Original Research Article

DISPARITIES IN THE SUSCEPTIBILITIES OF ABO AND RH BLOOD ANTIGENS TO SEVERE PLASMODIUM FALCIPARUM IN CHILDREN UNDER FIVE-YEARS; A CROSS-SECTIONAL STUDY AMONG RURAL DWELLERS, NORTH EAST REGION, GHANA

Abstract

Aim: To determine the association between ABO and Rh blood antigens, and severe *Plasmodium falciparum* malaria among children under five years.

Study Design: hospital-based cross-sectional study

Place and Duration of Study: Janga District Hospital, North East Ghana from April to August, 2021.

Methodology: The study recruited 410 children below five years of age. Three millilitres of venous blood were collected from each participant for haemoglobin (Hb) estimation, thick and thin blood films for malaria parasites, and blood antigens determination. The data were analyzed with STATA version 16.0. $P<.05$ was considered statistically significant.

Results: About one-third (32.68%) of the participants had severe *Plasmodium falciparum* malaria, and 264 (64.39%) had uncomplicated malaria. Blood group O Rh 'D' Positive (O+) was the most predominant blood antigen. Severe malaria was significantly higher in A Rh 'D' Positive (A+), 54/134 (40.30%) subjects than the other blood groups, while uncomplicated malaria was highest in the O+ group, 150/264 (56.82%). Complicated malaria patients were about seven times, and thrice likely to be of blood groups A+ and B Rh 'D' Positive (B+), respectively compared to blood group O+ (A+ vs O+: OR=7.60, 95% CI:4.27-13.51, $p<.001$; B+ vs O+: OR=3.11, 95% CI: 1.80-5.37, $p<.001$).

Conclusion: The study identified a relatively higher prevalence of *P. falciparum* malaria in children below five years in Janga district. About one-third of the participants had severe malaria. Individuals with blood groups A+ and B+ are more susceptible to severe *P. falciparum* infection than those with blood type O+. Incorporation of blood antigens determination into routine management of malaria is recommended.

Keywords: ABO and Rh antigens; *Plasmodium falciparum*; Parasitaemia; Susceptibilities; Thick and thin blood films

Introduction

Malaria is a life-threatening parasitic infection spread by the bites of infective female Anopheles mosquitoes [1]. Malaria is a major public health concern in Ghana and Africa at large, and its burden poses a threat to life, especially children. In sub-Saharan Africa, malaria causes over 2 million fever episodes and one million deaths [1]. The 2019 World Health Organization (WHO) report estimated 409,000 malaria deaths worldwide, of which 67% were children below five years of age [1]. The prevalence of malaria among children under five years is 21% in Arba Minch Zuria District, South Ethiopia [2], 33% in Mali [3], and earlier studies in Ghana have also revealed similar findings [4–6]. *Plasmodium falciparum* is the most common cause of severe clinical manifestations in Africa, out of the five species that infect humans [7]. According to WHO, *P. falciparum* contributed to 99.7% of malaria cases in African Region, from an expected 229 million cases worldwide in 2019 [8].

The ABO blood group is a collection of carbohydrate antigens found on human erythrocytes [9,10]. Studies have discovered the associations between the ABO blood group system and some disease conditions like skin cancer [11], schistosomiasis [12], onchocerciasis [13], hepatitis [14] and HIV infection [15]. There have also been reports linking the ABO blood group system to *P. falciparum* malaria susceptibility, resistance, and severity [16,17]. Changes in the clinical outcome of *P.* falciparum malaria may be related to the various erythrocyte polymorphisms [9]. The ability of infected red blood cells (RBCs) to cling to uninfected ones, resulting in cell rosetting, has been linked to *P. falciparum* pathogenicity [18]. The link between

ABO blood types and malaria parasitaemia has been reported in several studies. Severe malaria has largely been reported in blood group "A" individuals, whilst people with blood group "O" have been diagnosed with low parasitaemia and uncomplicated *P. falciparum* malaria [9,17,19–21]. A recent study in Ghana observed that, 16.1% of complicated cases had blood group "O" against 40.9% of uncomplicated controls [22]. In addition, a link between blood group 'AB' and severe malaria has been established in some populations, including Sri Lanka [21], Mali [23], and Ethiopia [10].

There are few hospital-based, comparative studies on the link between blood group types and the severity of malaria infections in Ghana and West Africa at large. Again, most of the studies in Ghana did not consider the Rh blood antigen. Thus, there is paucity of data regarding the relationship between blood group antigens and severe *P. falciparum* malaria in children under the ages of five in West Mamprusi District. This study examined the association between ABO and Rh blood antigens, and severe malaria among children below five years in West Mamprusi District.

Materials and Methods

Study Design & Setting

This hospital-based, cross-sectional study recruited children who had been diagnosed of *Plasmodium falciparum* malaria, from April to August, 2021, at Janga Hospital, West Mamprusi District of the North-East Region, Ghana. The hospital has 31-bed capacity and offers Out-patient and In-patient services, internal medicine, surgery, obstetric, laboratory, pharmaceutical, public health and reproductive health services. The hospital has male and female surgical and in-patient wards, labour ward, paediatrics ward and emergency ward. Janga hospital found in West

Mamprusi district is located in the central part of North East region. This region has estimated land size of 646.9 sqkm, and population size of 588,800 [24].

Study Population and Inclusion and Exclusion Criteria

The study included febrile children below five years of age who sought medical care at the outpatient department (OPD) of Janga District Hospital during the study period. These children were recruited into the study after they had further been diagnosed of *Plasmodium falciparum* malaria, and consented to the study. A total of 410 children were included into the study. Seriously ill patients and their caregivers, as well as *Plasmodium falciparum* malaria positive persons who had received anti-malarial medication, were excluded from the study. Recently blood-transfused children, and those who refused to give their informed consents were also excluded.

Sample Size Determination

The sample size was determined using the formula

n as proposed by Saunders and Lewis [25], where n is estimated sample size; Z is the critical score based on the desired degree of confidence; p is the prevalence rate; q is the compliment of the proportion (1-p) and E is the desired margin of error. A prevalence rate of malaria was 41.3% [22], Based on the above formula, an approximate value of 373 was obtained. However, an error margin of 10% was allowed for non-responses of questionnaires, and hence a total sample size was 410.

Sampling Technique and Procedure

Feverish children under the ages of five who sought medical care at Janga Hospital were selected using a convenient sample technique. Any feverish under-five child who consented to

participate in the study was sampled at the OPD unit. Each day, this process was repeated until the sample size was obtained.

Data Collection

Data was collected using a standardized questionnaire consisting primarily of closed-ended questions with a few open-ended ones. The survey questionnaire elicited information regarding socio-demographic background of the respondents (age, sex, and place of residence). The questionnaires were responded to by the children's guardians or care takers. A separate form was designed for recording the malaria status and blood group antigens of the respondents.

Blood Sample Collection

In the outpatient department, rapid diagnostic testing for malaria (CareStart Malaria Pf kit) was performed as an initial screening test using finger-prick capillary blood, and results verified by microscopy. Using a single-use, sterile butterfly needle kit, and trained phlebotomists aseptically collected approximately 3ml of venous blood from the antecubital fossa from each participant and the blood was quickly dispensed into evacuated EDTA blood collection tubes (Becton Dickinson, USA) for haemoglobin concentration (Sysmex XP-300 analyzer), malaria parasite identification and counts (microscopy), and ABO and Rh blood typing.

Preparation of Thick and Thin Blood Smears

The preparation, fixing and staining of blood smears were adopted from the White et al [26] and WHO [1], as described below. Thick and thin blood films were made on a microscope slide for each participant to identify, speciate and estimate parasitaemia. For the thick blood film preparation, a small aliquot of blood (6 uL) was placed 10 mm from the frosted end of the slide using micropipette and spread evenly in a circular motion to cover a diameter of 1.0-1.2cm. A second (2uL) blood was then placed 1.0 cm from the thick film and spread uniformly along the

length of the same slide using the edge of a second slide to make a thin film. The thin smear was fixed in absolute methanol after the slides were air dried. The thick and thin films on each slide were then stained in a freshly prepared 10% Giemsa stain solution using buffer of pH 7.2 for 15 minutes, and rinsed under a mild stream of water and air dried. Finally, the films were observed under the microscope using a X100 objective lens (Olympus CX 21 light microscope).

P. falciparum Parasitaemia and Severity Estimation

The number of parasites were counted against approximately 200 or 500 White Blood Cells (WBCs), depending on the number of malaria parasites counted under the microscope, to determine parasitaemia using hand tally counters, as adopted from Zerihun et al., [17]. The number of parasites per microliter of blood was then determined using the equation:

Parasite per µL =.

Parasitaemia was calculated from counts of *P. falciparum* infected RBC per 400 RBC when >1%, or else from counts of parasites per 200 WBC in the thick smear, and estimated to parasites per µl of blood using the measured RBC or WBC counts. Again, severe malaria was confirmed when participants exhibited any life-threatening symptom coupled with the presence of the *P. falciparum* in their blood.

Quality Control Measures

Following the manufacturer's instructions, the CareStart Malaria Pf kit test was done in parallel with a blood film analysis using the same blood sample. Three consecutive observers, the first of whom was always the one administering the tests, took readings after 20 minutes (the manufacturer's recommended reading time), in daylight with the help of a regular electric lamp. Observers two and three took their readings in about five minutes' intervals after the initial

readings by the first observer. The observers were unaware of each other's readings and the microscopy results. Consensus was used to determine the test results, which meant that a same result read by at least two out of three observers was withheld. In the event of a disagreement, the results of the first reader were taken into account. Positive and negative values, as well as line intensities, were used to test inter-observer agreement. A test was deemed invalid if no control line showed, and it was repeated. Again, two Microscopists examined the stained slides independently, and average counts were considered as the final count.

Determination of ABO and Rh Blood Antigens

ABO and Rh blood types were determined using anti A, B, and D reagents in an agglutination test. The tube method was used to identify blood groups. In a test tube, a suspension of washed red blood cells was mixed with antisera (or plasma, for reverse grouping). The mixture was centrifuged to separate the cells from the reagent, then gently agitated to resuspend the cells. The patient's blood type was; “A” if only the anti-A serum agglutinated; “B”, if only the anti-B serum agglutinated and the agglutination of RBC with both anti-A and anti-B test sera shows an “AB” blood type, whereas no agglutination in both test sera indicates a donor of blood group “O”. To determine Rh status of the patients, an anti-D blood-induced Rh-positive test of agglutinating RBCs was done [26].

Data Analysis

The statistical software STATA version 16.0 was used to analyze the data. The data was checked for normality using skewness and kurtosis, as well as Kolmogorov-Smirnoff. Categorical data were presented as frequencies and percentages, and normally distributed continuous data were presented as means and standard deviations, whilst skewed data, were

presented as medians and interquartile ranges (25^{th}-75^{th} percentiles). To examine the prevalence of other blood groups, such as 'A', 'B', 'AB', non-'O', and 'Rh', in severe and uncomplicated malaria, blood group 'O' was used as a reference [27]. To investigate the link between blood types and severe malaria or distinct clinical manifestations, the Fisher's exact test or Chi-square test was appropriately to perform. Statistical significance was set at $p<.05$. Additionally, odds ratios and 95% confidence intervals were calculated.

Results

Sociodemographic, Clinical and Haematological Characteristics of the Study Participants

The study recruited 410 children below five years of age as, with mean age of approximately 26±4.79 months. Most of the participants, 172/410 (41.95%) were aged 0-12 months, males 213/410 (51.95%) and resided in the rural setting 377/410 (91.95%). In addition, almost all participants 400/410 (97.56%) had symptoms of malaria, coupled with low Hb level 403/410 (98.23%). A higher proportion 182/410 (44.39%) of the participants were of blood group O+ and the mdian parasite density was 702.42 (658-836) p/µL of blood (Table 1).

Table 1: Sociodemographic, Clinical and Haematological Characteristics of the Study Participants

Characteristics	Category	Frequency (n=410)	Percentage (%)
Age in months		25.83 (8.62-48.23)	
	0-12	172	41.95
	13-24	82	20.00
	25-36	65	15.85
	37-48	91	22.20
Sex			
	Male	213	51.95
	Female	197	48.05

Residence			
	Urban	33	8.05
	Rural	377	91.95
Symptoms of fever, convulsion, etc			
	Yes	400	97.56
	No	10	2.44
Blood group			
	A+	90	21.95
	A-	7	1.71
	AB+	14	3.41
	B+	113	27.56
	O+	182	44.39
	O-	4	0.98
Hb level (mean±SD)		7.35±1.30	
	Normal Hb	7	1.71
	Low Hb	403	98.29
Parasite Density, p/µL		702.42 (658-836)	

A+ = A Rh (D) Positive; A- = A Rh (D) Negative; AB+ = AB Rh (D) Positive; B+ = B Rh (D) Positive; O+ = O Rh (D) Positive; O- = O Rh (D) Negative, Hb= Haemoglobin; p/uL= Parasites per microliter of blood. Categorical data are presented in frequencies, with corresponding percentages, and continuous data presented in medians (25th-75th percentiles).

Prevalence of Severe *Plasmodium falciparum* Malaria among the Study Participants at Janga Hospital, 2021.

Figure 1 portrays the prevalence of *Plasmodium falciparum* malaria severity among the study participants. There were 134 (32.68%) cases of severe *P. falciparum* malaria, and 264 (64.39%) cases of uncomplicated *P. falciparum* malaria. Apparently healthy individuals who had no malaria parasite (*P. falciparum*) seen after examining their blood samples accounted for 2.93%.

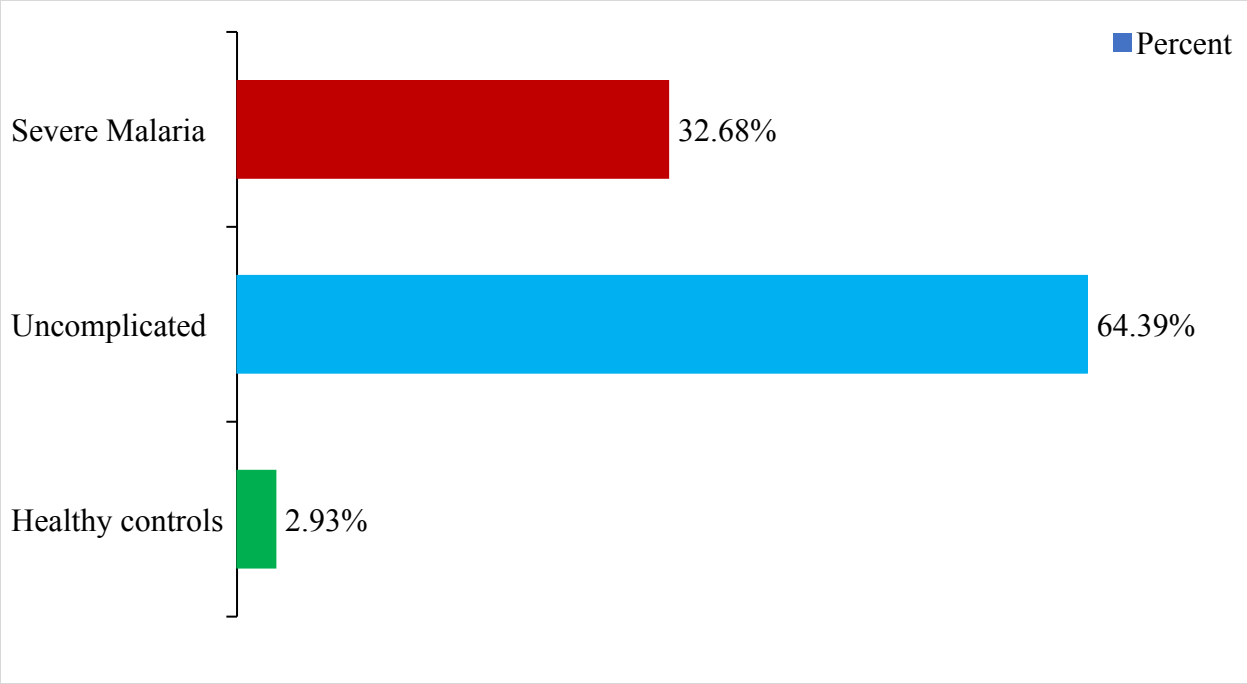

Figure 1: Prevalence of Severe *Plasmodium falciparum* Malaria among the Study Participants at Janga Hospital, 2021.

Age, Haemoglobin Concentration and Parasite Density Stratified by *Plasmodium falciparum* Malaria Severity among the Study Participants

There was no statistically significant difference in the ages of participants diagnosed with severe *P. falciparum* malaria and the uncomplicated malaria group (24.94 (12.33-41.52) months vrs 26.26 (12.86-46.66) months, p=.40). The children who had been infected with severe *P. falciparum* malaria had significantly reduced Hb level compared to those with uncomplicated malaria (6.40±0.86 vrs 7.82±1.22 g/dL, p<.001). The mean parasite density (% of Parasitized RBCs) in participants diagnosed with severe *P. falciparum* malaria was higher compared to participants with uncomplicated malaria (15.75±3.99 vs 2.79±1.1.60, p<.001) (Table 2).

Table 2: Age, Haemoglobin Concentration and Parasite Density Stratified by *Plasmodium falciparum* Malaria Severity among the Study Participants

Variables	*P. falciparum* Malaria Severity		p-value
	Severe Malaria	Uncomplicated Malaria	
Age (months)	24.94 (12.33-41.52)	26.26 (12.86-46.66)	.40
Hb (g/dL)	6.40±0.86	7.82±1.22	**<.001***
Parasite Density (% of Parasitised RBCs)	15.75±3.99	2.79±1.1.60	**<.001***

*Hb: Haemoglobin; g/dl: grams per decilitre. *Statistically significant at p<.05. Hb and Parasite Density compared using Student T-Test, and Age was compared with Mann-Whitney U-test*

Association between ABO and Rh Blood Antigens, and *Plasmodium falciparum* Malaria Status

There was a significant association between blood antigens and *P. falciparum* status of the children (p<0.0001) (Table 3). There was high percentage of blood group A+ patients, 54/134 (40.30%) in children with severe *P. falciparum* malaria compared to their counterparts with uncomplicated *P. falciparum* malaria, 35/264 (13.26%) and healthy controls, 1/12 (8.33%). Blood group B+ and O+ made up 32.09% (43/134) and 22.39% (30/134), respectively of severe *P. falciparum* malaria patients compared to 24.62% (65/264) and 56.82% (150/264) of the uncomplicated, respectively. There was low percentage of blood group AB+ (4/134, 2.99%), A- (3/134, 2.24%), and O- (0/134, 0.00%) patients in the severe malaria category than in uncomplicated malaria AB+ (6/264, 2.27%), A- (4/264, 2.24%), and O- (0,264, 0.00%).

Table 3: Association between ABO and Rh Blood Antigens, and *Plasmodium falciparum* Malaria Status

ABO/Rh	Malaria Status			*P*-value
	Healthy controls n (%)	Uncomplicated n (%)	Severe n (%)	
Blood group				**<.00 1***
A+	1 (8.33)	35 (13.26)	54 (40.30)	
A-	0 (0.00)	4 (1.52)	3 (2.24)	
AB+	4 (33.33)	6 (2.27)	4 (2.99)	
B+	5 (41.67)	65 (24.62)	43 (32.09)	
O+	2 (16.67)	150 (56.82)	30 (22.39)	
O-	0 (0.00)	4 (1.52)	0 (0.00)	

*A+ = A Rh (D) Positive; A- = A Rh (D) Negative; AB+ = AB Rh (D) Positive; B+ = B Rh (D) Positive; O+ = O Rh (D) Positive; O- = O Rh (D) Negative. Data are presented in frequencies with percentages in parenthesis. *Statistically significant at p<0.05.*

Binary Logistic Regression Analysis of Predictors of Severe *Plasmodium falciparum* Malaria Development

To assess whether blood antigens (ABO and Rh) were predictors of severe course of *P. falciparum* infection, binary logistic regression analysis was conducted. As shown in table 4, the odds of developing severe *Plasmodium falciparum* malaria were 7.60 times greater in persons with blood group A+ compared to blood group O+ (OR=7.60; 95% CI:4.27-13.51; p<.001).

Persons with blood group B+ were three times more likely to have complicated *Plasmodium falciparum* malaria compared to blood group O+ (OR=3.11; 95% CI: 1.80-5.37; $p<.001$).

Table 4: Binary Logistic Regression Analysis of Predictors of Severe *Plasmodium falciparum* Malaria Development

Variable	Odds Ratio	95% Confidence Interval		*P*-value
		Lower	Upper	
Blood groups				
A+	7.60	4.27	13.51	<.001*
A-	3.80	0.81	17.86	.09
AB+	2.02	0.60	6.89	.26
B+	3.11	1.80	5.37	<.001*
O+ (Reference)	1 (Ref.)			
O-	1	-		-

*A+ = A Rh (D) Positive; A- = A Rh (D) Negative; AB+ = AB Rh (D) Positive; B+ = B Rh (D) Positive; O+ = O Rh (D) Positive; O- = O Rh (D) Negative. Data generated by binary logistic regression model. * Statistically significant at p<.05.*

Discussion

Malaria is the leading cause of illness and mortality in Africa, especially among children, and accounts for the highest number of hospital visits [28]. Studies conducted in Malawi [29], Guinea [30], and Ethiopia [2], have all shown increased prevalence of *P. falciparum* malaria. The relationship between the severity of malarial infection and the patient's blood antigens has recently piqued attention in the search for solutions to the factors influencing the clinical course of the disease. This study investigated the susceptibilities of ABO and Rh blood antigens to severe *P. falciparum* malaria among children under five years in Janga District Hospital.

This study revealed that feverish and unwell children had considerably higher malaria parasitaemia than ostensibly healthy children who visited the Janga Hospital. Similarly, Onanuga and Lamikanra found that the prevalence of malaria infection was substantially greater among the unwell children than among the seemingly healthy children [31]. This could probably show that malaria is one of the primary causes of frequent hospital visits among children in these age groups, and that malaria parasites can make a person more susceptible to other diseases or disorders.

The overall prevalence of *P. falciparum* malaria in the study population was 97.07%, with 32.68% having severe *P. falciparum* malaria and 64.39% of uncomplicated *P. falciparum* malaria cases. The prevalence in this present study is higher than findings from previous studies in malaria endemic regions in Ghana: 22% by Afoakwah *et al*. [22], and 39% by Yankson *et al*. [6]. Studies in other parts of Africa had recorded relatively lower prevalence of *P. falciparum* malaria among children: 21.0% was reported in Arba Minch Zuria District, South Ethiopia [2], 22.8% in Ethiopia by Mengistu *et al*. [32], 33% in Mali by Zgambo *et al*. [3], and 19.0% in

Uganda [33]. This differences in malaria prevalence might be due to variations in season, variation in population characteristics, variation in the effectiveness of the implementation of existing and new malaria control prevention and chemotherapy programs.

The distribution of ABO blood groups varies from population to population in the world and the ratio of blood antigen O to A is higher in regions where there is present or history of malaria endemicity [10, 27]. A very high prevalence of blood group O is found throughout sub-Saharan Africa, where *P. falciparum* persists, whilst blood antigen A is the predominant blood group in the coastal regions of the Earth where malaria is endemic [28]. In a community-based study in a tribal population in Odisha, where malaria is widespread, blood group 'O' was shown to be more prevalent [34]. In other malaria non-endemic states in India, such as Maharashtra [20,35], and Uttar Pradesh [36], a decreased incidence of the 'O' blood type has been recorded, showing a selective advantage of this blood group in endemic areas. The ABO and Rh blood antigens distribution in this study showed blood group O+ was the predominant type. It occurred in more than twice the frequency of each of group's A+ and B+, while the O- blood group was the least encountered. Our study agrees with other studies in Nigeria [37,38] which reported the highest frequency of the ABO blood group as O, followed by groups A and B.

This study observed significant association between malaria status and blood group systems: ABO and Rh. The rosetting of parasitized erythrocytes and cyto-adherence have been implicated in the disparities in susceptibility and severity of *P. falciparum* malaria infection among the "A," "B," "AB," and "O" blood groups [18,39]. Previous studies observed that during *P. falciparum* infection, rosetting is reduced in blood group "O" erythrocytes compared to non-O blood groups (A, B, and AB) [40,41], even though, the protective mechanism is not well understood.

Interestingly, severe malaria was significantly higher in the A+ subjects than the other blood groups, while uncomplicated malaria was higher in the O+ group than the other blood types in this study. This confirms the claims that, individuals with blood group "A" are particularly vulnerable to *P. falciparum* malaria whilst those with blood group "O" are said to be protected against severe malaria [16,17,19]. Contrary to our findings, a study done in Ethiopia by Tekeste *et al*. [10] indicated that people with blood group O was the dominant blood type in malaria cases. A link between blood antigen 'A' and malaria severity has also been established in Gabon [42], Ethiopia [10] and Zimbabwe [43]. A similar study conducted in Ghana found that blood group O was present in few of complicated cases compared to uncomplicated controls [22]. In this study, more of the malaria cases were recorded among subjects with B+ blood group. The plausible explanation to this observation is not well understood. However, diverse rosetting capacity, heterogeneous population groups, and distinct infective strains may all contribute to the diversity of observations made for different blood groups [44]. Increased rosetting phenomena have been linked to blood groups 'A' in Uganda and Gambia [41,45] respectively, 'B' in Thailand [41], and 'AB' in Kenya [46].

The likelihood of developing severe *P. falciparum* malaria was 7.60 times greater in persons with blood group A+ compared to blood group O+. In addition, persons with blood group B+ were three times more likely to have complicated *falciparum* malaria compared to blood group O+. Previous studies [10,22], showed that individuals with complicated malaria were about twice as likely to be of blood groups A or B compared to group O. Again, the cross-sectional study in Awash, Ethiopia, by Tekeste and Petros discovered that severe malaria was almost twice more likely to be of blood antigens A or B than type O [10]. This therefore

reaffirms that ABO and Rh blood groups are associated with severity of *Plasmodium falciparum* malaria in children [10].

The study was limited by the inability to establish the exact pathophysiology of ABO and Rh blood antigens in the development of severe *Plasmodium falciparum* malaria.

Conclusion

The study recorded relatively higher prevalence of *P. falciparum* malaria among children below five years of age in Janga District Hospital. The blood antigen O+ was the predominant ABO and Rh antigen in the study area. Severe malaria was significantly higher in the A+ subjects than the other blood groups, while uncomplicated malaria was higher in the O+ group than the other blood types. The odds of developing complicated *P. falciparum* malaria were greater in persons with blood groups A+ and B+ compared to blood group O+. Incorporation of blood antigens determination into routine management of malaria is recommended. Further in-depth studies are recommended to clearly establish the roles of ABO and Rh blood antigens in the development of severe *P. falciparum* malaria in children.

Ethical Considerations

The 'Declaration of Helsinki-Ethical Principles for Medical Research' was adhered to strictly throughout the study. This study was approved by University for Development Studies Institutional Review Board (UDS/RB/027/22). Permission was sought from the Management of Janga District Hospital and the West Mamprusi Municipal Health Directorate. Caretakers or guidance of the children either thumb-printed or signed to confirm their consent, and participants were assured of the confidentiality of all data provided.

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