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ABO and Rhesus blood group variability and their associations with clinical malaria presentations

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Abstract

Background *Plasmodium falciparum* infection is associated with the human ABO blood group. However, there is a paucity of data on the role that ABO and Rhesus blood groups play in malaria clinical presentations. Therefore, the objective of this study was to assess the association of human ABO blood groups and the Rhesus blood (Rh) types with the severity of malaria.

Methods This cross-sectional study was carried out at the Suhum Government Hospital in the Eastern region of Ghana. Conveniently, study participants with malaria, diagnosed by microscopy, were selected into the study. Subsequently, their ABO and Rh blood groups were determined (Accucare ABO/Rh monoclonal antibodies, Chennai, India). Malaria severity was assessed using the criteria for assessing severe malarial anaemia published by the World Health Organization. According to the criteria, severe malarial anaemia was classified as having haemoglobin (Hb) < 5 g/dL for children < 12 years and in patients ≥ 12 years, Hb level < 7 g/dL, with parasitaemia > 10,000/µL in both cases. Severe malarial anaemia was also classified as having plasma bilirubin > 50 µmol/L with parasitaemia ≥ 100,000/µL, for all ages. Chi square statistical analysis was used to test the association between the blood groups and the clinical or laboratory findings, while multivariate analysis was performed to identify which blood groups were more vulnerable to develop severe malarial anaemia.

Results Of the total number of the study participants (n = 328), most of the patients had blood group O Rh positive (35.7%) while few of them had blood group AB Rh negative (2.1%). The types of Rhesus did not associate with malaria. However, compared to blood group O, the odds of developing severe malarial anaemia, in children < 12 years and in patients ≥ 12 years, were 16 times and 17.8 times higher among patients with blood group A, respectively. Furthermore, the odds of having bilirubin level > 50 µmol/L with parasitaemia ≥ 100,000 /µL was 10 times higher among patients with blood groups A and 2.6 times higher in patients with blood group B, compared to blood group O. Finally, in patients with blood group A majority (71.6%) of them developed high temperature (> 37.5 °C) while 43.3% of them vomited and had diarrhoea. However, pallor (group B = 46.2% vs group A = 37.3%), fever (group B = 84.6% vs group A = 79.1%) and nausea (group B = 46.2% vs group A = 25.4%) were more frequent in patients with blood group B than A.

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Conclusions This study found that people with blood groups A and B were severely affected by malaria, with group A being the most vulnerable. It is recommended that blood group assessment be performed for all patients with malaria. Patients found to have blood group A or B must be promptly and efficiently managed to avoid the development of severe malaria anaemia.

Keywords Severe malarial anaemia, Uncomplicated malaria, WHO malaria classification, ABO blood groups, Rhesus types, Ghana

Background

Studies conducted elsewhere on patients with malaria have shown that there is an association between malaria and blood groups of an individual [1]. These studies have indeed shown that people with the blood group O have a selective evolutionary advantage over malaria compared to people with non- O blood groups (A, B and AB) [2]. The mechanism by which blood group O confers selective resistance to malaria, while individuals with non-O blood groups show selective vulnerability, has been attributed to rosette formation [3]. Rosetting is a mechanism in which an infected erythrocyte with *Plasmodium falciparum* adheres to non-infected erythrocytes [4]. Therefore, in patients with malaria with the non-O blood group, invasion of uninfected red blood cells is enhanced, compared to the other red cell polymorphs. Rosetting may be mediated by a membrane protein, including PfEMP1 [5], RIFIN and STEVOR [3], which are expressed on the surface of the infected host cell. Rosettes formed by non-O blood groups are resilient to disruption [6].

However, there are several unanswered questions and unclear links about the various blood groups contribute to severe anaemia presentations. Furthermore, the role of Rhesus (Rh) types in *P. falciparum* infection remains unanswered, since available data on this subject are equivocal [7].

Despite the comparative vulnerability of blood groups A, B and AB to malaria, the association of these blood groups with malaria severity has not yet been fully studied. *Plasmodium* parasites are known to cause fever in patients with malaria [8]. However, it was interesting to note that the degree of fever, assessed by measuring body temperature, does not always correlate with *Plasmodium* parasitaemia. Furthermore, patients with malaria had a different set of clinical signs and symptoms, even with similar parasitaemia [9, 10]. Based on the foregoing, it was hypothesized that host genetic variants may influence clinical malaria presentations. To date, it remains unknown whether blood group variability plays a role in the observed differences in similar levels of parasitaemia. This evidence gap merits investigation. Therefore, the study aimed to explore and determine the association of malaria severity with the ABO and Rh blood groups. In this study, the severe malarial anaemia was defined using strict definitions of the World Health Organization (WHO) [11]. Per these definitions, the estimation of haemoglobin, plasma bilirubin and parasite count are required to assess severe malaria anaemia.

Methods

Study design, site, and participant recruitment

This cross-sectional study was done at the Suhum Government Hospital in the Suhum municipality (Latitude: 6.0379101, longitude: -0.4456668) in the Eastern Region of Ghana. Study participants were patients of all ages who had tested positive for malaria, by microscopy. The hospital is located in a malaria endemic district, where malaria is among the top five diseases recorded at the outpatient department in the study hospital. The hospital also serves as referral facility for other health facilities, both public and private. The healthcare professionals manage malaria according to the Ministry of Health guidelines [12]. Prospective study participants were patients with malaria classified as uncomplicated by the attending medical officer. After prior informed consent, the study participants were recruited. Study participants were selected based on their availability to the researcher.

Inclusion and exclusion criteria

Participants included in the study were individuals infected symptomatically with malaria, caused by *P. falciparum*, and had consented to participate in the study. Individuals who did not provide written consent, pregnant women and infants, were excluded from the study. Pregnant women are prone to both physiological and pathological anaemia of other aetiology and in infants it was difficult getting the desired volume of blood for the haematological and the bilirubin assays. In addition, patients who had tested for malaria only by rapid test kit were excluded. Furthermore, patients who had other common diseases (HIV and hepatitis B/C) and other red blood cell genetic disorders (sickle cell disease and G6PD deficient) were excluded from the study, because their co-morbidity could affect the outcome of the investigations. Finally, the microscopically detected *Plasmodium* parasites other than the *P. falciparum* species were also excluded from this study.

Sample size determination

Using the Cochrane's formula, $n = z^2p(1-p)/d2$, where n is the sample size, z is the confidence level at 95% (standard value of 1.96), d is the margin of error at 5% (standard value of 0.05), a minimum of 296 malaria patients were used, given that the prevalence of malaria in the Eastern region of Ghana among individuals suspected of malaria was 26% [13].

Collection of blood samples

With the consent of the patient, blood samples were collected by a trained Ghana Health Service phlebotomist. The area to be sampled (the antecubital fossa) was disinfected with 70% ethanol and allowed to air dry. Using a 23-gauge syringe, whole blood (approximately 4 ml) was collected into an EDTA tube and gently mixed. The punctured side was covered with cotton wool and covered with a phlebotomy plaster.

Laboratory diagnosis of malaria and confirmation of causative species

Malaria was diagnosed by microscopy. In doing that, a thick blood film was prepared using approx. 6µL of whole blood. The smear was air dried, stained with 10% Giemsa for 10 min and any parasite thereof was quantified according to WHO protocol [14]. Quantification was done by counting the number of parasites per at least 200 white blood cells. The number of parasites counted was then divided by the number of white blood cells counted, and the resulting figure was multiplied by 8000 (that is, the estimated total of white blood cells per microlitre of blood) as used elsewhere [15]. Subsequently, *P. falciparum* was specified by detecting the histidine-rich protein 2 (Pfhrp2) with CareStart TM rapid diagnostic test kit (Somerset NJ, USA). Pfhrp2 antigens are specific to *P. falciparum*. The confirmation of species was carried out placing exactly 5 µL of blood in the sample column and four drops of sample buffer placed in the appropriate column. The results were read after 15 min as recommended by the manufacturer.

Blood group and Rhesus factor determinations

Blood group and Rhesus factor typing were performed using Accucare ABO/Rh monoclonal antibodies (Chennai, India). The tile method was used. In summary, approximately 50 µL of antibodies was mixed with approximately 20 µL of whole blood, for about 30 s. After which the reactions were read. Samples with visible agglutinations were considered positive for the respective antigen and vice versa. Blood samples from known blood groups (A Rh D positive, B Rh D positive, and O Rh negative) were used as controls.

Haemoglobin estimation

Haemoglobin estimation was performed using a fully automated haematology analyser (Urit 5160; Guangzhou, China). The haemoglobin concentration was measured using the cyanide-free colorimetric method.

Bilirubin estimation

Bilirubin estimation was performed by using a Biobase Biochemistry analyser (Guangzhou, China) and Elitech reagents obtained locally but manufactured by the Elitech Group (Allées de Dublin, France). Total bilirubin was estimated on the basis of the modified Malloy-Evelyn endpoint method based on the following principle. Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. In the presence of accelerator (cetrimide), conjugated and unconjugated bilirubin reacts with diazotized sulfanilic acid to form azobilirubin. The increase in absorbance at 546 nm is proportional to the bilirubin concentration.

Data and statistical analyses

IBM SPSS data analysis software (version 27) was used to analyse the data. The demographic characteristics, clinical, laboratory findings and the various blood groups of study participants were presented as percentages, using the total number of participants as the denominator. Further, the Chi-square test was used to test the association between the laboratory findings, the different blood groups, and the association between the clinical presentations and the various blood groups. However, Fisher's exact test was used when the frequencies were less than 5. Further multinomial logistic regression analysis was conducted to verify the relationship between the multinomial outcome variable "blood groups and Rhesus types" and haemoglobin, bilirubin and parasite count variables that showed significance during the chi-square analysis. Blood group O was set as the reference for the blood groups for all regressions. The logistic regression analysis allowed for identifying significant predictors and quantifying their effects on the likelihood of a blood group being associated with severe malaria. Statistical significance was established at $p < 0.05$.

Results

Demographic characteristics of study participants

The study recruited 328 participants, of whom 67.1% were females and the males were 32.9%. The age range of the participants was < 1 and 89 years. The mean age of the participants was 19.4 years and the standard deviation was 20. Most of the participants, 142/328 (43.3%) were between < 1 and 9 years old. Additionally, the majority of the participants 170/328 (51.8%) were under marital age

with 68/328 (20.7%) in high school. Other details of the study participants are indicated in Table 1.

Clinical and laboratory findings of study participants

Most of the study participants (61.9%) enrolled in the study were recruited from the outpatient department of the hospital. Overall, the mean temperature ± standard deviation of the study participants was 37.4 °C ± 0.8. Furthermore, almost 40% of malaria patients recorded temperatures above 37.5 °C (severe hyperthermia). Surprisingly, 35.4% of malaria patients were hypothermic (temperatures below 37 °C). The overall mean level of haemoglobin was 9.5 g/dL ± 2.7. Among the study participants, 31.1% had no anaemia (Hb level > 11.0 g/dL) whereas 18.0%, 29.3% and 21.6% of the patients with malaria were mildly, moderately and severely anaemic, respectively. Using 17 µmol/L as the cut-off point of the normal human bilirubin level, the majority (69.8%) of the participants with malaria had elevated plasma bilirubin. The majority of the participants (53.0%) also had parasitaemia levels between 10,000 and 100,000 parasites/µL of blood. The signs and symptoms of malaria presented by the majority of the participants were fever (78.4%), chills (67.7%), and headache (59.5%). The other details are shown in Table 1.

Profiling of the ABO and Rhesus blood groups of study participants

A higher proportion of study participants (45.1%) were of blood group O with patients with blood group AB in the minority (10.7%). Furthermore, the majority of study participants 266/328 (81.1%) were Rhesus positive. Finally, in defining ABO / Rh types, most of the study participants (35.7%) were O positive in the blood group and few of them were AB negative (2.1%). The frequencies found in the other blood groups are presented in Table 1.

Association of laboratory findings with ABO blood groups in patients with malaria

The three laboratory variables used to assess the association between ABO variability and severe malarial anaemia were haemoglobin level, bilirubin level (jaundice), and parasite density (hyperparasitaemia). Analysis of the data revealed that in patients with malaria, anaemia, jaundice, and hyperparasitaemia are associated with the blood group. Significantly, a higher number of malaria patients with blood group O (42.5%) had no anaemia, while mild anaemia was associated with patients with blood group AB. Furthermore, most of the malaria patients with blood group B (37.2%) were moderately anaemic while those with blood group A (32.8%) were severely anaemic. Furthermore, hyperbilirubinaemia was significantly higher in patients with malaria with blood

Table 1 Demographic characteristics, clinical and laboratory findings, and ABO/Rhesus blood groups of study participants

Variable	Frequency	Percent (%)
Demographic characteristics		
Age range (years)		
0 – 9	142	43.3
10 – 19	67	20.4
20 – 29	43	13.1
30 – 39	26	7.9
40 – 49	14	4.3
50 – 59	12	3.7
60 – 69	14	4.3
> 70	10	3.0
Gender		
Male	108	32.9
Female	220	67.1
Marital status		
Under marital age (< 16 years)	170	51.8
Single	78	23.8
Married	66	20.1
Divorced	2	0.6
Widow/widower	11	3.4
Co-habitation	1	0.3
Highest education		
No formal education	8	2.4
Below pre-school	44	13.4
Primary	59	18.0
Junior high	54	16.5
Senior high	68	20.7
Tertiary	32	9.8
TVET[1]	63	19.2
Clinical and laboratory findings		
Patient type		
Ante-natal	20	6.1
Out-patient	203	61.9
In-patient	105	32.0
Temperature (mean ± SD)		
< 37 °C	116	35.4
37–37.5 oC	81	24.7
> 37.5 °C	131	39.9
Anaemia		
None (hb ≥ 11.0 g/dL)	102	31.1
Mild anaemia (10–10.9 g/dL)	59	18.0
Moderate anaemia (7–9.9 g/dL)	96	29.3
Severe anaemia (< 7 g/dL)	71	21.6
Jaundice classification		
Bilirubin level (≤ 17 µmol/L)	99	30.2
Bilirubin level (> 17 µmol/L)	229	69.8
Parasitaemia (/µL)		
< 10,000	89	27.1
10,000–100,000	174	53.0
> 100,000	65	19.8

Table 1 (continued)

Variable	Frequency	Percent (%)
Clinical presentations		
Pallor	100	30.5
Vomiting	148	45.1
Diarrhoea	102	31.1
Chills	222	67.7
Fever	257	78.4
Nausea	96	29.3
Headache	195	59.5
Fatigue	39	11.9
Muscle ache	113	34.5
Previous malaria		
Yes	266	81.1
No	62	18.9
If yes, how long ago		
< 6 months	61	22.9
6 months–1 year	85	32.0
> 1 year	120	45.1
ABO/Rhesus blood groups		
O	148	45.1
A	67	20.4
B	78	23.8
AB	35	10.7
Rhesus typing		
Positive	266	81.1
Negative	62	18.9
ABO Rh types		
O positive	117	35.7
O negative	31	9.5
A positive	57	17.4
A negative	10	3.0
B positive	64	19.5
B negative	14	4.3
AB positive	28	8.5
AB negative	7	2.1

[1] Technical and vocational education and training

group A (85.1%, p = 0.001). The Rh types did not associate with anaemia or jaundice, except for Rh positive that associated with hyperparasitaemia (17.4%, p = 0.036) (Table 2).

Association of clinical presentations to ABO blood groups in patients with malaria

Table 3 represents the association between the malaria clinical presentations and human blood types. Hyperthermia (> 37.5 °C) was significantly higher in blood group A (71.6%), while hypothermia was common in blood group O (48.6%). It was also observed that Rhesus types did not associate with degree of body temperature. Chills, headaches, fatigue, and muscle ache were not associated with any blood group. However, pallor (46.2%), fever (84.6%) and nausea (46.2%) significantly associated with blood group B, while vomiting (43.3%) and diarrhoea (43.3%) were commonly observed in patients with blood group A. Aside from pallor that associated with Rh positivity (p = 0.015), none of the clinical presentations was associated with any of the Rh types.

Relationship between ABO/Rh groups and severe forms of malaria

The World Health Organization (WHO) has a number of definitions to classify severe malarial anaemia. One of these definitions includes haemoglobin concentration < 5 g/dL together with parasite count > 10,000µL for children < 12 years of age. For patients with malaria ≥ 12 years, the same parasitaemia range together with haemoglobin concentration is < 7 g/dL is diagnostic. In Table 4, the prevalence of severe malarial anaemia among children classified as having uncomplicated malaria was 8% (26/328). In children, severe malarial anaemia associated with blood group B, while Rh types did not. In patients 12 years or above, the prevalence of severe malarial anaemia among those classified as having uncomplicated malaria was 4.3% (14/328). The incidence of severe malarial anaemia in patients ≥ 12 years associated with blood groups A and B, as well as Rh positivity. Using bilirubin as a criterion, WHO defined severe malarial anaemia as having bilirubin > 50 µmol/L with parasitaemia > 100,000 /µL. With this criteria, 17% (56/328) of the study participants with malaria were severe. Severe malarial anaemia was observed in patients with blood group A whereas Rh types did not associate with severe malarial anaemia (Table 4).

Prediction of malaria severity using ABO blood groups

The association of blood groups with the severity of malaria was compared to that of blood group O (reference blood group) (Table 4). Compared to blood group O, the odds of blood groups B (aOR = 1.6, 95% CI 0.6–2.7) and AB (aOR = 1.1, 95% CI 0.7–1.9) developing severe malarial anaemia in children under 12 years of age were higher but did not reach a significant level. However, children less than 12 years old with blood group A are approximately 16 times (p = 0.0005) more likely to develop severe malarial anaemia compared to those with the blood group O. For patients ≥ 12 years with malaria, the odds of developing severe malarial anaemia were higher among those with blood groups AB (aOR = 4.4, 95% CI 2.7–6.1, p = 0.0095) and A (aOR = 17.8, 95% CI 12.6–31.2, p = 0.0030), compared to blood group O. Using the bilirubin > 50 µmol/L with parasitaemia ≥ 100,000 /µL criteria, the odds of developing severe

Table 2 Association of laboratory findings with blood group variability

Variables	ABO blood groups, n (%)					Rhesus types		
	O (n = 148)	A (n = 67)	B (n = 78)	AB (n = 35)	p-value	Positive (n = 266)	Negative (n = 62)	p-value
Anaemia					< 0.001			0.115
None (Hb ≥ 11.0 g/dL)	63 (42.5%)	10 (15.0%)	14 (18.0%)	10 (28.5%)		82 (25.0%)	20 (6.1%)	
Mild anaemia (10 – 10.9 g/dL)	28 (19.0%)	11 (16.4%)	11 (14.1%)	14 (40%)		42 (12.8%)	17 (5.2%)	
Moderate anaemia (7 – 9.9 g/dL)	37 (25.0%)	24 (35.8%)	29 (37.2%)	6 (17.1%)		80 (24.4%)	16 (4.9%)	
Severe anaemia (< 7 g/dL)	20 (13.5%)	22 (32.8%)	24 (30.8%)	5 (14.3%)		62 (18.9%)	9 (2.7%)	
Jaundice classification					0.001			0.827
Bilirubin level (≤ 17 µmol/L)	57 (38.5%)	10 (15.0%)	18 (23.1%)	14 (40%)		81 (24.7%)	18 (5.5%)	
Bilirubin level (> 17 µmol/L)	91 (61.5%)	57 (85.1%)	60 (77.0%)	21 (60%)		185 (56.4%)	44 (13.4%)	
Parasitaemia (/µL)					< 0.001			0.036
< 10,000	41 (27.7%)	3 (4.5%)	36 (46.2%)	9 (25.7%)		77 (23.5%)	12 (3.7%)	
10,000 – 100,000	95 (64.2%)	31 (46.3%)	25 (32.1%)	23 (65.7%)		132 (40.2%)	42 (12.8%)	
> 100,000	12 (8.1%)	33 (49.3%)	17 (21.8%)	3 (8.6%)		57 (17.4%)	8 (2.4%)	

Table 3 Association of clinical presentation with variability in the blood group

Variables	ABO blood groups, n (%)					Rhesus types		
	O (n = 148)	A (n = 67)	B (n = 78)	AB (n = 35)	p-value	Positive (n = 266)	Negative (n = 62)	p-value
Temperature					< 0.001			0.119
< 37 °C	72 (48.6%)	6 (9.0%)	17 (21.8%)	21 (60%)		91 (%)	25 (7.6%)	
37.1 – 37.5 oC	39 (26.4%)	13 (19.4%)	21 (27.0%)	8 (22.9%)		72 (22.0%)	9 (2.7%)	
> 37.5 °C	37 (25%)	48 (71.6%)	40 (51.3%)	6 (17.1%)		103 (31.4%)	28 (8.5%)	
Clinical presentations								
Pallor	34 (23%)	25 (37.3%)	36 (46.2%)	5 (14.3%)	< 0.001	89 (27.1%)	11 (3.4%)	0.015
Vomiting	61 (41.2%)	42 (62.7%)	34 (43.6%)	11 (31.4%)	0.007	117 (35.7%)	31 (9.5%)	0.391
Diarrhoea	36 (24.3%)	29 (43.3%)	33 (42.3%)	4 (11.4%)	< 0.001	80 (24.4%)	22 (6.7%)	0.407
Chills	101 (68.2%)	45 (67.2%)	57 (73.1%)	19 (54.3%)	0.268	181 (55.2%)	41 (12.5%)	0.771
Fever	116 (78.4%)	53 (79.1%)	66 (84.6%)	22 (62.9%)	0.079	211 (64.3%)	46 (14.0%)	0.377
Nausea	38 (25.7%)	17 (25.4%)	36 (46.2%)	5 (14.3%)	0.001	84 (25.6%)	12 (3.7%)	0.057
Headache	97 (65.5%)	39 (58.2%)	39 (50%)	20 (57.1%)	0.152	159 (48.5%)	36 (11.0%)	0.805
Fatigue	16 (10.8%)	9 (13.4%)	10 (12.8%)	4 (11.4%)	0.943	31 (9.5%)	8 (2.4%)	0.784
Muscle ache	53 (35.8%)	23 (34.3%)	23 (29.3%)	14 (40%)	0.694	97 (29.6%)	16 (4.9%)	0.112

malarial anaemia were higher among patients with blood groups A (aOR = 10, 95% CI: 6.5–19.8, p < 0.0001) and B (aOR = 2.6, 95% CI: 1.1–6.0), p = 0.0232), compared to blood group O. However, individuals with the blood groups AB are less likely to develop severe malarial anaemia compared to those with the blood group O.

Discussion

Variabilities in blood groups play an important role in the pathogenesis of malaria. Several studies have linked malaria to non-O blood group [7, 16, 17]. Among the non-O group, malaria incidence is higher among individuals with blood group B [16, 18], some studies have also reported a higher probability of malaria incidence in blood group AB [19, 20]. Despite these associations, the impact of ABO and Rhesus (Rh) blood types on the severity of malaria has not been explored, especially in the Ghanaian population.

Among the participants with malaria studied, most of them were blood group O, followed by blood groups B, A and AB. The individuals who were in the O blood group were 54.9%. Among non-O blood groups, individuals with blood group B were more (43.3%), closely followed by blood group B (37.2%). This observation is consistent with previous publications on this subject matter, elsewhere [18, 19] and in Ghana [21]. However, among the

Table 4 Relationship between ABO blood groups and severe malarial anaemia

	Severe malarial anaemia	Uncomplicated malaria				
Age group less than 12	**Hb < 5 g/dL with parasitaemia > 10,000/µL (n = 26)**	**Hb > 5 g/dL with parasitaemia < 10,000/µL (n = 89)**	**Exclusions* (n = 213)**	**p-value**	**aOR (95% CI)**	**P-value**
ABO blood group				< 0.001		
O	7 (26.9%)	41 (46.1%)	100 (46.9%)		1	
A	8 (30.7%)	3 (3.4%)	56 (26.3%)		15.6 (9.3–20.6)	0.0005**
B	10 (38.4%)	36 (44.4%)	32 (15.0%)		1.6 (0.6–2.7)	0.3702
AB	1 (3.8%)	9 (10.1%)	25 (11.7%)		1.1 (0.7–1.9)	0.7040
Rhesus types				0.134		
Positive	23 (88.5%)	77 (86.5%)	166 (54.4%)			
Negative	3 (11.5%)	12 (13.5%)	47(45.6%)			
Age group more than 12	Hb < 7 g/dL with parasitaemia > 10,000/µL (n = 14)	Hb > 7 g/dL with parasitaemia < 10,000/µL (n = 88)	Exclusions* (n = 226)			
ABO blood group				< 0.001		
O	3 (21.4%)	40 (45.5%)	105 (46.5%)		1	
A	4 (28.6%)	3 (3.4%)	60 (26.5%)		17.8 (12.6–31.2)	0.0030**
B	4 (28.6%)	36 (41.0%)	38 (16.8%)		1.5 (0.3–2.6)	0.6222
AB	3 (21.4%)	9 (10.2%)	23 (10.2%)		4.4 (2.7–6.1)	0.0095**
Rhesus types				0.041		
Positive	14(100%)	76(86.4%)	176 (77.9%)			
Negative	0(0.0%)	12(13.6%)	50 (22.1%)			
	Bilirubin > 50 µmol/L with parasitaemia ≥ 100,000 /µL (n = 56)	Bilirubin < 50 µmol/L with parasitaemia < 100,000 /µL (n = 244)	Exclusions* (n = 28)	p-value		
ABO blood group				< 0.001		
O	12 (21.4%)	128 (52.5%)	8 (28.6%)		1	
A	28 (50%)	30 (12.3%)	9 (32.1%)		10 (6.5–19.8)	< 0.0001**
B	14 (25%)	57 (23.4%)	7 (25%)		2.6 (1.1–6.0)	0.0232**
AB	2 (3.6%)	29 (11.9%)	4 (14.3%)		0.7 (0.2–1.4)	0.6979
Rhesus types				0.288		
Positive	48 (85.7%)	198 (81.1%)	20 (71.4%)			
Negative	8 (14.3%)	46 (18.9%)	9 (32.1%)			

* Exclusions were individuals who did not fall into any of the definitions. Basically, they were uncomplicated cases according to WHO definitions

** Significant odds

general Ghanaian population, blood group O dominates, followed by blood group A, B and blood group AB in the minority [22].

The association of blood group B or A with malaria is attributable to resetting, which is enhanced in these blood groups compared to blood group O [3]. Rosette formation allows uninfected red blood cells to be attracted to infected red cells, mediated by the parasite protein called *Plasmodium falciparum* erythrocyte membrane protein (PfEMP1) [23]. Hyperparasitaemia was observed to be significantly higher in blood group A compared to the other blood groups. However, it was surprising to observe that low parasitaemia was higher in blood group B compared to blood group O. This observation could be due to the cytoadherence of the parasites in high parasitaemic situations in patients with blood group B, which could reduce the peripheral blood density of the parasites [23, 24]. Low parasitaemia after cytoadherence could be the case because even though low parasitaemia was associated with blood group B, more than 30% of them were moderately or severely anaemic, compared to blood group O. Notwithstanding the above, the effect of blood group variations on malaria parasite cytoadherence should be proven in a future study. In contrast, in group A blood, hyperparasitaemia corresponded to the severity of the anaemia, with concomitant hyperbilirubinaemia. Hyperparasitaemia and severe anaemia are linked due to the ability of the parasite to haemolyse infected cells,

mediated by enhanced resetting. Hyperbilirunemia is a direct consequence of intravascular haemolysis [24].

Furthermore, hyperthermia was observed at higher rates in patients with blood group A. This is explained by the associated hyperparasitaemia and severe anaemia. Hyperthermia occurs when infected red cells rupture and uninfected cells are being invaded [25]. This phenomenon is mediated by cytokines such as tumor necrosis factor (TNF) and interleukins (IL) 2 and IL6 [26]. Whereas pallor and nausea associated with blood group B of patients with malaria, vomiting, and diarrhoea associated with blood group A. According to the findings of this study, malaria in individuals in blood group A is a medical emergency. This is because the co-occurrence of vomiting and diarrhoea will eventually lead to dehydration, if parenteral fluids are not administered immediately. This will lead to hypovolemic shock and electrolyte imbalance, the result of which is mostly fatal. Due in part to hypoperfusion and hypoxia of the tissue. If left untreated, hypovolemic shock can cause ischemic injury to vital organs, leading to multi-organ failure [27].

In children, severe malarial anaemia due to low haemoglobin and parasite count > 10,000 per µL was significantly higher in blood group B, while in patients over 12 years of age, severe malarial anaemia was significantly higher in both groups A and B. On the other hand, severe malarial anaemia defined by hyperbilirubinaemia together with parasite count > 100,000 parasites/µL was significantly higher in individuals with blood group A. Therefore, individuals with blood groups A and B are vulnerable to developing severe malarial anaemia compared to blood groups O and AB. For these reasons, blood group assessments should be added to the list of investigations requested for patients suspected of malaria. This will help to promptly and adequately manage individuals who are likely to be severely affected by the disease, to ensure better treatment outcomes.

The majority of the participants studied in this publication were Rh positive (81.1%). However, Rh status was not associated with anaemia, jaundice, temperature, and severity of malaria. Furthermore, none of the clinical presentations associated with Rh status, except pallor. A review by Rattanapan et al. [28] found an inconsistencies in the vulnerability to severe malaria between individuals with Rh positive and negative. The review analyzed data from 36 eligible papers. Overall, 44.4% of the studies revealed that Rh positive individuals had a lower proportion of malaria than Rh negative individuals, while the remaining studies revealed a higher or no difference in the proportion of malaria between Rh positive and negative. The study then concluded that having Rh positive or Rh negative did not influence the development of severe malaria. This study adds to previous publications that Rh status did not have much impact on malaria clinical presentations.

Even though low levels of haemoglobin, high bilirubin levels with their attendant high parasitaemia were observed in blood groups A and B individuals with malaria, it must be stated that these levels could be confounded by various factors. Low levels of circulating erythropoietin is associated with low red blood cell and haemoglobin counts [29]. Further, bilirubin levels have also been found to be elevated in liver diseases [30]. In addition, anti-malaria immunity is likely to influence hyperparasitaemia seen in the study participant [31]. In addition, other factors such as poor nutrition, lower socioeconomic status and longer duration of symptoms were likely to affect the levels of the biomolecules reported in this study.

Limitations

The study participants were not tested for alpha thalassaemia. The G6PD screening was performed using the sodium nitrite-methylene blue method, which may not be of higher sensitivity, especially at lower haemoglobin levels. Other entero and haemoparasites, such as hookworms, filarial worms, and *Babesia* parasites, which could affect haemoglobin and bilirubin levels, were not screened. In addition, effect modifiers such as low levels of circulating erythropoietin, liver diseases, incompetent or low anti-malaria immunity, poor nutrition, lower socioeconomic status and longer duration of symptoms could directly or indirectly affect the levels of haemoglobin, bilirubin and/or the parasite counts recorded in this study. Finally, due to the convenient sampling technique employed, the outcome is limited to the population studied.

Conclusions

Out of the 328 participants, severe malarial anaemia was observed in 8% (26/328) children less than 12 years while in patients above 12 years or above, 4.3% (14/328) had severe malaria anaemia. Using hyperbilirubinaemia as a criterion, 17% (56/328) had severe malaria. When these low numbers were distributed among the blood group types, some of the frequencies were very low. However, the odds ratios were determined based on these figures. It was observed that, the odds of developing severe malarial anaemia, in children less than 12 years, was about 16 times higher, in patients with blood group A compared to patients with blood group O, with malaria. For patients ≥ 12 years, the odds of developing severe malarial anaemia was 4.4 times and 17.8 times higher among patients with blood groups A and AB, respectively, compared to patients with blood O. Finally, using the hyperbilirubinaemia with parasitaemia 100,000 /µL

criteria, the chances of developing severe malaria were 10 times higher among patients with blood groups A and 2.6 times higher in patients with blood group B, compared to blood group O.

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Author contributions
EA and RHA conceived, supervised and provided resources the study. PSA, PMA and SBA participated in participant recruitment and sample collection. RYM and FG performed laboratory analysis. EA wrote the initial draft. However, all authors approved the manuscript.

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Availability of data and materials
Request for the data can be obtained from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the University of Health and Allied Sciences Ethics Review Committee (UHAS-REC A.9 [14] 22–23). All participants provided written consent, either by themselves or on behalf of minors by accompanying adults, to participate in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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