Integrated Health Research Journal (IHRJ)

The Official Journal of the College of Health and Allied Sciences University of Cape Coast, Ghana

RESEARCH ARTICLE

Identification of High-Risk Groups of Falciparum Malaria in Western Region of Ghana: the predictive value of ABO Blood Group Typology

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Abstract

Background: Several studies have linked malaria to ABO blood groups with still others reporting insignificant association between ABO blood group system and malaria. Blood group 'O' has been shown to confer protection against severe malaria by studies in various populations but indecisive reports have been given about non-O blood groups in relation to their protection or vulnerability to severe malaria.

Objective: The present study sought to investigate the ABO blood group typology and the risk of developing severe malaria.

Materials and Methods: A total of 280 participants (140) with P. Falciparum malaria patients and 140 healthy controls) screened for ABO blood groups by Tile method were enrolled into the study. Thick and thin blood films stained with 10 % Giemsa were prepared for falciparum – infected individuals and their full blood counts obtained from the haematology analyzer (Cell Dyn 1800, Abbot Diagnostic Division, USA). Parasite counts were categorized into severe and uncomplicated malaria and further grouped into varying degrees of parasitaemia. The effects of parasite densities on some haematological parameters were then studied. Severe malaria was defined as hyperparasitaemia, malarial anaemia and thrombocytopenia.

Results: The frequency of blood group 'A' was significantly higher in patients with severe malaria compared to other blood groups ($p = 0.042$). Blood groups 'A' and 'B' showed higher parasite densities while 'A' and 'AB' blood groups revealed low platelet counts. Anaemia was severe in blood groups 'A' and 'O' ($p \leq 0.05$). Previous studies including this current one highlighted the protected nature of blood group 'O' to severe malaria ($p \leq 0.05$).

Conclusion: The present study provides the evidences that, individuals of blood group 'A' are highly susceptible to malaria infection and inferably with an increased risk of developing severe malaria than the other blood groups.

Keywords: Anemia, Blood group, Ghana, Malaria, *Plasmodium falciparum*.

Citation: Ampomah, P., Buadii, E., Aboagye, B. (2023) Identification of High-Risk Groups of Falciparum Malaria in Western Region of Ghana: the predictive value of ABO Blood Group Typology. Integrated Health Research Journal 1(1), 18-27. https://doi.org/10.47963/ihrj.v1i1.1177
Received January 13, 2023; **Published** April 20, 2023

Introduction

Malaria is a vector-borne disease caused by Plasmodium parasites which is transmitted through the bite of the infected female Anopheles mosquito or from mother to child or via blood transfusion (Ashley et al., 2014). It is a major cause of illness and death particularly among children and pregnant women. The disease is endemic in Ghana, accounting for 40% of all outpatient visits to hospital (Awine, Malm, Bart-Plange, & Silal, 2017) and it has been reported that households spent between US$5.70 on uncomplicated malaria and US$48.73 on severe in Ghana (Nonvignon et al., 2016). Statistics shows that between 3.1 and 3.5 million cases of clinical malaria are reported in public health facilities each year, of which 900,000 cases are in children under five years. On the whole children under five years and pregnant women constitute 20 % and 4 %, respectively, of the general

population affected by malaria (Kayentao et al., 2018), making malaria an infection of great worry.

Case management has been and continues to be one of the main strategies of controlling malaria in the country. There have been many large-scale initiatives undertaken during the last few decades with the goal of reducing or eradicating the burden of malaria in the developing world. These ambitious goals set by these programmes for reducing malaria burden in the near future however, appear unlikely to be met (Attaran et al., 2004). This is because mortality from malaria continues to be the major burden in developing countries, especially in children under five years of age. Effective treatment for malaria exists, but it must be administered promptly and timely by trained personnel in order to be effective as delay in treatment may be detrimental to the infected individuals (Alonso et al., 2011; Smith, Jones, Meek, & Webster, 2009)

It is generally accepted then, that most malaria deaths can be prevented when clinical cases are promptly diagnosed and effectively treated. In young children, malaria can progress from mild to severe cases within 24 hours after the onset of symptoms. Illness progression to the severe stages of malaria and mortality can be reduced if there is prompt diagnosis and timely malaria treatment within 24 hours after onset of first symptoms (Luz et al., 2013; Turuse, Gelaye, & Beyen, 2014).

Pathophysiologically, the response to malaria infection with P. falciparum is highly variable, depending on several different biological factors such as previously acquired immunity and blood polymorphisms (Modiano et al., 1996; Perkins et al., 2011). In light of this, researchers are investigating especially in developed countries on how best malaria infection and its progression can be curtailed through destruction of human cell-parasite interaction, most importantly, the destruction of the erythrocytic phase of the plasmodium cycle. One of such researches is the study of the interaction between ABO blood group antigens and plasmodium proteins, most especially, the Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) antigens, as it is believed to influence disease progression of malaria in different individuals (Cserti & Dzik, 2007). Blood group antigens A and B have been reported as co-receptors for P. falciparum rosetting whereas blood group 'O', lacking these antigens may offer some protection against severity of malaria by reduced rosetting (Barragan, Kremsner, Wahlgren, & Carlson, 2000). There are also increasing evidences that both the risk of acquiring P. falciparum malaria and the risk of developing severe complications are determined by host genetic factors (De Mendonça, Goncalves, & Barral-Netto, 2012; Fortin, Stevenson, & Gros, 2002). However, there are scarce studies and inconsistent data particularly in Ghana, relating a particular ABO blood group phenotype to the risk of developing severe malaria.

This study is therefore aimed at identifying the specific blood type(s) that is/are at a greater risk of developing severe malaria by determining the association between ABO blood groups and P. falciparum malaria.

Materials and Methods

Study site: This was a randomized cross-sectional study carried out from January to March 2013. The study took place at the medical laboratories of four (4) hospitals in the Sekondi-Takoradi Metropolis (STM); Effia-Nkwanta Regional Hospital (ENRH), Takoradi Hospital (TH), Kwesimintsim Hospital (KH) and Essikadu Hospital (ESKH).

Sekondi-Takoradi Metropolis, the administrative capital of the Western Region of the Republic of Ghana covers a land area of 385 km2 with Sekondi as the administrative headquarters. The Metro is bordered to the West by Ahanta West District, to the North by Mpohor Wassa East, to the East by Komenda-Edina Eguafo-Abrem and to the South by the Gulf of Guinea. The Metropolis is strategically located on the south-western coast of Ghana, about 200 km west of Accra and 130 km East of La Cote D'Ivoire (Sekondi – Takoradi Metropolitan Assembly, 2013). STM is a densely populated district having the largest share (23.5 %) of the region's total population of 2,376,021. The current population of the Metropolis stands at 559,548 with 273,436 males and 286,112 females (Fiave, 2017).

The central part of the Metropolis is low lying and occupied by muddy lagoons. It also has an equatorial type of climate and bi-modal type of rainfall. Temperatures are high with an average of 22 °C. With a mean annual rainfall of 1,380 mm, the metropolis experience heavy rainfall in March and July with minor rains occurring between August and November (Ministry of Food and Agriculture, 2013).

Study population: The study was cross-sectional and recruited two hundred and eighty (280) consenting individuals from patients who were referred to the laboratory for malaria test. One hundred and forty (140) malaria positive patients and another one hundred and forty (140) healthy individuals as control were enrolled into the study using a selection criterion described below. Malaria positive participants were confirmed Plasmodium falciparum infected patients of all age groups at the medical laboratories of the four hospitals. Healthy controls were non-malaria infected healthy individuals attending the outpatient department for routine check-up and the blood bank of ENRH for blood donation. Personal data included name, age and sex.

Ethical consideration: Ethical approval was given by the Department of Biomedical Sciences research committee students' research. Permission to undertake this study was obtained from the administration of all the four hospitals. The potential risk involved in the study was explained to the participants and finally, written consent from older study participants and permission from care givers of all younger participants were obtained prior to enrollment into the study. Records were kept strictly confidential hence there was no conflict of interest.

Inclusion criteria

- Patients who tested positive for malaria infection (irrespective of age and sex), in the medical laboratory of the hospitals and have not taken anti-malarial drugs before the study.

- Healthy non-malaria infected individuals at ENRH for routine check-up and for blood donation during the time of study.

Exclusion criteria

- Patients who took anti-malarial drugs before visiting the laboratory and those who were sickle cell positive were excluded.

Sample collection and processing

Sickle cell slide test: Sickle cell test was performed on each blood sample of malaria patients to exclude patients who were positive. One drop of blood was mixed on a slide with a drop of sodium metabisulphite, (di-sodium disulphite, Merck, Darmstadt, Germany) 0.2g in 10ml of distilled water covered with a cover glass and incubated at room temperature for up to 1 hour. This was observed under the microscope (Olympus; model CHA, USA) with 40x objective lens. A positive control blood from a known sickle cell trait person was set up.

Preparation, staining and examination of blood films for malaria parasite: Blood samples of both malaria patients and healthy individuals were taken into dipotassium Ethylenediamintetra- acetic acid (K2 EDTA) blood collection tubes (Cangzhou Yongkang, China). The blood samples were first confirmed by the trained microscopists at the laboratories to be either positive or negative for malaria. Confirmed malaria positive and negative blood samples for malaria infected patients and non- malarial healthy study participants respectively were then obtained from the laboratories for processing. Thick and Thin blood films were prepared on the same slide for malaria parasite quantification and identification respectively.

Thick film: Thick film was used to concentrate the parasite and to determine the degree of parasitaemia. The smear was prepared by placing 6 µl of blood on a lower half of a grease-free slide. The edge of another slide was used to spread the blood in a circular form of about 2 cm in diameter.

Thin film: Thin film consists of a single layer of red cell, which was prepared using 2 µl of blood on the lower part of the second half (about 1 cm apart from the thick smear) of the grease-free slide. A second slide with smooth but chopped edge was used as a 'spreader'. The 'spreader' was placed just before the 2 µl of blood and allowed to run along its edge. The spreader was then pushed along the slide at an angle of 30˚ to make the smear. The films were labeled correctly with the corresponding patient number and air dried.

Staining: Thin film was fixed with absolute (100 %) methanol after air drying both thick and thin films. The slides were placed on the staining rack, making sure that all thick films were at one end of the rack. A 1:10 or 10 % Giemsa stain solution (1 ml of Giemsa stock solution added to 9 ml phosphate buffered water of pH 7.2) was prepared and then filtered before it was used to avoid the introduction of dirt particles. The slides were covered with the stain for 10 minutes after which the stains were washed with excess buffered water. The back of the slides was blotted and air dried in a draining rack.

Examination of thick and thin smears: Plasmodium falciparum parasites were counted per 200 or 500 leucocytes, which were used to estimate the parasite density per microlitre of blood. The entire smear was first screened at a low magnification (40x objective lens) to examine first for stain reaction and in the case of thin film to select an area where the cells were evenly distributed and the parasites well seen. Smears were then examined using 100x oil immersion. White cells were symmetrically counted against the number of parasites in each field covered on the thick film using hand tally counters. Thin films were examined to confirm the species and stage identification on the thick film. A second opinion was sought when in doubt.

Estimation of parasite density: Parasite counts were done in the thin film (against 2,000 red blood cells) as a result of heavy parasitemia (greater or equal to 100 parasites on thick film per high power field), parasites counted were recalculated with 200 white blood cells (WBCs). The parasite density was estimated using the formula below (WHO, 1991):

Number of parasites per µl of blood (n)

n = (Number of parasites counted x Total white cell count)/200 WBCs counted

ABO blood grouping: Two spots of blood from each subject were made on the white plain tile and a drop of each antiserum A and B (Span Diagnostics Limited, Gujarat) was applied to each spot respectively. The mixture was further stirred with a plastic stirrer and rocked for some time. Signs of agglutination were observed.

Haematological analysis: The full blood counts (FBC) analysis for each malarial participant's sample was obtained from the automated haematology analyzer (Cell-Dyn 1800, Abbot Diagnostic Division. USA). Daily internal quality controls and the scheduled external quality assessment programme were adhered to as a quality measure.

Severity of infection: Development of severity of the malaria disease was determined based on the parasite density (parasitaemia) calculated as above, anaemia and thrombocytopenia (WHO, 1991). Malaria cases were further grouped into severe malaria (SM) and uncomplicated malaria (UCM).

Parasitaemia

- High parasitaemia was defined as parasitaemia> 10,000 parasites / µl.
- Moderate parasitaemia was defined as parasitaemia between 1000-9999 parasite /µl.
- Low parasitaemia was defined as parasitaemia between 1- 999 parasites /µl.

Anaemia

- Severe anaemia was defined as haemoglobin concentration of < 5g / dL or hematocrit < 15 % and RBC count <3.0 M / µL)

- Moderate anaemia was defined as haemoglobin concentration between 5 – 8 g / dL, hematocrit between 15 – 24 % and RBC count 3.0 - 4.20 M / µL).
- Mild anaemia was defined as haemoglobin concentration > 8 g / dL or hematocrit >24 % and RBC count >4.20 M / µL).

Thrombocytopenia

- Severe thrombocytopenia was defined as platelet count < 20,000 / µl.
- Moderate thrombocytopenia was defined as platelet count < 50,000 / µl but > 20,000 / µl.
- Mild thrombocytopenia was defined as platelet count < 150,000 / µl but > 50,000 / µl.

Severe malaria: Based on World Health Organization established criteria for severe malaria (WHO, 2000), severe malaria was defined as hyperparasitaemia> 250,000 parasites / µl of blood and / or hematocrit < 15 % or haemoglobin< 5 g / dl in the presence of parasite count > 10,000 / µl of blood.

Uncomplicated malaria: Uncomplicated malaria was defined as parasitaemia> 10,000 parasites / µl of blood and / or hematocrit >24 % or haemoglobin concentration > 8 g / dl in the presence of parasitaemia.

Severity was based on the presence of high parasitaemia, severe anaemia and severe thrombocytopenia. Normal reference ranges of the haematological parameters are shown in Appendix.

Statistical analysis: Data was entered in Microsoft Excel, checked for its correctness, and exported to and analyzed using SPSS version 16 (SPSS Inc. Chicago). Chi-square test was used to assess the difference between frequencies. One-way ANOVA was used to compare the mean scores of more than two groups. A probability value less than 0.05 ($p<0.05$) was considered statistically significant. The statistical evaluation of the data was done by mean, standard error and Chi square value (X2).

Results

The study enrolled 140 P. falciparum malaria patients representing the cases and 140 non- malaria infected individuals representing healthy controls. In general, the control group was significantly older, a mean age of 38.3 ± 1.17, than the malaria infected individuals with mean age of 18.0 ± 1.53 ($p = 0.001$). Of the malaria patients, 59 (42.1 %) were males and 81 (57.9 %) were females in contrast to 63 (45.0%) males and 77 (55.0%) females of the healthy controls. The ABO blood groups of both falciparum-infected patients and healthy controls were also determined. Correspondingly, 50 %, 23 %, 20 % and 6.4 % among the cases were found to be of blood types 'O', 'B', 'A' and 'AB' while 51 %, 22.9 %, 17.9 % and 7.9 % also of 'O', 'B', 'A' and 'AB' were found among the controls. Furthermore, among the patients with parasites, those below 5 years of age, 48 (34.3 %), represented the largest proportion whilst age group 37 – 51, 5(3.6 %), represented the smallest with 41 (29.3 %), 37 (26.4 %) and 9 (6.4 %) falling within age groups 5 – 20, 21 – 36 and greater than or equal to 52 years respectively **(Table 1)**.

Table 1: General characteristics of study participants.

Variables	Control (N = 140)	Case (N = 140)	p-value	(X2)
Age mean ± (SE)	38.3 ± (1.17)	18.0 ± (1.53)	0.001	
Gender				
Males	63(45.0)	59 (42.1)	0.63	0.232
Female	77(55.0)	81 (57.9)		
ABO Groupings				
A	25(17.9)	28 (20.0)	0.937	0.413
B	32(22.9)	33 (23.6)		
AB	11(7.9)	9 (6.4)		
O	72 (51.4)	70 (50.7)		
Age range (years)				
< 5	0 (0.0)	48 (34.3)	0.001	12.152
5 to 20	4 (2.9)	41 (29.3)		
21 – 36	76 (54.3)	37 (26.4)		
37 – 51	33 (23.6)	5 (3.6)		
≥ 52	27 (19.3)	9 (6.4)		
SE (Standard error); N (%): frequency (percentage). Differences between values are statistically significant at $p<0.05$				

In relating gender of malaria patients to their haematological parameters, males were found to be more infected with malaria than females, showing a higher mean white blood cell count of 8.4 ± 0.57 and mean parasite density of 30502 parasites / µl of blood though between them these differences were not statistically significant ($p>0.05$). Females however, recorded relatively lower mean values in the red cell indices; haemoglobin level, red blood cell count and hematocrit, compared to males. With the range of clinically normal platelet count to be 150 – 450 K / µl, it can be seen that males showed a near significant ($p=0.8$) lower platelet count (143.2 ± 13.5) than females (173 ±10.8) **(Table 2)**.

Table 2: Hematological characteristics of malarial subjects in relation to gender

Variables	Male	Female	p-value
	N = 59	N = 81	
Parasite density	30502 ± (581)	30084 ± (591)	0.97
Hb (g / dl)	10.6 ± (0.33)	10.2 ± (0.25)	0.32
RBC(M / µl)	4.02 ± (0.11)	3.9 ± (0.09)	0.34
HCT(%)	31.5 ± (1.01)	30.6 ± (0.74)	0.47
Platelet (K / µl)	143.2 ± (13.5)	173 ± (10.8)	0.08
WBC(109 / l)	8.4 ± (0.57)	7.9 ± (0.44)	0.51
Hb: Haemoglobin; RBC: Red blood cell; WBC: White blood cell; HCT: Hematocrit; N: Frequency. Values are in mean ± SE (Standard error). Differences between Mean values are statistically significant at $p<0.05$.			

Looking at the ABO distribution among malaria-infected participants, infection was more prevalent in blood group 'O' and less prevalent in blood group 'AB' in both males and females. The trend of high infection was then followed by blood group 'B' (15.00 %) before A (10.71 %) in females but took a reverse trend in males **(Figure1)**.

Degree of parasitaemia showed association with age as reported in most studies. From this study the highest prevalence of high parasite density was among children

under-five years of age (20.0%) as compared with older age groups but this difference was not statistically significant (p>0.05). Noticeably patients within the age group 32 – 57 years recorded the lowest incidence of parasitaemia (1.4 %) of the total population **(Table 3)**.

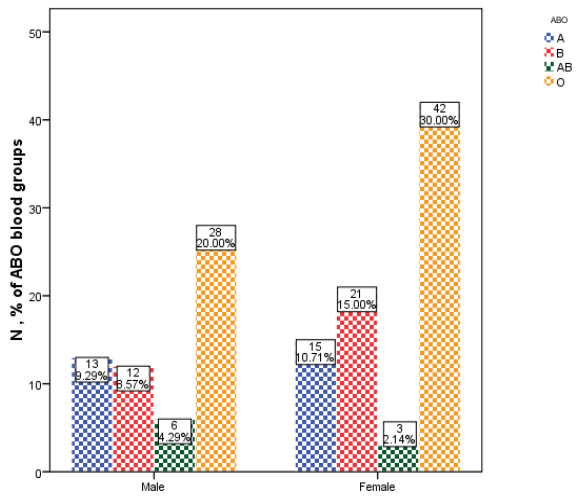

Figure 1: Frequency of ABO blood groups among malaria subjects per gender

Table 3: Relationship between age groups and the degree of parasitaemia

Age (years)	**LP**	**MP**	**HP**	**Total**
	N = 12 (8.6 %)	N = 63 (45.0 %)	N = 65 (46.4 %)	N = 140
< 5	3 (2.1)	17 (12.1)	28 (20.0)	48 (34.3)
5 – 20	3 (2.1)	22 (15.7)	16 (11.4)	41 (29.3)
21 – 36	6 (4.3)	17 (12.1)	14 (10.0)	37 (26.4)
37 – 51	0 (0.0)	3 (2.1)	2 (1.4)	5 (3.6)
≥ 52	0 (0.0)	4 (2.9)	5 (3.6)	9 (6.4)
Values in parentheses indicate percent values. N: Frequency; LP: Low parasitaemia; MP: Moderate parasitaemia; HP: High parasitaemia. Differences in percentages were analyzed using Chi-square statistical tool. Chi-square value (X2) = 8.764, df=6, p – value=0.363.				

To establish the possible association between ABO blood group system and severe P. falciparum malaria, the distributions of each ABO blood group in severe malaria and uncomplicated malaria patients were compared. Of the nine (9) patients with severe malaria, a significantly higher percentage (3.6 %) were blood group 'A' patients (X2 = 8.190, p = 0.042). Additionally, blood group 'O' patients were significantly associated with uncomplicated malaria than with severe malaria indicating its likely protective role **(Table 4).**

Parasite densities and their corresponding haematological parameters among each blood group were compared **(Table 5)**. In general, malaria infection displayed a significant association with blood groups (F = 5.573, p = 0.009), with the highest level of parasitemia observed among blood group 'A' patients (52318 parasites / µl of blood) and the lowest among blood group 'AB' (12086 parasites / µl of blood). Concerning the Mean platelet count, only a trend was observed between blood group 'AB' and the other blood groups (p = 0.06).

Table 4: Distribution of ABO blood group in severe and uncomplicated malaria

ABO blood group	SM	UCM	Total
	N = 9 (6.4%)	N = 131 (93.6%)	N = 140 (100.0 %)
A	5 (3.6)*	23 (16.4)	28 (20.0)
B	2 (1.4)	31 (22.1)	33 (23.6)
AB	0 (0.0)	9 (6.4)	9 (6.4)
O	2 (1.4)	68 (48.6)*	70 (50.0)
Values in parentheses denote percentages. N: Frequency; SM: Severe malaria; UCM: Uncomplicated malaria. Differences in percentages were analyzed using Chi – square statistical tool. Chi – square value (X2) = 8.190, df = 3, p – value = 0.042. ABO blood group frequency of healthy controls (N = 140): 'A' = 17.9 %, 'B' = 22.9 %, 'AB' = 7.9 % and 'O' = 51.4 %. * Shows the highest frequency in SM and UCM.			

On the whole, 4.3%, 2.1%, 1.4% and 0.7% of the 140 P. falciparum infected participants belonging to blood group 'B', 'O', 'A' and 'AB' respectively had low parasitemia (< 1000 parasites / µl of blood). Conversely 25.0 %, 9.3 %, 7.9 % and 2.9 % of them with blood type 'O', 'A', 'B' and 'AB' respectively had moderate parasitemia (1000-9999 parasites / µl of blood). High parasitemia (>10,000 parasites / µl of blood) was also most prevalent (22.9%) among blood group 'O' patients, followed by blood group 'B' (11.4 %), 'A' (9.3 %) and 'AB' (2.9 %). This difference was statistically insignificant (p = 0.356, X2 = 6.636) **(Table 6)**.

Severe malarial anaemia was defined as haemoglobin (Hb) value of <5 g / dl or hematocrit (HCT) < 15 % in the presence of parasitaemia. Severe anaemia was prevalent among blood group 'A' and 'O' patients; both recording 1.43 %. Moderate anaemia (Hb: 5 – 8 g / dl and / or HCT: 15 – 24 %) and mild anaemia (Hb: ≥ 8 g / dl or HCT: ≥ 24 %) were both prevalent among blood group 'O' malarial subjects (5.71 % and 42.86 % respectively) compared to non-O subjects. This high prevalence was followed by blood group 'A' (3.57 %) in moderate anaemia and blood group 'B' (21.43%) in mild anaemia **(Figure 2)**.

Thrombocytopenia is common in malaria. In finding the association between thrombocytopenia and ABO blood groups, their distribution in relation to platelet counts was studied. Platelet counts among the patients were stratified into low (< 150,000 / µl) and normal (> 150,000 / µl) platelet counts. Low platelet count (Thrombocytopenia) was further classified as severely (<20,000 / µl), moderately (< 50,000 / µl but > 20,000 / µl) and mildly (< 150,000 / µl but > 50,000 / µl) low. Among these categories just a patient each for blood group 'A' and 'O' reported with severe thrombocytopenia representing 0.7 % of the total population of malaria patients. Furthermore, blood group 'O' patients were associated with moderate and mild thrombocytopenia with still the highest percentage of them observed among patients with normal platelet counts. Among the non-O blood groups, blood group 'A' recorded the highest prevalence of severe (0.7 %) and moderate (2.1 %) thrombocytopenia whilst blood group B showed the

Table 5: Haematological characteristics of malarial subjects stratified by ABO blood groups

Variables	A (N = 28)	B (N = 33)	AB (N = 9)	O (N = 70)	Total (N = 140)
Parasite density	52318 ± (514)a	36890 ± (462)b	12086 ± (169)c	20647 ± (405)d	30260±(413)
Hb (g/dl)	9.5 ± (0.49)a	11.3 ± (0.43)b	10.4 ± (0.63)a	10.3 ± (0.23)a	10.4±(0.19)
RBC (M/µl)	3.6 ± (0.19)a	4.3 ± (0.14)b	4.1 ± (0.25)a	3.9 ± (0.09)a	3.9±(0.07)
HCT (%)	28.5 ± (1.51)a	33.9 ± (1.31)b	31.6 ± (2.01)a	30.6 ± (0.7) a	31.0±(0.61)
Platelet(K/µl)	131.8 ± (18.03)c	171.1 ± (17.6)a	99.7 ± (13.12)b	174.0 ± (12.4)a	160.3±(8.49)
WBC (109/l)	9.65 ± (0.83)a	7.83 ± (0.73)a	6.64 ± (0.91)a	7.78 ± (0.48) a	8.104±(0.34)

Differences between mean values of the various groups were analyzed with one-way ANOVA. Mean values in row with different superscripts are statistically significant ($p < 0.05$). Parasite density, ($F = 5.573$, $p = 0.009$); RBC ($F = 3.349$, $p = 0.021$); Hb ($F = 3.132$, $p = 0.028$); HCT ($F = 3.114$, $p = 0.028$); Platelet ($F = 2.811$, $p = 0.061$); WBC ($F = 1.893$, $p = 0.134$). Parasite density is in parasites / µl of blood.

highest prevalence in both mild thrombocytopenia (12.1 %) and normal platelet count (11.4 %). Blood group AB however displayed the lowest percentages across all stratifications. These associations though comparable were not statistically significant ($p > 0.05$) **(Table7).**

Table 6: Relationship between the ABO blood groups and the degree of parasitemia

ABO blood Groups	LP (N = 12)	MP (N = 63)	HP (N = 65)	Total (N = 140)
	-8.60%	-45.00%	-46.40%	-100%
A	2 (1.4)	13 (9.3)	13 (9.3)	28 (20.0)
B	6 (4.3)	11 (7.9)	16 (11.4)	33 (23.6)
AB	1 (0.7)	4 (2.9)	4 (2.9)	9 (6.4)
O	3 (2.1)	35 (25.0)	32 (22.9)	70 (50.7)

N: Frequency. Chi-square value (X2) = 6.636, df = 6, p – value = 0.356. Healthy control participants ABO blood group frequency (N = 140): 'A' = 17.9%, 'B' = 22.9 %, 'AB' = 7.9 % and 'O' = 51.4 %.

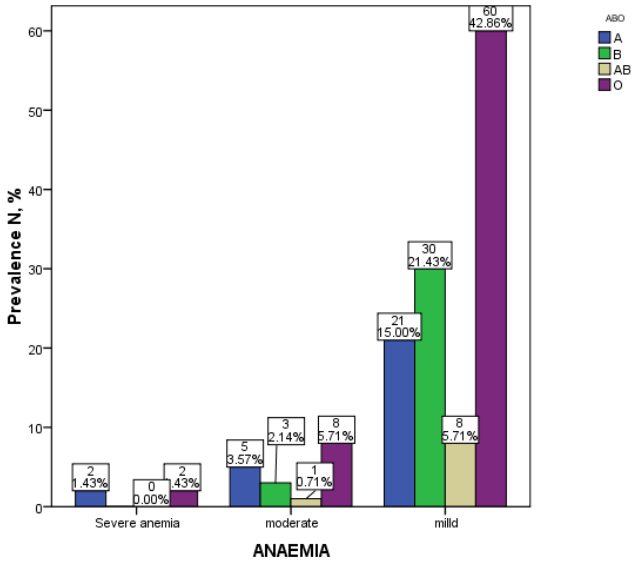

Figure 2: Frequency of ABO blood group among malaria subjects in relation to anaemia

Discussion

This study was conducted to establish the possible relationship between ABO blood groups and the risk of developing severe malaria among malaria patients of all ages. The distribution of blood groups among healthy control individuals revealed a higher percentage of blood group 'O' (51.4 %) followed by blood group 'B' (22.9 %), 'A' (17.9 %) and 'AB' (7.9 %). Matching the 140 healthy controls to 140 malaria infected cases, blood group 'O' represented 50.7 % of the cases which is lower than 51.4% represented by the control group. This is in consistence with the findings of Rowe et al., (J. A. Rowe et al., 2007) who also found a lower percentage of blood group 'O' among severe malaria infected Malian children compared with healthy controls. Conversely, a significantly lower frequency of blood group 'O' have been observed in other malaria non-endemic areas (Chavhan, Pawar, & Baig, 2011; RAI & KUMAR, 2011) which indicates the selective advantage of blood group 'O' in malaria endemic places. Blood group 'O' has been reported by several studies to confer protection against severe malaria. It has therefore been hypothesised that the prevalence of blood group 'O' would be higher in malaria endemic areas by natural selection due to its capacity to stay protected from severe malaria. This study being carried out in a malaria endemic region also confirms this hypothesis. The observations and hypotheses on the protective advantage of blood group 'O' and the less protective role of non-O blood groups are therefore in line with the ABO blood group distributions among both healthy controls and malaria cases recorded in this study. This could be further supported by the higher prevalence of blood type 'O' and lower prevalence of blood types 'A', 'B' and 'AB' globally, where malaria is more prevalent (Cserti & Dzik, 2007; Tekeste & Petros, 2010).

By gender, the mean parasite density was found to be higher in males (30502 parasites /µl of blood) than in females (30084 parasites /µl of blood). This finding is in agreement with previous findings of Akanbi et al (Akanbi, Badaki, Adeniran, & Olotu, 2010; Akanbi, Odaibo, Olatoregun, & Ademowo, 2010) who attributed this to the reason that men expose their bodies more than females when the weather is hot, thus increasing their chances of being bitten by mosquitoes more than females. Moreover, both males and females showing low levels of haemoglobin concentrations suggest the impact of parasitaemia on the haemoglobin molecules. Malaria parasites feed on haemoglobin of infected host in order to grow (Kulkarni, Suryakar, Sardeshmukh, & Rathi, 2003). The increase in malaria parasites present in males (evident by the higher mean parasite density) caused the critical reduction in their haemoglobin level. This effect had a greater impact on the males than females considering the fact that males naturally have a higher haemoglobin level (13 - 18 g / dl)

Table 7: Relationship between the ABO blood groups and platelet count

ABO blood group	Low platelet count			Normal	Total
	Severe	Moderate	Mild	Platelet count	
	N = 2 (1.40)	N = 8 (5.70%)	N = 67 (47.90%)	N = 63 (45.0 %)	N=140 (100%)
A	1(0.7)	3(2.1)	16(11.4)	8(5.7)	28(20.0)
B	0(0.0)	0(0.0)	17(12.1)	16(11.4)	33(23.6)
AB	0(0.0)	1(0.7)	6(4.3)	2(1.4)	9(6.4)
O	1(0.7)	4(2.9)	28(20.0)	37(26.4)	70(50.0)
Chi-square value (X2) = 9.786, df = 9, p – value = 0.368.					

than females (12 - 15 g / dl), a feature more attributable to the increased body exposure of males to mosquito bites than females. The mean differences of parasite densities and haemoglobin concentration together with hematocrit and red blood cell counts between both sexes were however not statistically significant ($p < 0.05$). Gender, thus did not influence the severity of malaria in this study.

The study showed a significant difference between the mean ages of healthy controls and that of malaria cases ($p = 0.001$). The healthy controls can be perceived as an adult-aged group (mean age, 38.3 ± 1.17) and the malaria cases as a young-aged group (mean age, 18 ± 1.53). It could therefore be inferred that malaria is more prevalent among children than adults. To assess how age influence parasitaemia and inferably the risk of developing severe malaria, the relationship between age groups and degree of parasitaemia was established. Prevalence of parasitaemia differed between age groups, with a very high percentage of children under five years old (20 %) recording high parasitaemia in contrast to lower percentages of high parasitaemia recorded among adults. This could be due to the increased number of exposures to mosquito bites among adults by virtue of age, a fact which is found to be coherent with similar studies (Akanbi, Badaki, et al., 2010; Kuadzi, Ankra-Badu, & Addae, 2011). These researchers attributed this to lack of protective immunity in children compared to adults who by their increased previous exposure to malaria have developed an acquired immunity to the disease stage of malaria and its severe complications thereof. The frequencies of parasitaemia found to be highest among patients less than five years of age, and decreased with increasing age respectively; 20 % among <5 years, 11.4 % among 5 – 20years, 10 % among 21 – 36 years and 2 % among 37 – 51 years. As indicated by Akanbi et al (Akanbi, Badaki, et al., 2010), the exposure of persons to mosquito bites has been reported to increase with age which confirms the relation of increased level of immunity against malaria and the decreased level of parasitaemia with increasing age. Almost all adults living in malaria-endemic areas experience repeated malaria infections (Doolan, Dobaño, & Baird, 2009). This might help in the development of antibodies against many sporozoite, liver-stage, blood-stage and sexual-stage malaria antigens that protect the adults from severe forms of malaria complications.

It was however observed in this study that patients above 52 years though older than patients within the ages of 37 – 51years presented with a comparably higher parasitaemia. This may be due to the functional degeneration of the immune system as one ages. Older age is commensurate with weakened immunity more importantly when the thymus is not producing enough T cells to respond to the numerous ill conditions. Therefore, suppressed immunity in old age renders them most vulnerable to malaria infections just as in children. It can be emphasized then, that age influence parasitaemia in relation to malaria severity.

Looking at the overall ABO distribution among malaria-infected participants, infection was more prevalent in blood group 'O' and less prevalent in blood group 'AB' (Satti, 2017; Singh, Urhekar, & Singh, 2015). The trend of high infection was then followed by blood group 'B' (15 %) before 'A' (10.71 %) in females but took a reverse trend in males. This should give a clue that parasite densities will be very high among blood group O and very low in blood group AB patients, but this wasn't so for 'O' blood groups. The high frequency of 'O' blood groups is therefore due to their high distribution in general (Anjomruz et al., 2014; Zerihun, Degarege, & Erko, 2011) Hyperparasitaemia among non-O blood groups was more prevalent among blood group 'A' and 'B' malaria patients (Fig. 1) (Panda et al., 2011; J. A. Rowe et al., 2007; Chigozie Jesse Uneke, Ogbu, & Nwojiji, 2006). A significantly high prevalence (3.6 %; p – value = 0.042) of severe malaria was observed to be among blood group 'A' malaria patients compared to the other blood groups. This indicates the higher vulnerability of blood group 'A' individuals to severe malaria and also confirms the reports by other studies that severe malaria is more implicated in blood group 'A' individuals (Deepa, Rameshkumar, & Ross, 2011; Degarege, Gebrezgi, Ibanez, Wahlgren, & Madhivanan, 2019; Pathirana et al., 2005; A. Rowe, Obeiro, Newbold, & Marsh, 1995; Zerihun et al., 2011), but contradicts earlier reports of high prevalence of severe malaria among blood group 'AB' patients in Sri Lanka (Panda et al., 2011) and blood group 'B' patients in Odisha, India (Deepa et al., 2011). High prevalence and increased risk of placental malaria in blood group O primigravid pregnant subjects has also been recorded(Gupte, Patel, & Patel, 2012).

The variability of observations made considering the different blood groups may be attributed to varied infective strains and probably to different rosetting capacity (Cserti & Dzik, 2007; Loscertales et al., 2007). Blood group 'O' showing the highest frequency (68 / 140; 48.6 %) among uncomplicated malaria cases indicates that majority of blood group 'O' patients are much more protected from severe malaria than non-O blood groups. Blood group 'A' patients again showed significantly increased susceptibility to *P. falciparum* malaria infection evidenced by the highest

mean parasite density they recorded, followed by blood group 'B' (Table 5) and agree with previous findings (Lell et al., 1999; C J Uneke, 2007), which implicate the presence of A or B antigens on rbcs and increasing frequency of malarial episodes in Brazil. Blood group 'AB' showed the lowest parasite density in this study though many previous reports suggest that individuals with blood groups 'A', 'B' and 'AB' are more susceptible to P. falciparum infection than those with blood group 'O' (Gupta & Chowdhuri, 1980; J. A. Rowe, Opi, & Williams, 2009). Similar observation has been made in Sars Cov2 infections 45. However, some previous studies have reported blood group 'O' type to be highly susceptible malaria infection (Singh et al., 2015). Conversely, no differential susceptibilities in blood groups to malaria infection were found in India (J. A. Rowe et al., 2009; Tadesse & Tadesse, 2013). This could be attributed to the smaller sample size of this study and the universally low prevalence of blood group 'AB' compared to other blood groups.

P. falciparum is a parasite of blood and so induce haematological changes. It was observed generally that, haemoglobin concentration decreases with increasing parasitaemia explaining the feeding effects of parasite numbers on the haemoglobin molecule. Blood group 'A' patients were seen to be more "anaemic" than the other blood groups because they showed lowest haemoglobin level, hematocrit and RBC counts (Table 5) owing to their higher mean parasite density (52318 parasites / µl of blood). They are thus, likely to be more susceptible to malarial anaemia, a complication of malaria infection. This finding supports that of Fischer and Boone 48 but contrary to low hematocrit in blood group O in Vivax malaria (Resende et al., 2017). Platelets counts were significantly reduced in blood group 'A' and blood group 'AB'. This may be attributed to increased rosetting reported to be greater in non- O individuals than blood group 'O (Carlson & Wahlgren, 1992; Udomsangpetch, Thanikkul, Pukrittayakamee, & White, 1995) and immune mediated platelet destruction due to hyperparasitamia as reported in vivax malaria(S. Akhtar, Gumashta, Mahore, & Maimoon, 2012). Blood group antigens A and B are trisaccharides attached to variety of glycoproteins and glycolipid on the surface of erythrocytes. These antigens are absent on blood group O erythrocytes but are also found on platelets and to a lesser extent on von Willebrand Factor (vWF) and endothelial membrane (Cserti & Dzik, 2007). These trisaccharides are thought to act as receptors for rosetting on uninfected erythrocytes binding to parasite rosetting ligands such as Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) and sequesterin on infected red blood cells. Cserti and Dzik explained that infected RBCs adhere to uninfected RBCs via A or B antigens and also to endothelium either by binding to blood group antigens on endothelial cells or by binding to blood group antigens on platelets or vWF (Cserti & Dzik, 2007). Thereby causing clumping and sequestration of erythrocytes in small blood vessels as indicated by Barragan et al.(Barragan et al., 2000). Hence, binding of A and B antigens on platelets could be the cause of thrombocytopenia observed in blood group 'A' and 'AB'. Thus, reduction in the number of platelets in circulation could be as a result of platelets sequestration. Thrombocytopenia could also result from increased platelet destruction through an immune mechanism during when increased immune complexes generated by malarial antigens lead to sequestration of deformed platelets by macrophages in the spleen (M. N. Akhtar, Jamil, Amjad, Butt, & Farooq, 2005; Batool et al., 2019). This could also explain why 'A' blood groups showed thrombocytopenia because they showed the highest parasite densities among the blood groups. Platelet survival is reduced in severe P. falciparum malaria through increased utilization of platelets and reduced compensation by bone marrow production. The evidence is that, rosetting in blood group 'A' and 'AB' caused thrombocytopenia when compared to the higher frequency of normal platelet counts among 'O' blood groups (Table 7). Because blood type 'O' lacks A and B antigens on erythrocytes and platelets, which are not involved in binding with PfEMP1, hence, platelets are free in circulation, which is evidenced by the higher distribution of blood group 'O' patients with normal platelet counts. The iRBCs of blood group 'O' patients are not sequestered but are easily destroyed by phagocytes in circulation for elimination by the spleen. It can therefore be said that splenic destruction of iRBCs accounts for the degree of anemia observed among blood group 'O' while hyperparasitaemia and rosetting accounted for severe anemia and thrombocytopenia in non-O blood groups. Thrombocytopenia causes bleeding observed in severe P. falciparum malaria. Clumping of infected and non-infected red blood cells together with platelets may cause vaso-occlusion which induce clotting that may lead to death through disseminated intravascular coagulation (DIC), cerebral malaria, ischaemia and coma.

Conclusion

Hyperparasitaemia, thrombocytopenia and severe malarial anemia are all associated with severe malaria. Children under five years of age were significantly more vulnerable to developing severe malaria. Parasitemia was highest in 'A' blood groups while platelet count, hemoglobin together with hematocrit and red blood cell count were lowest in blood groups 'A' and 'AB'. This study therefore provides the evidences that individuals of blood group 'A' are more susceptible to malaria infection and at a comparably higher risk of developing severe malaria than other blood groups.

The study recommends based on these current findings that ABO blood group identification should be included in malaria test requests to further help in directing course of treatment and prioritization of patients for intensive care. Also, further studies should be conducted in Ghana on the impact of ABO blood groups on the risk of developing severe malaria recruiting a larger sample size to attain more statistical significance. Further studies should take a longer span including more laboratory markers and other blood polymorphisms that might have protective role against severe malaria.

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