios, Relative Risks and Likelihood Ratios of being infected with 2+ *Plasmodium falciparum* Malaria based on Genotype, Blood Group and Gender Differences

Variables	Odds Ratio	Relative Risk	Likelihood Ratio	p-value
Genotype AA	1.21 CI 0.519 to 2.712	1.12 CI 0.617 to 2.319	1.03	0.8322^{NS}
Genotype AS	0.76 CI 0.294 to 1.893	0.81 CI 0.389 to 1.575	0.80	0.6651^{NS}
Genotype SS	1.64 CI 0.111 to 14.35	1.43 CI 0.260 to 3.665	1.63	0.5539^{NS}
Blood Group A	1.16 CI 0.568 to 2.414	1.12 CI 0.629 to 1.906	1.11	0.7039^{NS}
Blood Group B	1.38 CI 0.554 to 3.283	1.27 CI 0.664 to 2.257	1.30	0.4932^{NS}
Blood Group AB	0.00 CI 0.000 to 0030	0.00 CI 0.000 to 3.600	0.00	>0.9999^{NS}
Blood Group O	0.75 CI 0.396 to 1.452	0.81 CI 0.487 to 1.345	0.88	0.4935^{NS}
Blood Group Rh D+	0.74 CI 0.150 to 3.839	0.80 CI 0.333 to 2.841	0.98	0.6628^{NS}
Blood Group Rh D-	0.00 CI 0.000 to 1.355	0.00 CI 0.000 to 1.193	0.00	0.2208^{NS}
Females	1.34 CI 0.260 to 6.640	1.25 CI 0.352 to 2.999	1.33	0.6628^{NS}
Males	0.80 CI 0.411 to 1.57	0.85 CI 0.491 to 1.427	0.87	0.6040^{NS}

3.6: Comparison of Odds Ratios, Relative Risks and Likelihood Ratios of 1+ Plasmodium falciparum Malaria based on Genotype, Blood Groups and Gender Differences

Based on their odds the likelihood of having 1+ infection with *Plasmodium falciparum* malaria for the studied variables at $p > 0.05$, were in the order of: Genotype – AS >AA > SS with odds of 1.13 > 0.84 > 0.76 respectively; ABO blood group – AB > O > A > B with odds of 1.54 > 1.14 > 0.87 > 0.84 respectively; Rh blood group – Rh D+ > Rh D- with odds of 1.09 > 0.92 respectively; and Gender – Male > Female with odds of 1.14 > 0.87 respectively. Details are shown in Table 5.

Table 5. Odds Ratios, Relative Risks and Likelihood Ratios of being infected with 1+ *Plasmodium falciparum* Malaria based on Genotype, Blood Group and Gender Differences

Variables	Odds Ratio	Relative Risk	Likelihood Ratio	p-value
Genotype AA	0.84 CI 0.454 to 1.563	0.90 CI 0.650 to 1.325	0.96	0.6384^{NS}
Genotype AS	1.13 CI 0.591 to 2.095	1.08 CI 0.728 to 1.520	1.11	0.7469^{NS}
Genotype SS	0.76 CI 0.052 to 6.638	0.84 CI 0.154 to 2.061	0.76	>0.9999^{NS}
Blood Group A	0.87 CI 0.489 to 1.554	0.92 CI 0.625 to 1.300	0.90	0.7644^{NS}
Blood Group B	0.92 CI 0.625 to 1.300	0.90 CI 0.546 to 1.364	0.86	0.7149^{NS}
Blood Group AB	1.54 CI 0.080 to 29.36	1.27 CI 0.238 to 2.396	1.53	>0.9999^{NS}
Blood Group O	1.14 CI 0.707 to 1.980	1.11 CI 0.814 to 1.545	1.07	0.5965^{NS}
Blood Group Rh D+	1.09 CI 0.280 to 4.202	1.05 CI 0.552 to 2.921	1.00	>0.9999^{NS}
Blood Group Rh D-	0.92 CI 0.238 to 3.560	0.95 CI 0.342 to 1.810	0.92	>0.9999^{NS}
Females	0.87	0.92	0.95	0.6931^{NS}

	CI 0.522 to 1.479	CI 0.680 to 1.264		
Males	1.14 CI 0.676 to 1.914	1.08 CI 0.791 to 1.469	1.08	0.6931^{NS}

4. Discussion

The study revealed that 2.7% of the studied subjects were infected with 3+ *falciparum* malaria, subjects infected with 2+ was 30.6%, and 66.7% had 1+ *falciparum* malaria infection. The reason for having more of 1+ infection may be as a result of anti-malaria medication that have reduced the parasite load.

Based on the relationship of ABO/Rh blood groups and haemoglobin genotypes with malaria parasitemia, odd ratios find its usefulness in comparing the relative odds for the occurrence of the possible outcome of a disorder or disease (in this case, malaria). The odd ratio establishes the risk factor for malaria parasitaemia in relation to the presence of the different blood group antigens, haemoglobin genotypes and gender. An odd ratio (OR = 1) indicates that the result, malaria parasitaemia, will not be impacted by the presence of that antigen and haemoglobin genotype. An odd ratio (OR > 1) indicates that a blood group antigen's and haemoglobin genotype's presence is linked to an increased level of malaria parasitaemia. A blood group antigen and haemoglobin genotype with an odd ratio (OR < 1) suggests that having it is linked to a lower level of malaria parasitaemia. The likelihood ratio gives the usefulness of the blood group outcomes in ascertaining which blood group and genotype is more likely prone to be at risk of malaria. Confidence interval finds usefulness in estimating the precision of odd ratios; a large confidence interval is indicative of a low level of precision of the odd ratios, while a small confidence interval is indicative of a higher precision. So, the combination of odd ratios and confidence interval will give a better interpretation of the degree of malaria parasitaemia risk.

For haemoglobin variants consideration, the risk of being infected with 3+ *plasmodium falciparum* malaria, indicated that individuals with AA genotype were more infected with 3+ *Plasmodium falciparum* malaria than individuals with the AS haemoglobin genotype. The study carried out by Bougouma *et al.* (21) revealed AS genotype was associated with lower incidence of clinical malaria relative to AA genotype among children aged 2–3 years, and their findings are not in deviation from the findings in this study, though with younger subjects. Similar to AS, individuals with the SS haemoglobin genotype have a very low chance of infection. For ABO blood group as a factor, considering 3+ *plasmodium falciparum* malaria, the order of infection was: B > A > O > AB, however considering the fact that the p-value and confidence intervals were not statistically significant, this finding calls for a more robust research. The finding from this study is contradictory to a study presented by Zerihun *et al.* (22) which indicated that *P. falciparum* infection showed significant association with blood types (P<0.05) and the chance of having *P. falciparum* infection in patients with blood groups A, B and AB was 2.5, 2.5 and 3.3 times more than individuals having blood O phenotypes, respectively. The variation observed could arise from variations in gene frequencies. For Rh D factor, its presence (Rh D+) indicated a high infection rate as seen from the study. However, considering the number of Rh D- subjects (3 subjects), the comparison is not robust enough to draw a statistical conclusion; this is in agreement with a research carried out by Rattanapan *et al.* (23) where they reported no link between the Rh D blood group and malaria. Based on gender, the females may likely be infected with 3+ *falciparum* malaria than males, but considering the confidence interval and p-value which is >1 and >0.05 respectively, gender may not be considered as a risk factor for malaria in the studied population.

Consideration statistical outcomes for being infected with 2+ *plasmodium falciparum* malaria, the study revealed that subjects with the AA haemoglobin genotype had slightly increased numbers. Subjects with the AS genotype recorded a slightly decreased outcome in terms of number of infections, but again, this difference is not statistically significant. The

odd ratios (OR) for the blood groups under consideration of associating them as a risk factor of being infected with 2+ *plasmodium falciparum* malaria, indicated that none of the blood groups (A, B, AB, O) showed strong association with the risk of 2+ *falciparum* malaria infection, however, the infection trend indicated (B > A > O > AB) but with p-values and confidence intervals that are not statistically significant. Both Rh D+ and Rh D- individuals do not show a significant difference in the risk of infection with 2+ malaria. Based on gender, there is difference in numbers; the trend indicates that the female gender was more infected with *falciparum* malaria than their male counterpart; however, considering the outcomes of p-values and confidence intervals, this may not be considered clinically relevant in the epidemiology of the disease.

For 1+ *plasmodium falciparum* malaria, the study revealed that subjects with the AA genotype have a slightly decreased outcome in terms of being infected with 1+ *Plasmodium falciparum* malaria than subjects who are of the AS. Individuals with AS haemoglobin variants were more compared to other variants – this may be as a result of the reduced ability of *Plasmodium falciparum* parasites to grow and multiply in HbAS red blood cells, and also because they are less likely to fall ill compared to AA individual; thus, they do not take malaria prophylaxis as often as subjects who are AA, who whenever they are attacked, their response to *falciparum* malaria seems to be more severe than AS individuals, and the prophylaxis likely to have reduced the parasite load. For the blood groups under consideration, none of the blood groups (A, B, AB, O) indicated a strong association with the risk of 1+ *falciparum* malaria infection. The differences in odds, risk, and likelihood ratios were not statistically significant; the confidence intervals were large; and based on these outcomes, there are no association; and this is also applicable to gender disparity (there is no association found between gender and malaria infection). However, a research conducted by Okiring *et al.* (24) indicates a higher likelihood of females contracting malaria compared to males. Possible reasons for the difference in this study to that of theirs could be as environmental factors and variations in sample size.

5. Conclusions

Conclusively, 3+ *Plasmodium falciparum* malaria infection is common amongst individuals with AA haemoglobin genotype, blood group B, Rh D+ and females; 2+ *P. falciparum* infection is common amongst individuals with haemoglobin genotype AA, blood group B, Rh D- and females; while 1+ *P. falciparum* malaria infection is common amongst individuals with AS haemogobin genotype, blood group AB and Rh D+, and amongst males than females.

6. Recommendation

We recommend a larger sample size be considered for further studies, and the use of molecular techniques for malaria diagnosis and quantitation.

Author Contributions: This work was carried out and approved in collaboration between all authors who take responsibility for its intellectual contents, accuracy and integrity. SGC designed the study; PNO sourced for funding; SGC wrote the protocol; PNO and DTR contributed in literature search; SGC, PNO, BBB and DTR did laboratory experiments; SGC did the statistical data analysis; SGC, PNO, BBB and DTR contributed in the discussion; SGC and PNO drafted the manuscript; SGC supervised the study.

Informed Consent Statement: "Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Okoroiwu, I. J., Obeagu, E. I., Christian, S.G., Elemchukwu, Q. & Ochei, K. C. Determination of the haemoglobin, genotype and ABO blood group pattern of some students of Imo State University, Owerri, Nigeria. International Journal of Current Research and Academic Review, 2015;3(1), 20-27.
2.. Mitra, R., Mishra, N. & Rath, G. P. Blood groups systems. Indian Journal of Anaesthia, 2014;58(5), 524-528.
3. Lögdberg, L., Reid, M. E., Lamont, R. E. & Zelinski, T. Human blood group genes 2004: Chromosomal locations and cloning strategies. Transfusion Medical Review, 2004;19, 45–57.

4. Lögdberg, L., Reid, M. E. & Zelinski, T. Human blood group genes 2010: Chromosomal locations and cloning strategies revisited. Transfusion of Medical Review, 2011;25, 36-46.
5. Westhoff, C. M. The Rh blood group system in review: A new face for the next decade. Transfusion, 2004;44, 1663–1673.
6. Levine, P., Celano, M. J., Wallace, J. & Sanger, R. A human 'D-like' antibody. Nature, 1963;198, 596-597.
7. Landsteiner, K. & Wiener, A. S. (1940). An agglutinable factor in human blood recognized by immune sera for rhesus blood. Procedure and Societal Experimental Biological Medicine, 1940;43, 223-228.
8. Fisk, R. T. & Foord, A. G. Observations on the Rh agglutinogen of human blood. American Journal of Clinical Pathology, 1942;12, 545-552.
9. Lukin, J. A. & Ho, C. The structure function relationship of haemoglobin in solution at atomic resolution. Chemical Reviews, 2004;104(3), 1219-1230.
10. Safo, M. K., Ahmed, M. H. A., Ghatge, M. S. & Boyiri, T. Haemoglobin–ligand binding: Understanding Hb function and allostery on atomic level. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 2011;1814(6), 797-809.
11. Scott, T. Introduction to Medical Laboratory Technology. British Journal of Biomedical Science, 1999;56(2), 154 - 158.
12. Tamarin, R. H. Principles of genetics. (7th ed.), USA: The McGraw Hall Companies, Inc. 2002.
13. Christian, S. G., Eze E. M. & Briggs, S. K. Haemoglobin genotype, ABO and Rhesus blood group distribution in Briggs family of Abonnema, Rivers State, Nigeria. Asian Journal of Medicine and Health, 2018;13(2), 1–8.
14. American Academy of Pediatrics. Red Book: Report of the Committee on Infectious Disease, 32nd Edition. Illinois, Academy of Pediatrics, 2021.
15. Rietveld, A. E. C. & Newman, R. D. (2015). Malaria. In: Heymann DL, editor. Control of communicable diseases manual. 20th ed. Washington, DC: American Public Health Association, 2015;372-389.
16. Centers for Disease Control and Prevention (CDC). Malaria. Available from: https://www.cdc.gov/malaria, 2017.
17. Afoakwah, R., Aubyn, E., Prah, J., Nwaefuna, E. K. & Boampong, J. N. Relative susceptibilities of ABO blood groups to Plasmodium falciparum malaria in Ghana. Advanced Haematology, 2016;5(3), 68793.
18. Luzzatto, L. Sickle Cell Anaemia and Malaria. Mediterranean Journal of Haematology and Infectious Diseases, 2012;(1), 213 – 218.
19. Chauvin, E. D., & Weil, D. N. Malaria and Early African Development: Evidence from the Sickle Cell Trait. Economic Journal, 2018;128(610), 1207–1234.
20. Cheesbrough, M. District Laboratory Practice in Tropical Countries, Second Edition Update, Part 2. Cambridge: Cambridge University Press, 2010.
21. Bougouma, E. C., Tiono, A. B., Ouédrago, A., Soulama, I., Diarra, A., Yaro, J., Ouédraogo, E., Sanon, S., Konaté, A. T., Néblé, I., Watson, N. L., Sanza, M., Dube, T. J. & Sirima, S. B. Haemoglobin variants and Plasmodium falciparum malaria in children under five years of age living in a high and seasonal malaria transmission area of Burkina Faso. Malaria Journal, 2012;11, 154.
22. Zerihun, T., Degarege, A. & Erko, B. Association of ABO blood group and Plasmodium falciparum malaria in Dore Bafeno area, Southern Ethiopia. Asian Pacific Journal of Tropical Biomedicine, 2011;1(4), 289–294.
23. Rattanapan, T., Duangchan, K., Wangdi, A., Mahittikorn, M. & Kotepui, A. Association between rhesus blood groups and malaria infection: A systematic review and meta-analysis. Tropical Medical Infectious Disease, 2023;8(4), 190-106.
24. Okiring, J., Epstein, A., Namuganga, J. F., Kamya, E. V., Nabende, I., Nassali, M., Sserwanga, A., Gonahasa, S., Kamya, M. R., Nankabirwa, J. I., Briggs, J., Jagannathan, P. & Dorsey, G. (2022). Gender difference in the incidence of malaria diagnosed at public health facilities in Uganda. Malaria Journal, 2022;21, 22.