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 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 immunoagglutination-based blood group phenotyping 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: In 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, 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. **They are very important clinical tools that are commonly used in blood transfusion assessment [5]. ABO/Rhesus blood group system has been associated with 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 population**

The study location was Sagbama. Sagbama 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 participants for the study [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 Temperature measurement

Axillary method (under the armpit) was used for temperature measurement following the manufacturer`s protocol for Royal care diagnostics digital thermometer (Royal Care Diagnostic, Indiral 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 Erythrocyte ABO/Rhesus antigen phenotyping

The principle of **erythrocyte ABO/Rhesus antigen phenotyping** was based on immunoagglutination [21] and was carried out using commercially available ABO/Rhesus blood group test kit (BioLab Diagnostics (1) Private Limited, Mumbau, 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 Total genomic DNA extraction and purification

gSYNC^{TM} 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].

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

The presence of *Plasmodium falciparum* DNA in the extracted total genomic DNA of each study participant was determined using HOT FIREPol® EvaGreen® HRM Mix-ROX (SolisBiodyne, Tartu Estonia) **following the manufacturer's protocol, as previously described [3].**

Malaria parasite 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. 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].

2.6 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 phenotypes and malaria susceptibility 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, and with certain locations only accessible by boats [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.

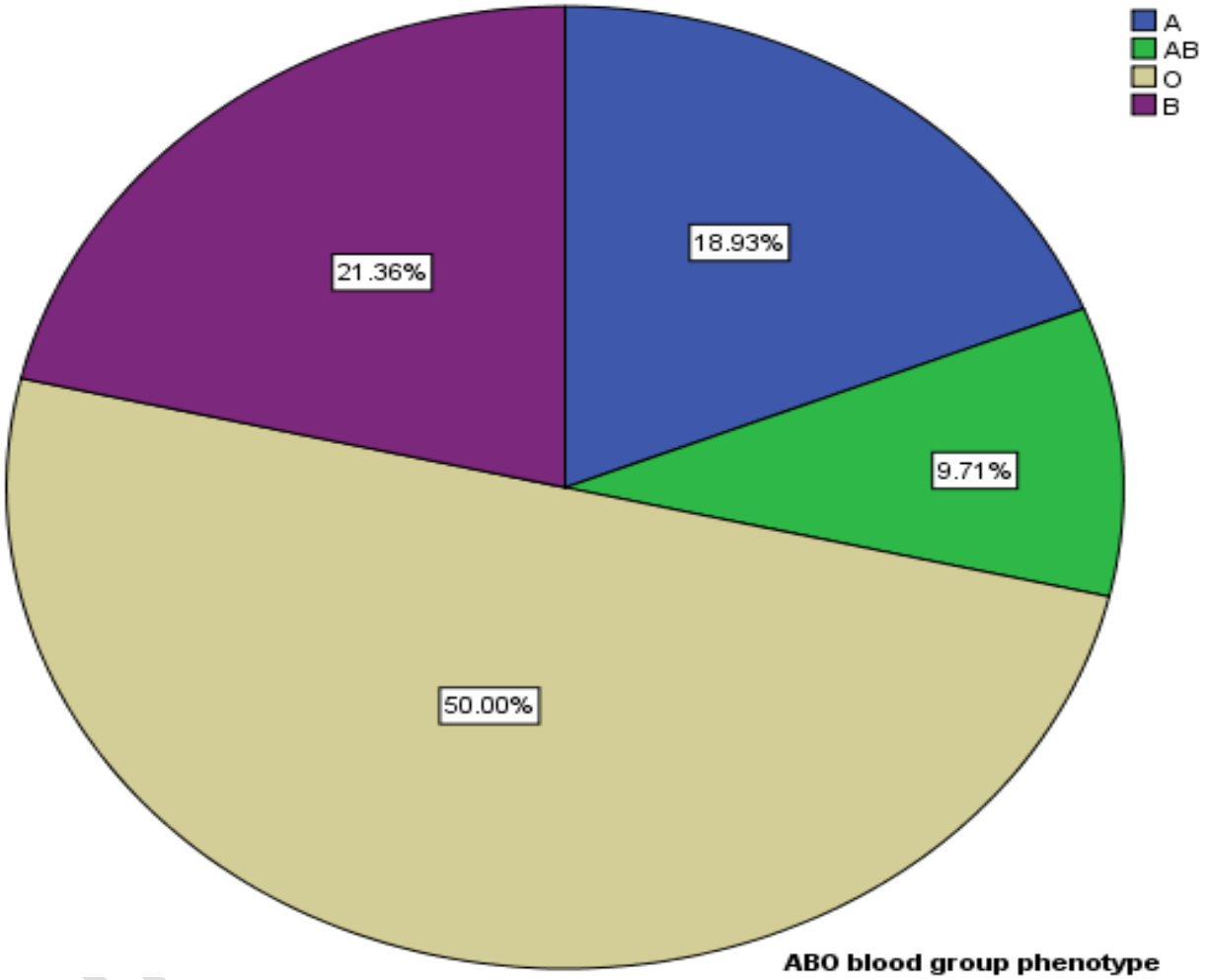

Figure 1 Distribution of ABO polymorphism in Sagbama LGA, Bayelsa State, Nigeria

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. This finding nearly agrees with findings of previous studies where the prevalent Rhesus antigen was 95.58% among Mexican population [**31] and 98% in Uganda [32]. These findings show that geographical variations in Rhesus antigen distribution is minimal compared with geographical variations in ABO antigen polymorphism distribution.**

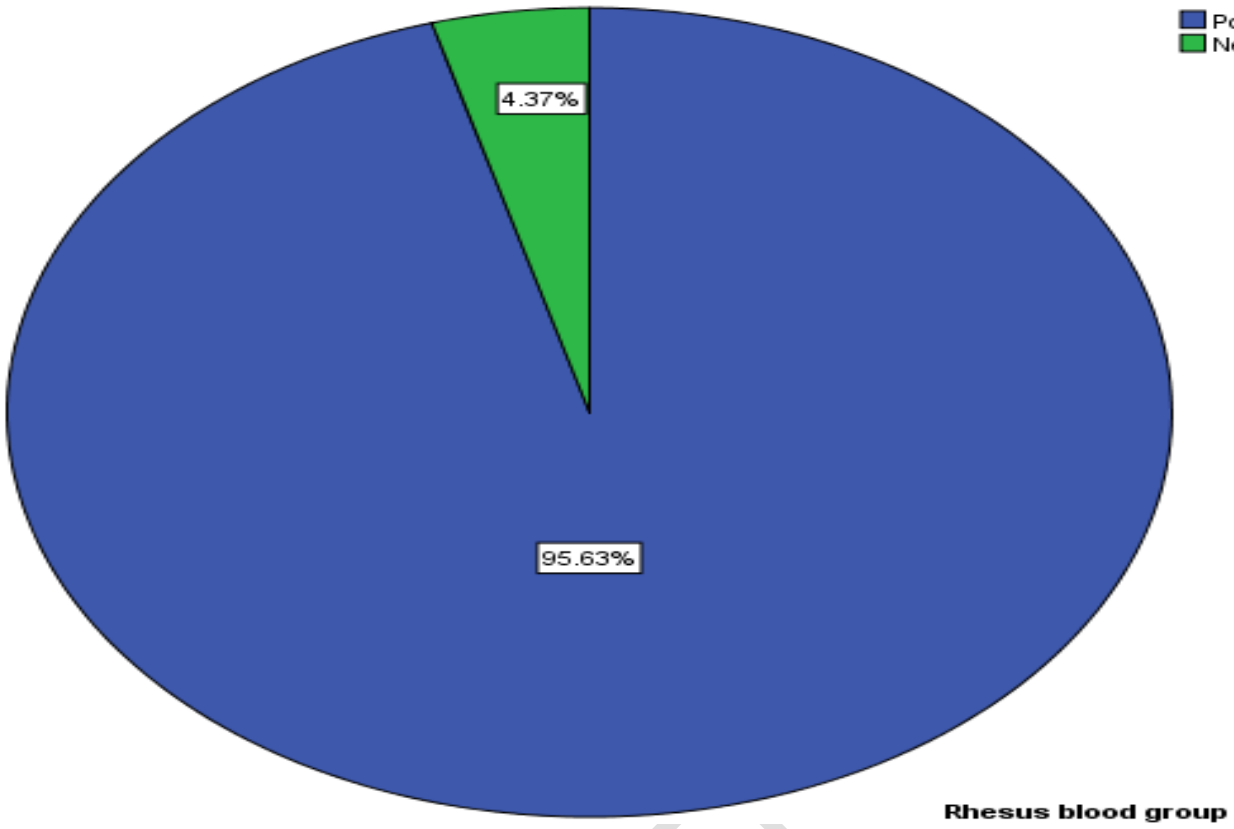

Figure 2 Distribution of Rhesus blood group antigens among adults in Sagbama LGA, Bayelsa State

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 carried out by Apecu et al., [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.

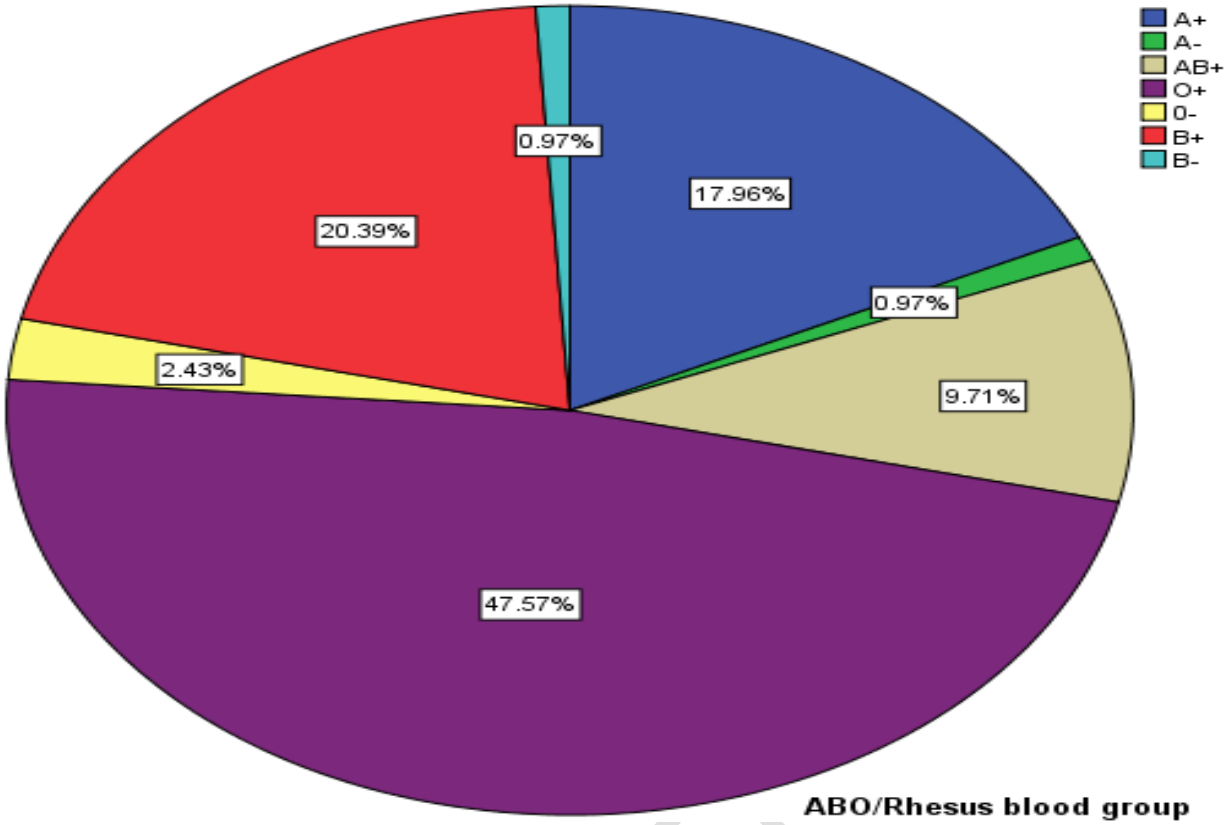

Figure 3: Distribution of ABO/Rhesus blood group antigens among adults in Sagbama LGA, Bayelsa State

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 show the association of ABO blood group polymorphism with malaria using multivariate logistics regression analysis. As showed in Table 3, compared with the uninfected group (reference category), blood group O was significantly associated with the likelihood of acquiring asymptomatic malaria infection (*P* value; .000), Blood group O was however not significantly associated with the risk of mild (*P* value = .147) nor severe malaria (*P* value; .484). This suggests that blood group O is protective against mild and severe malaria but not against acquiring the infection. On the other hand, blood groups A, B and AB were associated with the likelihood of acquiring asymptomatic malaria infection [(*P* values: .008 (A), .008 (B), .037 (AB)] as well as with the risk of mild malaria [(*P* values: 0.015 (A), 0.022 (B) and .037 (AB)]. Only blood group B was associated with the risk of severe malaria (*P* value: .032). In addition, as showed in Table 3 compared with other ABO blood group antigens, blood group O

had a lower *P* value of .000 under the asymptomatic malaria study group. This suggests that blood group O subjects were more likely not to fall ill of malaria after acquiring *Plasmodium falciparum* infection compared with other ABO antigen subjects. Although some blood group O subjects still had mild malaria but there was no significant association compared with the significant association found among blood group A, B and AB for mild malaria. This implies that after infection, blood group O subjects might **harbour the malaria *Plasmodium* parasite without translation from infection to clinical manifestation of malaria. Also,** 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. **Findings of the present study corroborates the findings of a previous study carried out by Tazebew *et al*., [33] in Ethiopia, where it was found that blood group A was associated with about four times the risk of having malaria compared with blood group O. on the contrary, another previous study contradicts findings of the present study [7]. These inconsistencies in previous findings underscores the essence of the present study. The present study has thus helped to clarify this inconsistencies by characterizing the ABO antigens associated with malaria using house-house community based study population.**

Table 3 ABO antigen polymorphism associated with malaria in Sagbama LGA, Bayelsa State

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 $P < 0.05$.

3.6 Association of malaria parasitemia density with ABO antigen polymorphism in Sagbama

Presented in Table 4 is the association between malaria parasitemia and ABO antigens in Sagbama. As shown in Table 4, no association was found between malaria parasite density and ABO blood group antigen polymorphism. This is in disagreement with previous studies where association of ABO blood group with malaria parasitemia density was found [9, 10]. This disagreement might be due to the fact that the present study was carried out in a region where malaria transmission is very high due to the swampy nature of the terrain which provides breeding ground for mosquitoes and enables all year round transmission of *Plasmodium* in the study area. The result of the present study probably indicates that in highly endemic areas, the association of ABO polymorphism with malaria might not be based on the influence of malaria parasitemia. Repeated exposure of residents in endemic areas to *Plasmodium* transmission by mosquitoes may have conferred partial tolerance and partial immunity to residents in the study location for the present study as previously reported [12].

Table 4 Association of malaria parasitemia density with ABO antigen polymorphism 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 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. Furthermore, t**he present study found no relationship between Rhesus D blood group and malaria.** Prevention and treatment of malaria in relation to ABO blood groups is implicated in the present study.

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