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Assessing the association of severe malaria infection and ABO blood groups in northwestern Ethiopia

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ABSTRACT

Background & objectives: There is lack of adequate information on the association between severe malaria and some human genetic markers like ABO blood types. The study was undertaken to evaluate the association between severe malaria infection and ABO blood types among febrile patients attending Felegeselam Health Center, northwestern Ethiopia.

Methods: A total of 398 febrile patients were examined for malaria and tested for ABO blood groups in December 2011. The blood samples were collected by finger pricking, stained with Giemsa and slides were examined microscopically. ABO blood group was determined by agglutination test using agglutinating A and B monoclonal anti-sera together with parasite load count. Chi-square and ANOVA tests were used to assess the difference between frequencies and means, respectively.

Results: Out of 398 acute febrile patients, 201 (50.5%) were found to be infected with *Plasmodium* parasites. Of which 194 (48.74%) and 7 (1.76%) belong to *Plasmodium falciparum* and *P. vivax*, respectively. The distribution of ABO blood groups was O (46%), A (27.1%), B (23.1%) and AB (3.8%). The percentage of severe malaria with respect to blood group A, B, AB and O was found to be 40, 34.1, 14.3 and 5.1%, respectively. The association of severe malaria with non 'O' blood types was statistically significant ($\chi^2 = 31.246$, $p < 0.01$).

Interpretation & conclusion: The present findings indicate that individuals with blood groups A, B and AB are more susceptible for severe malaria infection than blood group O.

Key words ABO blood group; acute febrile illness; Ethiopia*; Plasmodium falciparum; P. vivax;* severe malaria

INTRODUCTION

The ABO blood grouping system consists of the A, B and H carbohydrate antigens produced by a series of enzymatic reactions catalyzed by glycosyltransferase and antibodies against these antigens. ABO blood grouping is based on the presence or absence of A and B blood group antigens on the surface of red blood cells (RBC) derived from inherited gene[1].

In clinical practice, ABO is the most important system for blood group compatibility and ABO antigen associations with infections[2]. The relationship between ABO and malaria was first suggested 40 years ago[3]. There is a hypothesis that *Plasmodium falciparum* malaria has shaped the distribution of ABO blood groups in humans[3].

Malaria has been the most important selective force on the human population, and several erythrocyte polymorphisms have evolved that confer the resistance to severe malaria. *Plasmodium falciparum* rosetting is reduced in blood group 'O' but the contribution of the ABO blood group system to protect severe malaria has received little consideration[4–5]. Most of the time individuals great parasite density in their peripheral blood have a higher risk of developing severe malarial disease. However, some individuals develop severe and even fatal malaria with a very low peripheral parasitaemia due to sequestration of the parasite in the deep tissue capillaries[6].

Many investigations have been conducted to find out whether or not ABO blood group antigens are associated with susceptibility, resistance, or severity of *P. falciparum* malaria. *Plasmodium falciparum* infections can be linked to the most severe forms of human malaria and virulence is associated with parasite reproduction rate and erythrocyte invasion mechanism[7].

However, some studies reported the absence of significant association between severe malaria and ABO antigens[8]. On the other hand, few studies have shown that high frequency of malaria episodes has been observed among blood group 'O' individuals as compared to other blood group individuals[9]. Large numbers of severe malaria cases were also reported among blood group 'A' individuals[10]. Furthermore, low parasitaemia was observed among uncomplicated malaria cases and blood group 'O' individuals[4, 10–11].

Variations in reports on the association of ABO blood groups and disease progression of *P. falciparum* malaria show the complexity of the interaction between the parasites and host immune responses. In addition, studies have shown the impact of other RBC polymorphisms including hemoglobin (Hb) abnormalities such as HbS, HbC and thalassemia[9, 12–14], deficiency in erythrocyte complement receptor (CR) or glucose-6-phosphate dehydrogenase on *P. falciparum* malaria susceptibility and severity[15–16] and lack of Duffy antigen[17] protection developing severe malaria in endemic areas. This makes it difficult to make a clear analysis on the association of severe malaria and ABO blood groups. Therefore, this study aims to assess the relationship of severe malaria infection with their ABO blood types among acute febrile patients who sought medical attention at Felegeselam Health Center, northwestern Ethiopia where *P. falciparum* is the dominant species.

MATERIAL & METHODS

Study area

A cross-sectional study was conducted at Felegeselam Health Center, northwestren Ethiopia, to assess the association of ABO blood group antigens with severe malaria in December 2011. Felegeselam is located at about 570 km away from Addis Ababa. The area has an elevation between 1000 and 1050 m above sea level. The study area has annual temperature ranging from 28–43°C with annual rainfall of 1050 mm. The total population of the study area is 50,307; of which 26,984 are males and 25,323 are females[18]. The study population comprised of febrile outpatients who sought medical attention at Felegeselam Health Center in December 2011. A total of 398 acute febrile patients were selected as study participants, excluding individuals who took antimalarial drugs within two weeks before the blood test, patients who were not living permanently in the area and who refused to participate in the study.

Clinical and laboratory diagnosis

Health staff members were trained about how to collect sample and explanation was given prior to the data collection. Capillary blood was collected by finger pricking using 70% alcohol and sterile disposable lancet. Thick and thin films were prepared on the same slide. Thin films were fixed with methanol. The blood films were stained with 6% Giemsa for 10 min. Finally, the films were examined under an oil immersion microscope objective (100×). Parasitaemia was determined for febrile patients who tested positive for *P. falciparum* and *P. vivax* by counting the number of parasites (asexual forms only) against 200 WBCs using hand tally counters. Then, the number of parasites per microliter (µl) of blood was calculated. If the parasitic load was greater than 10,000 parasites/µl of blood, the infection was said to be severe. On the other hand, the infection was considered as uncomplicated if the parasite load was lesser than 10,000 parasites/µl. This can be used to give a more accurate figure with appropriate adjustment of the multiplication factor[19]. In addition, the blood group of the study participants was determined by direct slide method, using agglutinating A and B monoclonal Eryclone® antisera together with the former procedures.

Data analysis

Data were entered into Microsoft Excel, exported to SPSS version 16 and analyzed. Chi-square test was used to assess the difference between frequencies (the associations between blood groups and *P. falciparum* malaria cases). ANOVA was used to test the difference between parasitaemia means. Observed difference was considered to be significant for $p < 0.05$.

Ethical approval

The study obtained ethical clearance from Microbiology, Immunology and Parasitology Department Ethical Review Committee, College of Health Science, Addis Ababa University and from the Pawe Woreda Health Office. Written informed consent was obtained from every study participant and guardians in case of children. Malaria positive cases were treated with antimalarial drugs based on the current national treatment guidelines of Ethiopia.

RESULTS

Malaria infection

Out of 398 acute febrile cases who visited Felegeselam Health Center for medical attention, 201 (50.5%) were found to be infected with *Plasmodium* parasites as determined by microscopy. Of which *P. falciparum* and *P. vivax* accounted for 194 (48.7%) and 7 (1.8%), respectively (Table 1). The prevalence of malaria

Table 1. Prevalence of malaria by age and sex among acute febrile cases

Age (yr)/ Sex	No. of cases examined	*Pf*	*Pv*	Total	χ^2, *p*-value
1–4	52	23 (44.2)	1 (1.9)	28 (53.8)	1.26, 0.87
5–17	166	86 (51.8)	3 (1.8)	77 (46.4)	
≥ 18	180	85 (47.2)	3 (1.7)	92 (51.1)	
M	176	83 (47.2)	3 (1.7)	90 (51.1)	0.22, 0.896
F	222	111 (50)	4 (1.8)	107 (48.2)	
Total	398	194 (48.7)	7 (1.8)	197 (49.5)	

Figures in parentheses indicate percentages.

was highest among 5 to 17 yr as compared with under five and older age groups but the difference was not significant (p >0.05). Similarly, the prevalence was higher among females (51.8%) than males (48.9%) and this difference was not statistically significant (χ^2=0.22, p >0.05) (Table 1).

ABO blood groups and malaria infection

All febrile patients examined for malaria were also tested for ABO blood groups. Accordingly, 46, 27.1, 23.1 and 3.8% were found to be blood types of O, A, B and AB (Table 2). All of them were Rh positive. There were 200 volunteer blood donors used as a control to assess the distribution of blood types among the community. The donors who lived in that specific community and donated blood in Pawe Hospital were selected based on their address. The distribution of ABO phenotypes among blood donors was O (60%), A (26%), B (12.5%) and AB (1.5%). Only one blood donor with blood group O was Rh (–)ve.

In general, malaria infection was observed with the highest proportion (53.6%) among individuals with O blood group, followed by those with blood group A (50.9%) (Table 2). In all blood groups of acute febrile illness (AFI) cases that have body temperature above normal, the prevalence of *P. falciparum* malarial infection was higher than that of *P. vivax* infection. Prevalence of *P. falciparum* infection was high (51.9%) among individuals with O blood group, followed by individuals with blood group A (50.9%) (Table 2). The percentage of *P. falciparum* infection among blood groups A, B, AB and O was 50.9, 41.3, 40 and 51.9%, respectively (Table 2). The proportion of A or O was higher in individuals with *P. falciparum* infection compared with non-infected individuals (Table 2) who were screened. *Plasmodium falciparum* infection did not show significant association (p >0.05) with the age among different blood groups.

Table 2. Prevalence of malaria among the study participants based on their blood types

Blood group	No. of cases examined	*Pf* (%)	*Pv* (%)	Non-infected (%)	Total (%)	χ^2, *p*-value
A	108	55 (50.9)	0 (0)	53 (49.1)	55 (50.9)	7.96, 0.24
B	92	38 (41.3)	3 (3.3)	51 (55.4)	41 (44.6)	
AB	15	6 (40)	1 (6.7)	8 (53.3)	7 (46.7)	
O	183	95 (51.9)	3 (1.6)	85 (46.4)	98 (53.6)	
Total	398	194 (48.7)	7 (1.8)	197 (49.5)	201 (50.5)	

Figures in parentheses indicate percentages.

ABO blood type and P. falciparum parasitaemia

There were 42 severe malaria (parasitic load >10,000 parasites/µl) and 159 uncomplicated malaria (parasitic load <10,000 parasites/µl) cases isolated in this study. Parasitic load was used as a marker of differentiation between severe and uncomplicated malaria. Prevalence of severe malaria among blood groups A, B, AB and O was 39.3, 35, 14.3 and 5.1%, respectively (Table 3). All severe malaria cases were caused by *P. falciparum*. In general, severe malaria infection showed significant association (χ^2= 30.54, p <0.01) with non 'O' blood groups. The highest proportion of severe malaria was observed among participants with blood group A (39.3%) but the least proportion was found to be in O blood group (5.1%) (Table 3).

The median parasitic count for all positive, uncomplicated malaria and severe malaria cases were 82, 54 and 396 parasites/µl, respectively. Similarly, the median parasitic count for each blood group A, B, AB and O was 128.5, 140, 84 and 56 parasites/µl, respectively. On the other hand, the mean parasitic count for all positive, mild malaria and severe malaria cases were 144, 169.2 and 539.4 parasites/µl of blood, respectively. The mean parasitic count for each blood group A, B, AB and O was 228.9, 125, 108.7 and 55.7 parasites/µl of blood, respectively.

There were also three severe malaria cases whose parasitic load was greater than 100,000 parasites/µl of blood and two of them had the blood type 'A' where as one had blood group 'O'. The frequencies of A, B, AB and O blood groups in uncomplicated malaria cases was 21.4, 16.5, 3.8 and 58.9%, respectively (Table 3). The prevalence of uncomplicated malaria among blood groups A, B, AB and O was 60.7, 65, 85.7 and 94.9%, respectively (Table 3). This indicated that most uncomplicated malaria cases had blood group O followed by blood group AB.

The chance of having severe malaria infection in patients with blood groups A, B and AB was 12 (χ^2= 28.801,

Table 3. Percentage distribution of malaria characters based on their ABO blood types

Malaria characteristics	Blood group types				Total	χ^2, *p*-value
	A	B	AB	O		
Severe	22 (39.3)	14 (35)	1 (14.3)	5 (5.1)	42	30.54, 0.0
Uncomplicated	34 (21.4)	26 (16.4)	6 (3.8)	93 (58.5)	159	
Total	56 (27.9)	40 (19.9)	7 (3.5)	98 (48.6)	201	

Figures in parentheses indicate percentages.

$p = 0.0$, OR=12.04, 95% confidence interval (CI) = 4.222–34.306), 10 ($\chi^2 = 21.387$, $p = 0$, OR = 10.02, 95% CI = 3.301–30.385) and 3.1 ($\chi^2 = 2.284$, $p = 0.335$, OR = 3.10, 95% CI = 0.311–30.929) times higher than individuals showing blood group O phenotypes, respectively.

The parasite load for all malaria positive cases was ≥ 100 parasites/µl. About 21.8% of *P. falciparum* infected study participants of blood group A, 12.2% of blood group B, 28.6% of blood group AB and 21.4% blood group O had shown parasitic load of ≤ 1000 parasites/µl of blood. On the other hand, 78.2% (43/55), 85.4% (35/41), 71.4% (5/7) and 77.6% (76/98) *P. falciparum* infected individuals of blood group A, B, AB and O, respectively had parasite density of ≥1000 parasites/µl of blood. Only 3.6% (2/55) and 1% (1/98) of *P. falciparum* infected patients with blood group A and O respectively had a parasite density of ≥100,000 parasites/µl of blood.

DISCUSSION

In the present study, high proportion of blood group O (46%) phenotype was observed among the study participants. This agrees with some previous reports (51.3%) in southern Ethiopia[10], (45.7%) in Awash, Metehara and Ziway areas of Ethiopia[11], (55.83%) in Amazon region of Brazil[20] and 54.4% in Zimbabwe[21] which showed high frequency of group 'O' than non 'O' phenotypes in tropical regions where malaria is prevalent.

Significantly higher proportion of individuals with blood groups A, B and AB were found to have severe *P. falciparum* infection than blood group O. This was consistent with previous reports[4, 10–11, 21] which emphasised that non O blood groups were more susceptible to *P. falciparum* infection than those with O blood group.

The lowest mean parasitaemia was observed among individuals with blood group O as compared to blood groups A, B and AB in this study. The results of the present study is in agreement with reports from southern Ethiopia[10] and Awash, Metehara and Ziway areas of Ethiopia[11] which showed that blood group O had the lowest mean parasitaemia.

In contrast to the present study, previous reports indicated high prevalence of blood group A and low prevalence of blood group O phenotypes in colder regions where malaria has not been endemic[22]. Hence, the present finding seems to confirm the hypothesis about a selective survival evolutionary advantage of *P. falciparum* infection in blood group O compared with non O blood groups in malaria endemic areas.

The present study revealed that there was a difference in the frequency of ABO blood groups between controls and those with uncomplicated malaria cases. Similarly, individuals with severe malaria also had significantly higher parasite count than patients with uncomplicated malaria. This study is consistent with previous reports[5, 10]. In contrast to the present study, other authors reported the absence of significant difference in the frequency of parasitic load between severe and uncomplicated malaria cases[11]. However, this finding can be explained by the fact that in severe falciparum malaria infection, parasitized erythrocytes at schizont stage are known to be sequestered in deep tissue capillaries and may result in low parasite count in the peripheral blood[23]. In the present study, the severity of infection with falciparum malaria may have been greater.

The mechanism by which blood group 'A' promote susceptibility and blood group 'O' confers a relative protective effect against severe malaria is not well understood; however, different studies have done on the basis of rosette formation. Several reports supported that blood group A stands for a risk factor for high chance of rosetting, which is usually characterized by high *P. falciparum* parasitaemia during malaria infection and a reducing effect of blood group 'O' on rosetting[5, 24–25]. The presence of several glycosylated intracellular adhesion molecules and chondroitin sulfate A[26], CD36[27] and Duffy antigen[17] in blood group 'A' cells promote a high chance of binding with the rosette-forming surface molecules of *P. falciparum* and leads to development of severe malaria.

The present study only employed parasitic load as a laboratory marker to determine the association of ABO blood groups and severe malaria. The study also did not consider factors like hemoglobin S, hemoglobin C, iron status of the host, place of residence of the study population which could affect the nature of *P. falciparum* infection among the study population. Nevertheless, the findings indicated that severe malaria is associated with blood groups and individuals of blood group A, B and AB are more susceptible to severe malaria infection as compared with individuals with the blood type O. Further, indepth studies are needed to clearly assess the role of ABO blood groups in severe malaria cases to minimize mortality and morbidity of malaria in endemic areas.

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