Original Research Article

CHARACTERIZATION OF ABO/RHESUS ANTIGEN POLYMORPHISM ASSOCIATED WITH MALARIA IN A MALARIA HOTSPOT IN BAYELSA STATE, NIGER DELTA, NIGERIA

ABSTRACT

Aim: The aim of the present study was to characterize the role of ABO/Rhesus blood group antigen polymorphism in malaria among adults in Bayelsa State, Niger Delta, Nigeria. The pattern of distribution of ABO/Rhesus antigen polymorphism in the area was also assessed.

Materials and methods: Two hundred and six adults (91 males and 115 females) were randomly selected for the study and they were examined for the presence of malaria parasite infection and illness as well as for type of ABO/Rhesus antigen polymorphism using real time PCR-high resolution melting analysis, clinical malaria indicators and agglutination methods respectively. The density of malaria parasitemia was evaluated using microscopy and socio-demographic variables were collected using a structured questionnaire. Multinomial *logistics regression* and one way ANOVA tests were used to assess the difference between frequencies and means, respectively.

Results: showed that the distribution of ABO/Rhesus antigen polymorphism were in the following order: blood group O, 50% (O^+ 47.57%; O^- 2.43%), blood group B, 21.36% (B^+, 20.39%; B^-, 0.97%), blood group A, 18.93% (A^+, 18.93%, A^-, 0%), and blood group AB (AB^+, 9.71%, AB^-, 0%). Among severe malaria subjects, ABO antigen polymorphism distribution were A, 29%; B, 57%; AB, 5% and O, 10%. Among mild malaria subjects, ABO antigen polymorphism distribution were A, 36%; B, 33%; AB, 23% and O, 8%. Among asymptomatic malaria subjects, ABO antigen polymorphism distribution were A, 12%; B, 12%; AB, 7% and O, 69% of the subjects respectively. Among uninfected subjects, ABO antigen polymorphism distribution were A, 20%; B, 20%; AB, 7% and O, 53%. Each ABO blood group antigen was associated with asymptomatic malaria, while blood group A, B, and AB were associated with mild malaria and only blood group B was associated with severe malaria ($P < 0.05$). Parasite density showed no association with ABO Blood group.

Conclusion: ABO blood group antigen polymorphism was associated with malaria. Blood group O conferred protection against mild and severe malaria, while blood group B conferred susceptibility to severe malaria. Prevention and treatment of malaria in relation to ABO blood groups is implicated in the present study

Keywords: [Blood groups, HRM-analysis, malaria, Sagbama, Bayelsa]

1. INTRODUCTION

Malaria is a global public health problem. It is an infectious disease caused by *Plasmodium* and transmitted by the female Anopheles mosquitoes [1]. The symptoms of malaria include, fever, headache and chills. The onset is usually 10 – 15 days after been bitten by *Plasmodium* infected mosquitoes and complications include seizures, coma, as well as anaemia. An estimated 247 million cases of malaria was recorded globally in 2021 against 245 million cases in 2020 with a global mortality of 619 000 attributed to malaria in 2021. Out of this estimate, sub-Saharan Africa had the highest number of cases and deaths [1].

Nigeria, the most populated country in Africa has a malaria prevalence of 22% [2]. In Nigeria, malaria accounts for 60% of outpatient visits to health facilities, 30% of childhood deaths, 11% of maternal death and 25% of deaths in infants. The prevalence of malaria has been increasing with age and is higher in rural areas compared with urban areas [2]. Nigeria has the highest prevalence of malaria globally and contributes 27% to global malaria cases and 32% to global malaria deaths [1]. The distribution of malaria varies across different states in Nigeria. A previous study found that the prevalence of malaria parasite infection in Bayelsa State was 92.72%. This high prevalence makes Bayelsa State a malaria hotspot. The previous study revealed that the 92.72% prevalence of malaria *Plasmodium falciparum* found in Bayelsa State was defined by high clinical malaria and high asymptomatic malaria carriage [3]. Thus, eradicating malaria requires community ownership and active participation [4].

ABO blood group antigens remains the most immunogenic of all the blood group antigens and **are very important clinical tools that are commonly used in blood transfusion assessment [5]. ABO blood groups have been implicated in COVID19 susceptibility and severity [6],** cancer, cardiovascular diseases, infections, hematologic disorders, cognitive disorders, circulatory diseases and metabolic diseases [7]. However, studies on the association of ABO blood group antigen polymorphism with malaria has yielded conflicting results. A previous study found no significant association between ABO blood groups and susceptibility to malaria [8], while another study found association of malaria parasitemia with ABO blood group polymorphism [9] In another study carried out by Onanuga and Lamikanra [5], no association between malaria and ABO blood group antigens was found. Furthermore, another study found higher parasite density among blood group O individuals compared with other blood groups [10]. Geographical variations in the distribution of ABO blood group antigens have also been previously reported [9, 11, 12]. These variations and conflicting results suggest the need for further studies on the relationship between ABO/Rhesus blood group and malaria susceptibility. Thus, the aim of the present study was to evaluate the malaria protective role of ABO/Rhesus blood group antigen polymorphism among adults in Niger Delta, Nigeria. The pattern of distribution of ABO/Rhesus antigen polymorphism in Niger Delta, Nigeria was also assessed.

2. MATERIAL AND METHODS

2.1 **Study location and study population**

The study location was Sagbama, it is a local Government Area (LGA) in Niger Delta, Nigeria under Bayelsa State and it lies partly within the Bayelsa National Forest [13, 14]. A house-to-house community based cross sectional study design was employed to randomly select 206 study population [15] and they were grouped into uninfected, asymptomatic malaria, mild malaria and severe malaria groups [2, 16, 17]. Each group was further stratified based on ABO/Rhesus antigens blood groups. Sample size was determined by Cochran formula [18] as previously described [19]. Approval for the study was obtained from the Ethics and Research Committee under the Bayelsa State Primary Health Care Authority in Sagbama Local Government Area as well as from the community development chairman of every selected community. Voluntary informed consent was obtained from each participant and they were assured of confidentiality. Sociodemographic variables of the study participants were obtained using a structured questionnaire [20].

2.2 Inclusion and exclusion criteria for the study

Individuals below 18 years of age were excluded from the study. Adults negative for *Plasmodium falciparum* infection were included in group 1 (uninfected group). Adults positive for *Plasmodium falciparum* infection but with no febrile illness were included in group 2 (asymptomatic malaria group) [17]. Adults positive for *Plasmodium falciparum* infection, with febrile illness but with no anaemia (haemoglobin concentration above 8 g/dl) were included in group 3 (mild malaria group). Adults positive for *Plasmodium falciparum* infection, with febrile illness as well as with anaemia (haemoglobin concentration below 8 g/dl) were included in group 4 (severe malaria group) [2, 3].

2.3 Assessment of febrile illness

Temperature measurement using axillary method (under the armpit) was used for the determination of febrile illness following the manufacturer`s protocol for Royal care diagnostics digital thermometer (Royal Care Diagnostic, Indira colony, Chennai, India). The thermometer was placed under the armpit of participants and they were asked to hold their arms down tightly at their side until the digital thermometer beeped, then the thermometer was removed and the temperature value displayed on the thermometer window was read and recorded.

2.4 ABO/Rhesus blood group test

Agglutination method was used for ABO/Rhesus blood group test [21] using commercially available ABO/Rhesus blood group test kit (Bio Lab Diagnostics (1) Private Limited, Mumbai, India) following the manufacturers' instructions Briefly, blood samples from each participant was sectioned into three on a flat tile and monoclonal sera anti-A, anti-B, and anti-O specific IgM immunoglobulin was directed against the human red blood cell antigens A, B and Rhesus D respectively, by adding each antisera separately on the sectioned blood sample of each participant. The presence of agglutination on any of the blood sections was taken as confirmatory positive test for the presence of the respective ABO/Rhesus blood group antigen.

2.5 Human DNA extraction and purification

gSYNC™ extraction kit (Geneaid, Biotech. Ltd, New Taipei, Taiwan) was used for genomic DNA extraction and purification following the manufacturer's protocol as previously described [3]. Briefly, 200 μl of whole blood was mixed with 20 μl of proteinase K, and was incubated at 60^{0}C for 5 min. Then 200 μl of GSB buffer was added to the mixture, followed by incubation at 60^{0}C for 5 minutes and vigorous mixing of the mixture after the addition of 200 μl of absolute ethanol. Absolute ethanol was added to precipitate the DNA Thereafter, centrifugation at 14,000×*g* was carried out in order to extract the precipitated DNA from the mixture. Extracted DNA was purified in a two-step process. First, 400 μl of W1 buffer was added to the extracted DNA in a spin column followed by centrifugation and decantation of the flow through. Secondly, a mixture of wash buffer and 600 μl of absolute ethanol was added to the extracted DNA in the spin column followed by centrifugation and decantation of flow through. The column matrix was dried by centrifugation and the purified DNA was eluted with a 100 μl of pre-heated elution buffer.

2.6 Plasmodium falciparum detection using real time PCR-high resolution melting analysis

HOT FIREPol® EvaGreen® HRM Mix-ROX kit (SolisBiodyne, Tartu Estonia) was used to detect **the presence of *Plasmodium falciparum* in the extracted human DNA of the study population following the manufacturer's protocol, as previously described [3].** Briefly, 4 μl of genomic DNA template, 4 μl of HOT FIREPol® EvaGreen® HRM Mix-ROX, 11 μl of nuclease-free water and 1 μl primer mix were added to a PCR tube. The forward primer sequence was: 5`TTAAACTGGTTTGGGAAAACCAAATATATT3` while the reverse primer sequence was 5`ACACAATGAACTCAATC ATGACTCCCGTC 3` [22]. If *Plasmodium falciparum* is present in the sample, the primer pair will amplify the 18S small subunit (SSU) of the rRNA gene of *Plasmodium falciparum* in the sample. Polymerase chain reaction (PCR) was carried out in a StepOnePlus™ Real-Time PCR Thermal Cycler Systems (Applied Biosystem, Foster City, USA) under the following conditions: an initial activation at 95 ^{0}C for 15 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 65 ^{0}C for 20 s, extension at 72 ^{0}C for 20 s and a post-amplification high resolution melting curve prepared from 65^{0}C to 90^{0}C for 10 minuetes using a default setting. Positive and negative controls were included in the assay and the reaction was carried out in duplicates. Samples with melting temperatures (T_m) aligning with the T_m of positive control (with a T_m shift interval of ± 0.4 ^{0}C) and with fluorescent signal within 30 cycles (C_t) were considered positive for *Plasmodium falciparum*. HRM curves were generated using high resolution melt software v 3.0.1 (Applied Biosystems, step one plus) [17, 23]. A previous study showed that high resolution melting analysis (HRM-analysis) was more sensitive than microscopy for *Plasmodium falciparum* determination [3] that was why the present study employed HRM-analysis for *Plasmodium falciparum* determination.

2.7 Malaria parasitemia density determination by microscopy

Six microliter of blood from each participant was used to prepare thick film, while two microliter was used to prepare thin film and the thin film was fixed with absolute methanol. The thick and thin blood films were stained with 10% and 3% Giemsa stain respectively and were examined using immersion oil with a magnification of x100. *Plasmodium falciparum* parasites were counted per 500 leukocytes for parasite count less than 100 or per 200 leukocyte for parasite count equal to 100 and above. This count was used to estimate parasite density (parasites per microliter of blood) using an assumed parasite count of 8000 white blood cells/µl [24, 25].

2.8 Statistical analysis

SPSS software version 24 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Chi square test and multinomial logistic regression were used to test for significant association between categorical variables. One way-ANOVA was used to test for significant difference between means. Significant level was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Sociodemographic determinants of malaria in Sagbama LGA

Table 1 shows the sociodemographic phenotypes associated with susceptibility or protection against malaria among adults in Sagbama LGA, Bayelsa State, Nigeria. All sociodemographic phenotypes were associated with susceptibility to asymptomatic malaria ($P < .05$). This shows that *Plasmodium* infection has no respect for age, sex, occupation, marital status nor level of education. Thus possessing any of the sociodemographic variables does not guarantee the likelihood of protection against the malaria *Plasmodium* ($P < .05$). This suggests that exposure is the underlying factor for infection. Thus, as shown in the present study, once an individual is exposed to the *Plasmodium,* by way of finding oneself in a locality of high transmission, the likelihood of acquiring asymptomatic infection is very high. The present study also found that some of the socio-demographic variables were associated with mild malaria ($P < .05$). However, no sociodemographic variable was associated with severe malaria. This suggests that factors other than exposure and sociodemographic variables are responsible for the transition from infection to malaria illness. Although previous studies have found an association of malaria illness with age and sex [25, 26], the present study found no association. This suggest the need for further study on the impact of socio-demographic variables on malaria. The present study was a house-to-house community based study in rural locations were standard hospital and sewage disposal facilities are hardly ever available, with certain locations only accessible by boats and almost every household surrounded by standing body of water [27, 28]. Thus, larger sub-populations of sociodemographic variables unlikely to be present in hospital based malaria studies [25, 26], were captured in the present study.

Table 1 Sociodemographic characteristics of adults in Sagbama LGA, Bayelsa State, Nigeria

Sociodemographic Characteristics	**Uninfected N (%)**	**Asymptomatic) N (%; *P*-value)**	**Mild N (%; *P*-value)**	**Severe N (%; *P*-value)**
Sex				
Male	**7 (47%)**	**55 (42%; .00)**	**19 (49%; .02)**	**10 (48%; 05)**
Female	**8 (53%)**	**76 (58%; .00)**	**20 (51%; .03)**	**11 (48%; .05)**
Age (years)				
18 – 25	3 (20%)	28 (21%; .00)	9 (23%; .10)	9 (43%; .10)
26 – 35	3 (20%)	43 (33%; .00)	10 (26%; .07)	3 (14%; 1.00)
36 – 45	3 (20%)	30 (23%; .00)	10 (26%; .07)	6 (29%; .33)
46 – 55	2 (13%)	16 (12%; .01)	4 (10%; .42)	3 (14%; .66)
≥56	**4 (27%)**	**14 (11%; .03)**	**6 (15%; .53)**	**0 (-; -)**
Marital status				
Single	5 (33%)	50 (38%; .00)	17 (44%; .02)	13 (62%; .07)
Married	10 (67%)	81 (62%; .00)	22 (56%; .04)	8 (38%; .64)

Occupation				
Farming / fishing	7 (47%)	71 (54%; .00)	22 (56%; 01)	14 (67%; .13)
Petty trading	3 (20%)	28 (21%; 00)	13 (33%; .03)	5 (24%; .48)
Civil servants	3 (20%)	17 (13%; .01)	4 (10%; .48)	2 (10%; .66)
Craftsmanship	2 (13%)	15 (11%; 01)	0 (-; -)	0 (-; -)
Formal education				
None	4 (27%)	29 (22%; 00)	17 (44%; .01)	8 (38%; .26)
Primary	3 (20%)	20 (15%; 00)	14 (36%; .02)	5 (24%; .48)
Secondary	5 (33%)	52 (40%; 00)	4 (10%; 74)	4 (19%; .74)
Tertiary	3 (20%)	30 (23%; 00)	4 (10%; .71)	**4 (19%; .71)**

N = number of participants

3.2 Distribution of ABO blood group polymorphism in Sagbama

Figure 1 shows the distribution of ABO polymorphism among adults in Sagbama LGA, Bayelsa State. Blood group O was the most frequent (50%), followed by blood group B (21.36%), A (18.93%) and AB (9.71). A previous study carried out in Chongjing, China, found the same distribution order of ABO antigen polymorphism, but with different proportions as follows; blood group O 35.54%, B, 31.90%, A, 24.14% and AB, 8.42% [29]. Also in another previous study in India the distribution order for ABO polymorphism was similar to what was found in the present study but the proportion was also different and it was blood group O, 34.56%; B, 34.10%; A, 23·16%; 34·10%, and AB, 8·18% [30]. However, contrary to the distribution order found in the present study, a previous study carried out among the Mexican population, found blood group A as the second most prevalent, O, 61.82%; A: 27.44%; B: 8.93%; and AB: 1.81% [31], unlike the present study where blood group B was the second most prevalent. This reemphasizes the fact that around the world, ABO blood group distribution varies and underscores the need for the present study. Furthermore, given that ABO polymorphism has been associated with various diseases [6, 7], this variation in ABO blood group distribution suggest a basis for the possible variation in response to diseases around the world.

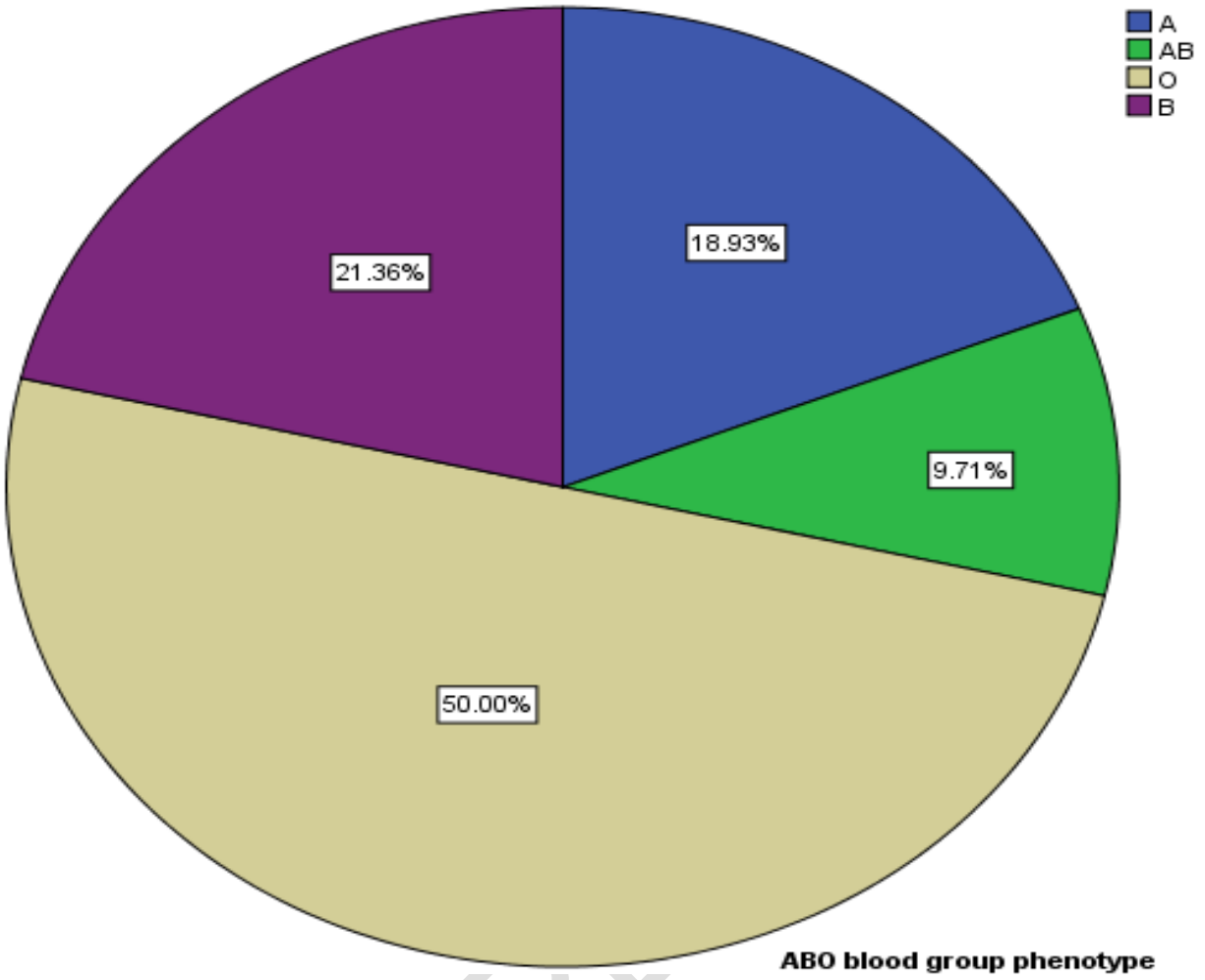

Figure 1 Distribution of ABO polymorphism in Sagbama LGA, Bayelsa State, Nigeria

3.3 Distribution of Rhesus blood group polymorphism in Sagbama

The distribution of Rhesus blood group polymorphism in Sagbama is presented in Figure 2. As shown in Figure 2, 95.63% of the study population were Rhesus positive. A previous study found that the prevalence of Rhesus positive was 95.58% among Mexican population [**31], while another previous study found that the prevalence of Rhesus positive was 98% in Uganda [32], These findings show that little or no geographical variation exist with respect to Rhesus antigen distribution.**

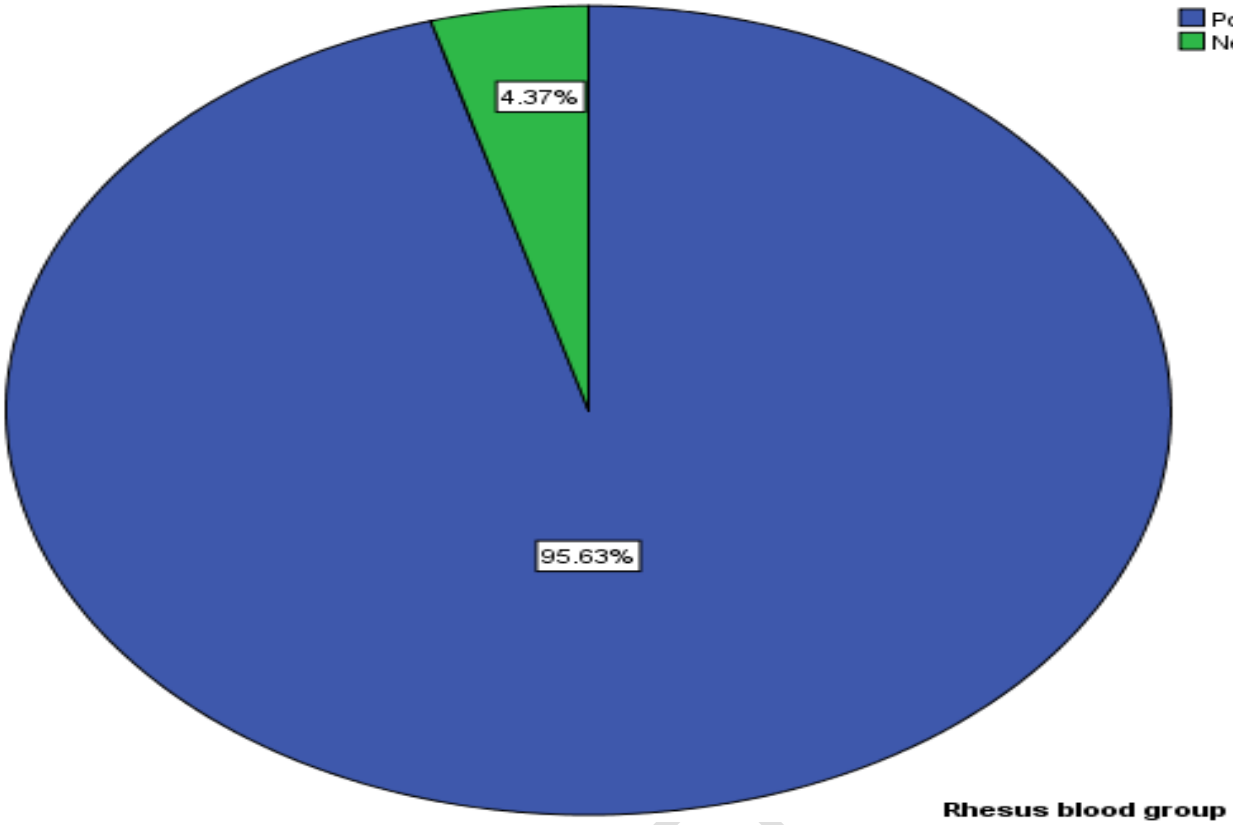

Figure 2 Distribution of Rhesus blood group antigens among adults in Sagbama LGA, Bayelsa State

3.4 Distribution of Rhesus D among the different ABO blood groups in Sagbama LGA

The pooled ABO and Rhesus (Rh) antigen distribution in Sagbama LGA is showed in Figure 3. Blood group O^+ (O positive) was the most frequent (47.57%), while B^- (B negative) was the least frequent. Blood group AB^- (AB negative) was not found among the study population. In a previous study, it was also found that blood group O^+ (O negative) antigens were the most frequent [31]. However in contrast with the present study, blood group AB^- (AB negative) was found in the Canizalez-Román et al., [31]. Furthermore, as shown in Table 2, Rhesus blood group distribution did not vary to a large extent with ABO blood group, but was almost equally distributed among all the ABO blood groups (A^+. 94.87%; B^+, 95.45%; and O^+. 95.15) except for blood group AB where only AB positive (100%) was found. This probably suggests that Rhesus antigens has little or no influence on the clinical role of ABO antigen polymorphism. This finding corroborates the finding of a **previous study [32].** Studies on the geographical variations in blood group distribution is fundamental to health and clinical practice. This underscores the importance of the present study.

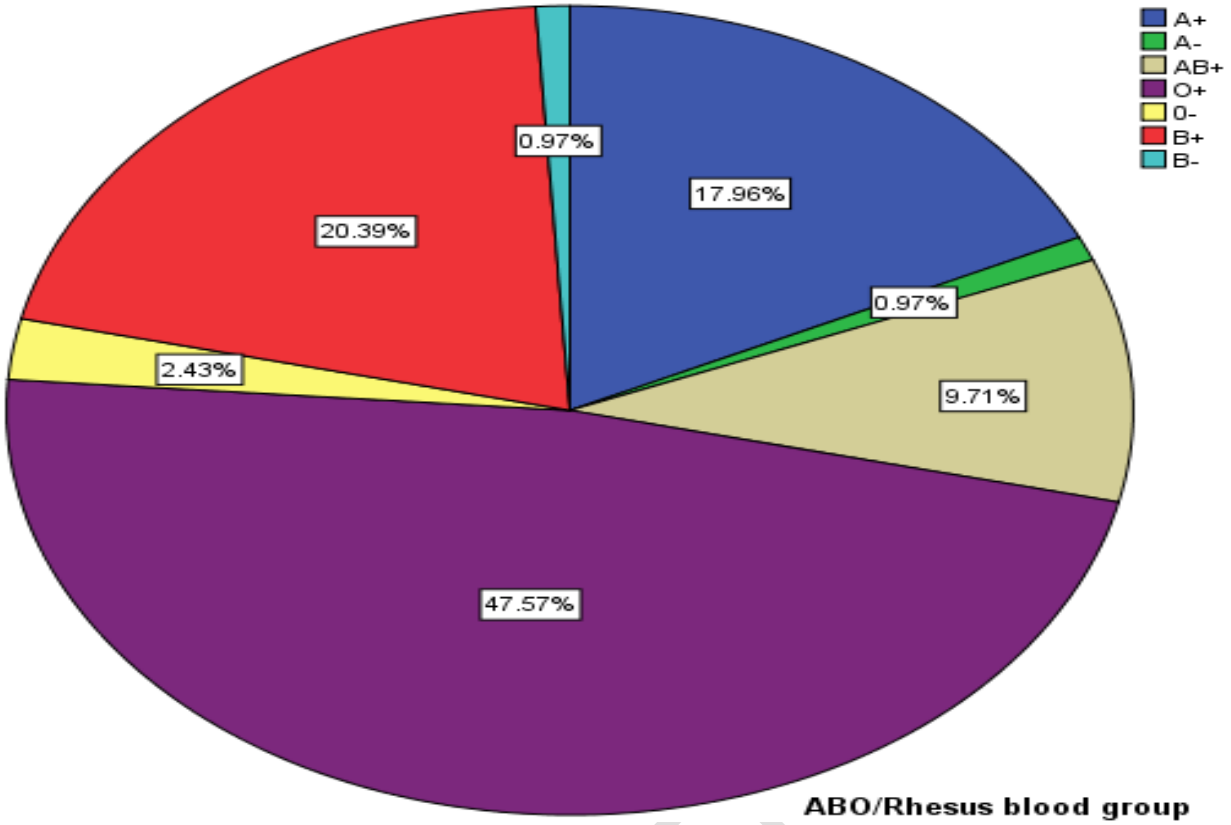

Figure 3: Distribution of ABO/Rhesus blood group antigens among adults in Sagbama LGA, Bayelsa State

Table 2 Distribution of Rhesus D among the different ABO blood group polymorphism among adults in Sagbama LGA, Bayelsa State, Nigeria

ABO polymorphism	Rhesus D positive N (%)	Rhesus D negative N (%)
A	37 (94.87)	2 (5.13)
B	42 (95.45)	2 (4.55)
AB	20 (100)	0 (0.00)
O	98 (95.15)	5 (4.85)

N = number of participants

3.5 ABO antigen polymorphism associated with malaria in Sagbama LGA, Bayelsa State

Table 3 shows the ABO blood groups associated with malaria. As showed in Table 3, blood group O was significantly associated with asymptomatic malaria infection (*P* value; .000), but was not associated with mild malaria (*P* value = .147) nor with severe malaria (*P* value; .484). This suggests that blood group O is protective against mild and severe malaria but not against asymptomatic malaria. As showed in Table 3, blood groups A (*P* value: .008), B (*P* value: .008) and AB (*P* value: .037) were all associated with asymptomatic malaria and mild malaria. Only blood group B was associated with severe malaria (*P* value: .032). Furthermore, as shown in Table 3 and Figure 4, Rhesus factor D was associated with both asymptomatic malaria and mild malaria (*P*-values: .001 and .000 respectively). Thus no definitive protective or susceptibility role could be ascribed to Rhesus D in relation to malaria in the

present study. The role of Rhesus D in malaria requires further investigation. Previous **findings [33], corroborates the findings of the present study.**

Table 3 Characterization of ABO/Rhesus antigen polymorphism associated with malaria in Sagbama LGA, Bayelsa

Study group [a]	Blood group	*P* value	OR	95% confidence interval
Asymptomatic	A (12%)	.008	5.333	1.554 – 18.304
	B (12%)	.008	5.333	1.554 – 18.304
	AB (7%)	.037	9.000	1.140 – 71.038
	O (69%)	.000	11.250	5.459 – 23.184
Mild	A (36%)	.015	4.667	1.341 – 16.239
	B (33%)	.022	4.333	1.235 – 15.206
	AB (23%)	.037	9.000	1.140 – 71.038
	O (8%)	.147	.375	.099 – 1.414
Severe	A (29%)	.147	.375	.099 – 1.414
	B (57%)	.032	4.000	1.129 – 14.175
	AB (5%)	1.000	1.000	.063 – 15.988
	O (10%)	.484	1.667	.398 – 6.974
Asymptomatic	Rhesus+	.000	9.923	5.610 – 17.552
	Rhesus-	1.000	1.000	.141 – 7.099
Mild	Rhesus+	.001	2.923	1.557 – 5.487
	Rhesus⁻	.571	.500	.045 – 5.514
Severe	Rhesus+	.292	1.462	.722 – 2.959
	Rhesus⁻	1.000	1.000	.141 – 7.099

[a]. the reference category is: uninfected. OR = Odd ratio; association significant at $P < 0.05$.

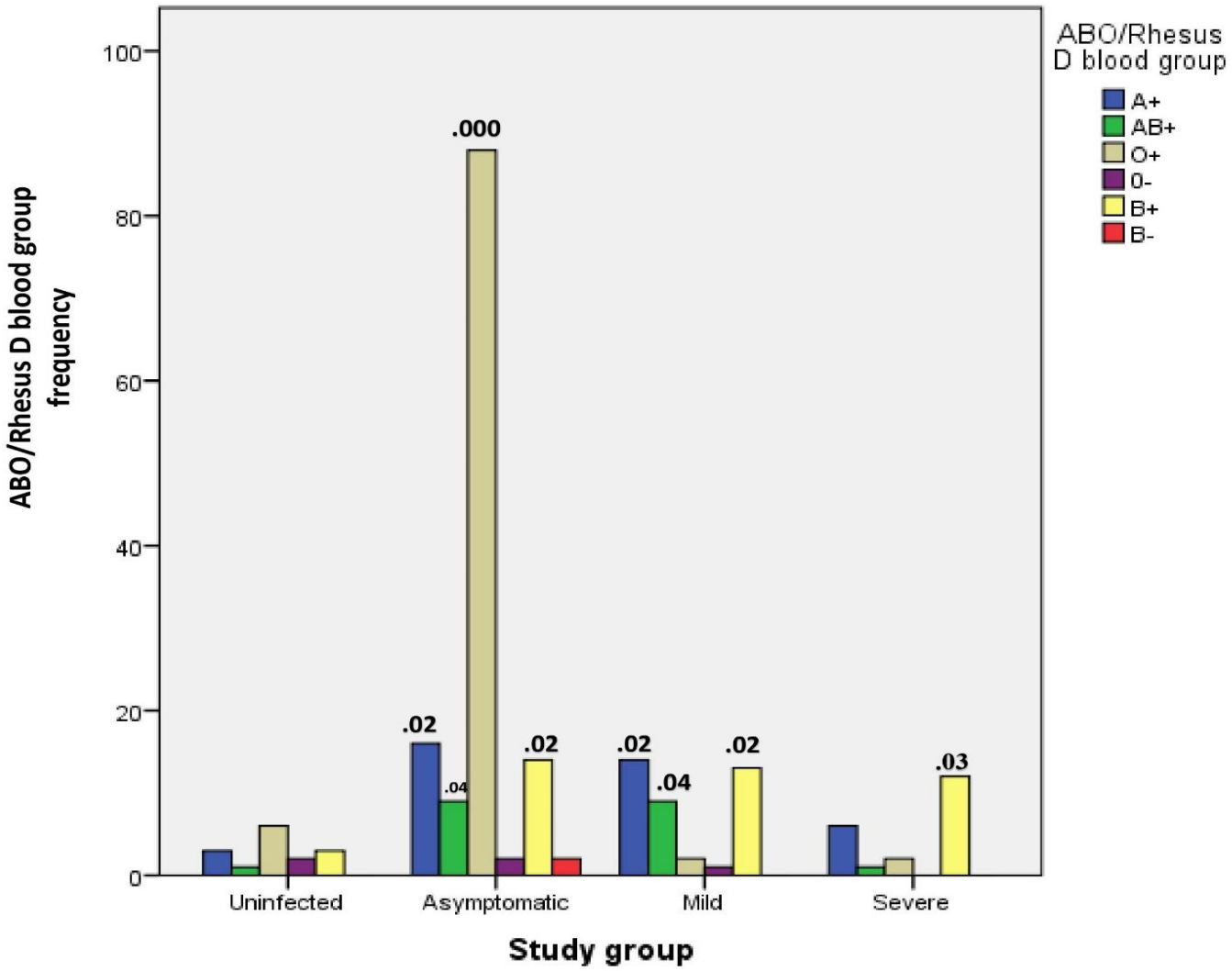

Figure 4 ABO/Rhesus antigens associated with malaria infection. Values represent *P*-values. Bars with *P*-values less than .05 are significantly associated with malaria

3.6 Association of malaria parasite density with ABO blood groups in Sagbama LGA

Presented in Table 4 is the association between parasite density and ABO blood groups in relation to malaria in Sagbama LGA. As shown in Table 4, although differences in parasite density was found among the different blood groups, however, no significant difference (*P* value < .05) was found. This shows that factors other than parasite density were responsible for the observed difference in malaria outcome among the different blood groups.

Table 4 Association of malaria parasite density with ABO antigen polymor in Sagbama LGA

Blood group	Malaria parasite density (parasites/μl of blood)			
	Uninfected	Asymptomatic	Mild	Severe
ABO				
A	0	19672 ± 337[a]	83330 ± 12476[b]	101622 ± 1108[c]
B	0	20217 ± 267[a]	79587 ± 8898[b]	103515 ± 1558[c]

AB	0	20083 ± 726[a]	82467 ± 1651[b]	112000 ± 0000[c]
O	0	19495 ± 495[a]	77340 ± 8229[b]	105600 ± 4600[c]
Rhesus D				
Positive	0	19927 ± 0000	74185 ± 5985[a]	103779 ± 1244[b]
Negative	0	0	80000 ± 0000[a]	102600 ± 2400[b]

Results presented as mean ± SEM; Values with different superscript on a column are significantly

4. CONCLUSION

In conclusion, the present study found the possible role of ABO antigen polymorphism in malaria among adults in Bayelsa State, Niger Delta, Nigeria. Blood group O conferred protection to malaria while blood group A, B and AB conferred susceptibility to malaria. The pattern of distribution of ABO antigen polymorphism found in the present study in decreasing order was blood group O > B > A > AB. Prevention and treatment of malaria in relation to ABO blood groups is implicated in the present study.

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