J Vector Borne Dis 48, March 2011, pp. 7–11

Research Articles

ABO blood groups and malaria related clinical outcome

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ABSTRACT

Objectives: The study was undertaken to correlate the blood groups and clinical presentations in malaria patients and to understand the differential host susceptibility in malaria.

Methods: From October 2007 to September 2008, malaria positive patients' samples were evaluated in this study. Hemoglobin, total leukocyte count, and platelet count of each patient were done on an automated cell counter. After determining the blood groups, malarial species and the severity of clinical course were correlated.

Results: A total of 100 patients were included in the study, of which 63 cases were positive for *Plasmodium falciparum* and 37 cases were positive for *P. vivax* infection and 11 patients had mixed infection. The results of the blood groups showed 22 – 'A' group, 42 – 'B' group, 35 – 'O' group and 1 was 'AB' group. When the clinical courses between different groups were compared using the following parameters for severe infection—a parasitic load of >10/1000 RBCs, severe anemia with hemoglobin < 6 g%, platelet count of <10,000/mm^3, hepato or splenomegaly or clinical signs of severe malaria such as fever >101^{o}F and other organ involvement, it was observed that 'O' group had an advantage over other the groups. The difference in rosetting ability between red blood cells of different 'ABO' blood groups with a diminished rosetting potential in blood group 'O' red blood cells was due to the differential host susceptibility.

Conclusion: 'O' group had an advantage over the other three blood groups. Based on literature and the results of this study, the diminished rosetting potential in blood group 'O' red blood cells is suggested as the basis for the differential host susceptibility.

Key words Blood groups; host susceptibility; malaria

INTRODUCTION

The association of genetic markers with malaria has been the subject of numerous investigations, since the protection afforded by sickle-cell hemoglobin against infection by falciparum malaria parasite. A broad range of available evidence suggests that the origin, distribution and relative proportion of ABO blood groups in humans may have been directly influenced by selective genetic pressure from *Plasmodium falciparum* infection[1]. Clinical reports of ABO blood groups and *P. falciparum* infection, reveals a correlation between disease severity and ABO groups. However, several studies undertaken have been unable to link ABO blood groups to the incidence of malaria or to the repeat attacks of malaria[2,3].

Recent studies of the pathogenesis of malaria have shown that parasite triggered red blood cell rosette formation is associated with the severity of clinical disease and malaria[4,5]. Rosetting was established as a *P. falciparum* virulence factor, the expression of which is modified by a variety of host factors. Anti-rosetting activity, presumably mediated by antibodies, was found in sera from patients in malaria endemic areas, and it was demonstrated that such activity was more abundant in individuals with uncomplicated malaria than in those with cerebral disease, suggesting that humoral immunity protects against rosette formation *in vivo*. Erythrocytes from individuals with sickle-cell trait, α- and β-thalassemia trait or with HbE formed smaller and weaker rosettes than did normal HbAA red blood cells. Recently, even *P. vivax* infection has been reported with clinical severity. There is a paucity of hospital-based, comparative studies to investigate the relationship between blood group types and severity of malarial infections. This study was undertaken to fill up the lacunae in understanding the relationship between blood group phenotypes and malaria in a hospital environment. The objectives of the present study were: (i) to screen and estimate the incidence of malaria in a hospital environment; and (ii) to correlate the blood groups and the clinical presentations including outcome in malaria patients.

MATERIAL & METHODS

This study was conducted during period from October 2007 to September 2008 on blood samples from patients presented with malaria and confirmed as positive in St. John's Medical College Hospital. The diagnosis was based on peripheral smear and quantitative buffy coat method (QBC). Blood group was determined by forward and reverse method. Hematological parameters which included hemoglobin, total leukocyte count and platelet count of each patient were done on automated cell counter. The demographic details of the patients and clinical details were obtained from case records of the patients. Malarial species and the severity of clinical course were correlated with blood groups.

The clinical course between the different groups was compared using the following parameters for severe infection:

- a parasitic load of >10/1000 RBCs
- severe anemia with hemoglobin <6 g%
- platelet count of <10,000/mm^3
- hepato or splenomegaly or
- clinical signs of severe malaria such as fever >101^o F and
- other organ involvement

RESULTS

Among 11,552 smears screened during this period, 100 patients turned out to be positive and all the 100 samples were evaluated by both QBC method and thick and thin Leishman stained smears. The species of malarial parasites were *P. falciparum* (*Pf*) (63%); *P. vivax* (*Pv*) (37%) and 11 patients had mixed infection of both *Pv* and *Pf*.

Among 100 patients, 22 were 'A' positive, 42 'B' positive, 35 'O' positive and 1 'AB' positive. Irrespective of the blood group, the number of patients affected by *P. falciparum* was more than *P. vivax*. The only patient who had AB positive group had *P. falciparum* and was hospitalized as Intensive Care Unit patient. Among the patients who had mixed infection, 6 had 'A' group, 3 'B' group and 2 'O' group.

Malaria affected all age groups and the age ranged from 1 to 71 yr old (M : F 8 : 2). The number of adults affected was more (n = 82) than the children (n = 18). Among the adults, the prevalence was more in the younger age group. Among 18 in the pediatric age group, 6 were treated in ICU, while the rest were treated as inpatients. One year old child, though affected by *Pv,* was in ICU as the hemoglobin was low (4.9 g%).

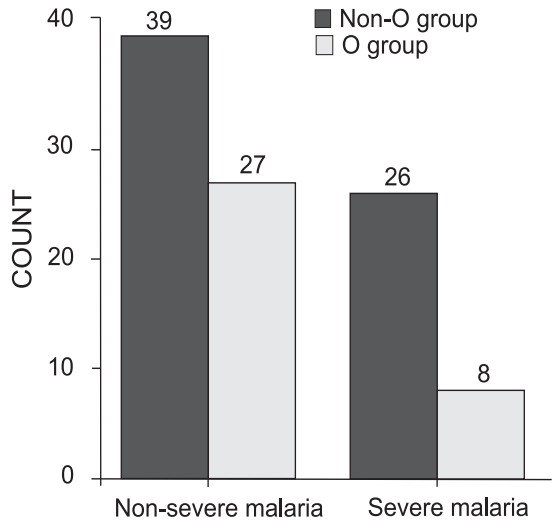
Although the majority of them were inpatients (98%) and only 2 were managed as outpatients, both of them were affected by *P. falciparum.* Among 98% hospitalized patients, 37% were affected by *P. vivax* and 61% by *P. falciparum.* When the clinical severity was compared with blood groups, it was observed that 'O' group had an advantage (Fig. 1). The laboratory investigations are summarized in Table 1.

DISCUSSION

Malaria has been known since antiquity. Much new information has emerged since a relationship between ABO and malaria was first suggested >40 years ago[6]. However, the correlation of severity of malarial infection to the patient's blood group has been of recent interest in the quest for the answers to the factors influencing clinical course of the disease. The observation by Miller *et al*[7] that human erythrocytes lacking the Duffy blood group antigens are refractory to invasion by *P. vivax* parasites indicate the usefulness of studying the association of blood group with malaria. In the Indian scenario, the literature relating to malaria and the blood groups are sparse and have mixed results. Thakur and Verma[8] in their study, concluded that ABO blood groups do not show differen-

Table 1. Summary of the salient laboratory features and blood group phenotype

Details	Parasite species	'A'	'B'	'O'
Number affected	*Pf*	14	27	21
	Pv	8	15	14
Adult	*Pf*	12	23	14
	Pv	8	13	7
Hb range (g%)	*Pf*	5.2–15.2	6.7–13.9	6.5–14.6
	Pv	7.6–13.5	8.9–14.1	7.4–14.4
Total WBC count (per mm^3)	*Pf*	2300–24,000	2900–28,700	3500–11,400
	Pv	3200–9800	3200–6400	2800–10,000
Platelet count (per mm^3)	*Pf*	10,000–1.4 lakh	9000–2.6 lakh	13,000–2.8 lakh
	Pv	35,000–1.3 lakh	29,000–1.7 lakh	13,000–1.7 lakh



Cross table

Severity of malaria*	Blood group		Total
	Non-O Gr	O Gr	
Non-severe malaria	39	27	66
Severe malaria	26	8	34
Total	65	35	100

*Severe malaria is defined as: Hyperparasitaemia >10% parasitaemia; severe anaemia with Hb <6 g%; clinical signs of severe malaria with both hepatomegaly, splenomegaly or thrombocytopenia

Chi-square tests

	df	Asymp. sig (2-sided)	Exact sig (2-sided)	Exact sig (1-sided)
Pearson chi-square	1	0.062		
Continuity correlation	1	0.099		
Likelihood ratio	1	0.057		
Fisher's Exact			0.080	0.048

Fig. 1: Comparison of O and non-O blood group with clinical severity. By applying chi-square test, it was observed that proportion of O group was 76% (26) compared to 24% (8) in Non-O group which was significant ($p<0.05$), suggesting that O group confers. When it was further calculated, the severity was less in A than in B group.

tial susceptibility to malaria. Joshi *et al*[9] reported no correlation between ABO blood groups and malaria in Delhi. Other studies indicated a possible relationship[10]. The present study was attempted to correlate the blood groups and the clinical presentations in malaria patients.

Among the adults, this study showed different age groups had no significant correlation with incidence of malaria. All age groups and both genders were affected. However, the course of the disease in children was severe than that among adults. In a cross-sectional study by Eli *et al*[11], influence of age and other factors that affect clinical outcome of *P. falciparum* malaria in non-immune patients was evaluated. In their study of 135 patients with *P. falciparum* malaria, 84 (62%) were <40 yr old, and only 5% developed severe malaria, compared with 18% who were ≥40 yr old (odds ratio, 4.29); moreover, all deaths occurred in the latter group. Male subjects did not differ from female subjects with regard to severity of disease. Similarly, in this study, the severity did not differ between males and females. Red blood cells of children with severe malaria-associated anemia (SMA) have acquired deficiencies in the complement regulatory proteins complement receptor 1 (CR1, CD35) and decay accelerating factor (DAF, CD55). Deficiencies in red blood cell CR1 and CD55 in children with SMA were accompanied by a marked decline in immune complex binding capacity and increased C3b deposition *in vivo* and *ex vivo*. Importantly, these changes were specific because they were not seen in red blood cells of children with cerebral malaria or their controls. These data suggest that the decline in red blood cell CR1 and CD55 seen in children with SMA were of physiologic significance and may predispose erythrocytes to complement-mediated damage and phagocytosis *in vivo*.

All patients presented with fever and chills. The initial presentation of fever and chills was present irrespective of the blood groups. However, the number of ICU admissions was more in 'B' group than other groups, and also associated with *P. falciparum* infection. Among the hospitalized adult patients, 4% had hemoglobin below 6 g/dl including both *P. falciparum* and *P. vivax* infections. Though the predominant population was adults, in the children who were affected, hemoglobin ranged from 4.9 to 13.8 g%. The reason for the anemia was because macrophages not only clear infected erythrocytes but also phagocytes destroyed uninfected red blood cells during malaria infections. It has been observed in a prospective study conducted in Orissa that the clinical features in Indian children differed from those reported in most studies that involved an African population. Multiple organ dysfunctions emerged as an important presenting feature and a new predictor of death in childhood malaria though anemia causes morbidity[12] with malaria. Anemia was also seen with *P. vivax* infection. A one year old baby with *P. vivax* had a hemoglobin value of 4.9 g% was treated in ICU indicating other factors also played a role.

Leucocytosis was observed only in 5% of the patients, though malaria is considered to be an infection. Areas that cannot afford even simple laboratory diagnostic tests often use only a history of fever which is subjective as the indication for malaria. Using Giemsa stained blood smears

from children in Malawi, one study showed that unnecessary treatment for malaria was significantly decreased when clinical predictors including temperature, pallor and splenomegaly were used as treatment indicators[13] (sensitivity increased from 21 to 41%).

Platelet count was decreased in 80% of patients. In *P. falciparum* infection platelet count dropped to <20,000/mm^3 irrespective of the blood group. When correlated with parasitic load, it did not show any correlation. It has been noted that the presence of thrombocytopenia in an endemic area should alert malaria infection. Both non-immunological destruction and immune mechanisms are implicated in the pathogenesis of thrombocytopenia.

- Specific platelet-associated IgG antibodies that bind directly to the malarial antigen in the platelets play a role in the lysis of platelets.
- Elevated M-CSF levels in malaria, by increasing macrophage activity may mediate platelet destruction.
- Oxidative stress damage-decreased platelet superoxide-dismutase and glutathione peroxidase activity and high platelet lipid peroxidation.

Clinical severity, rather than incidence or prevalence of detectable parasitemia, is a more relevant outcome to assess ABO group and survival. Studies reporting clinical features such as cerebral malaria carry more weight than those reporting only laboratory markers such as percent parasitemia, because the latter does not always predict survival. Among those with a well-developed humoral immunity, there is little correlation between high circulating parasitemia and severity of illness. *Plasmodium falciparum* infection increases the serum levels of IgM and IgG antibodies, and also IgE in individuals living in endemic areas. The association of high anti PF IgE levels with a reduced risk of developing clinical malaria suggests the involvement of IgE in protection[14].

In 1998, Fischer and Boone[15] reported favourable outcomes for group 'O' individuals compared with group 'A' among 489 patients in Zimbabwe with *P. falciparum* malaria. They studied 209 outpatients and 280 severely ill inpatients. Coma was 3-times more common among group 'A' individuals compared with non-A persons (9 of 104 group 'A' *vs* 11 of 385 with non-A blood, $p = 0.008$; odds ratio, 3.6). Because patients with coma are at a higher risk for death, this study supports the hypothesis that group 'O' individuals may have a survival advantage in severe malaria. However, the sample size was insufficient to observe an effect of 'ABO' group on survival[15].

Lell *et al*[16] compared 100 cases of severe *P. falciparum* malaria with 100 cases of mild malaria in Gabon. Severe malaria was defined as either hyperparasitemia with $>0.25\times10^{12}$ infected red blood cells (RBCs) per L or >10% parasitemia; or severe anemia with Hb <5 g/L; or clinical signs of severe malaria. Mild malaria cases had minimal laboratory abnormalities and were treated as outpatients. The ratio of group 'A' to group 'O' in patients with severe malaria was 0.50, but was only 0.17 among those with mild malaria (3-fold relative risk). Among all group 'A' individuals, 71% had severe malaria and only 29% mild malaria ($p<0.01$). In contrast, among all group 'O' cases, 46% had severe malaria and 54% mild malaria ($p<0.21$). In Sri Lanka, Pathirana *et al*[5] assessed 243 adult cases (mean age, 29.8 yr) of *P. falciparum* malaria (163 mild, 80 severe) compared with 65 control patients with other infections[5]. The proportion of group 'O' in mild malaria cases was 48%, but only 24% in severe malaria cases. The distribution of ABO groups was significantly different in severe malaria syndromes compared with uncomplicated malaria or the control population ($p<0.001$). Once again, a case of severe malaria was nearly 3-times as likely to be in group 'A' as O ($p = 0.005$). This study provided the strongest statistical evidence of an association between ABO and disease severity in *P. falciparum* infection.

Udomsangpetch *et al*[17] showed a strong association between rosette formation and ABO blood group, with group 'A' and group 'B' RBCs (A > B) forming rosettes more than group 'O' cells in each of 8 tested strains ($p < 0.001$). Rowe *et al*[18] confirmed that among 154 isolates, RBCs from group 'O' patients rosetted less (median rosette frequency, 2%; range, 0–45%) than those from group 'A' (median, 7%; range, 0–82%; $p < 0.01$) or group 'AB' (median, 11%; range, 0–94%; $p<0.03$). Chotivanich *et al*[19] found the highest relative rosette ratios *in vitro* among RBCs from healthy donors who were group 'A' (2.7 ± 1.4) and 'B' (2.4 ± 1.1) compared with group 'O' (1.6 ± 0.7; $p = 0.05$). Most recently, Barragan *et al*[20] confirmed that group 'A' targets formed the strongest rosettes. In addition, they reported that RBCs of group 'A' enzymatically converted to RBCs, of group 'O' and Bombay RBCs rosetted minimally and to the same degree. Thus, potentiation of rosetting appears specific (but not exclusive) to A and B antigens.

The adherence of parasitized RBCs to other cells is central to the pathophysiology of severe malaria syndromes including cerebral malaria, respiratory failure, multiorgan failure, and death. Parasitized RBCs adhere to the vasculature through a process termed "sequestration," closely mimicking inflammatory leukocyte attachment. Furthermore, half of infected RBC isolates form occlusive intravascular aggregates, which consist not only of infected RBCs bound to each other "autoagglutinates" but also

infected RBCs bound to uninfected RBCs "homotypic RBC rosettes" and/or to platelets "heterotypic RBC rosettes". Sequestration and rosette formation impair blood flow, causing tissue ischemia and cell death. Not surprisingly, *in vitro* rosetting is more pronounced in parasite strains derived from patients with severe disease, particularly in cases of cerebral malaria. In addition, host ABO group may affect other aspects of malaria pathogenesis.

Malaria is known to have affected many erythrocyte genes, including those concerned with globin synthesis, membrane proteins, and RBC enzymes. Given the importance of RBCs in malaria, an influence on genes encoding the most abundant antigens on the RBC membrane such as blood groups is expected . It is also difficult to dissociate the role played by ABO sugars from the contribution of other glycosylated adhesion molecules. The present study including both *P. falciparum* and *P. vivax* infections provides supporting evidence in favour of an effect of ABO group on disease severity as 'O' group provides advantage over non 'O' groups.

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Received: 8 June 2010 *Accepted in revised form:* 13 October 2010