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Original Article

Association between blood group and susceptibility to malaria and its effects on platelets, TLC, and Hb

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Abstract

Introduction: According to the World Health Organization, the estimated number of malaria cases in Pakistan is about 1.5 million. Hematological variables like platelets, total leukocyte count (TLC), and hemoglobin (Hb) need to be evaluated to diagnose malaria in suspects. This study aimed to investigate the association between blood group and susceptibility to malaria and effects on platelets, TLC, and Hb. Methodology: This was a case-control study with a sample size of 446, of which 224 were malarial cases and 222 were controls. A designated questionnaire was developed to know age, gender, malarial strain, Hb, TLC, platelets, and blood group. Results: Of 224 malarial cases, 213 were *P. vivax*, and 11 were *P. falciparum*. There were 58 patients with blood group A, 72 with group B, 69 were O and 23 were AB. There was no significant difference in the blood group of controls compared to malarial patients ($p > 0.05$). Mean Hb level was 11.5mg/dL in malaria patients and 12.5mg/dL in controls. There was significant difference ($p<0.01$) in the mean platelet count in malarial (11,7000/µL) and control (24,5000/µL) patients. All blood groups showed similar falls in Hb and platelet levels, showing no significant difference among blood groups ($p = 0.79$ and $p = 0.52$, respectively). TLC was not significant between malarial and control groups ($p = 0.072$). Males were two times susceptible to malaria. Conclusions: There was no significant association between the type of blood group and susceptibility to malaria or developing anemia or thrombocytopenia.

Key words: Malaria; hemoglobin; platelet.

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Introduction

Even in today's industrialized and modern era, malaria is a threat to over two billion people – approximately 40% of the world's population – residing in hundred different countries [1]. Its severity in the African continent can be judged by the fact that out of all the endemic areas, 90% of the clinical burden lays there [2]. Pakistan falls in the World Health Organization (WHO) category 3 with Afghanistan, Djibouti, Somalia, South Sudan, Sudan, and Yemen. These countries account for 95% of the total regional malaria burden, with an estimated 1.5 million cases annually [3]. In 1961, a nationwide campaign nearly eradicated malaria, but financial and administrative setbacks led to a resurgence in the 1970s. In 1975, the strategy switched from eradication to control when malaria control interventions integrated into the primary healthcare system [4]. In a 2004 study conducted in the Balochistan area of Musakhel and Loralai, of 7,899 subjects tested, 28.8% were malaria positive [5]. Another study conducted in Sindh in 2006 concluded that malaria was endemic throughout the year, with greater risk in coastal areas in rainy and post-rainy seasons [6].

Malaria is usually associated with various degrees of reduced blood count and mild to moderate thrombocytopenia, the cause of which is poorly understood [7]. Much new information has emerged since a relationship between ABO and malaria was first suggested more than 50 years ago [8]. However, the correlation of severity of malarial infection to the patient's blood group has been of recent interest. The observation by Miller *et al.* [9] 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. No study of this sort has been conducted in Pakistan, but similar studies have been undertaken in India, Sri Lanka, and other countries around the world, with mixed results. Knowledge of risk factors is vital for the prevention of malaria, and blood group is one of the important factors along with gender. No study is currently available that analyzes the relationship between blood group and contracting malaria in our

setting, and there is a need to investigate this association.

Methodology

This was a case-control study conducted with the collaboration of Dr. Essa's Laboratory in three zones of Karachi: District Central (North Nazimabad), District East (Gulshan e Iqbal), and District South (Saddar). Cases and controls were sought in these districts from the period October 2012 to December 2012. The method of sampling used was convenience sampling, and cases and their controls were collected for the above-mentioned time period. There were 224 cases and 222 controls. The selection of cases was on the basis of malaria status; cases were positive and controls were negative. The following parameters in all the cases and controls were recorded, based on a questionnaire: age, gender, locality, malarial strain, hemoglobin (Hb), total leukocyte count (TLC), platelets, and blood group. Age was divided into five groups: infants 0–1; children 2–12; adolescents 13–18; adults 19–55; and old adults >55. The reported Hb, TLC, and platelets were noted and then categorized accordingly. Hb for males was categorized as low (<13.5 mg/dL), normal (13.5-17.5mg/dL), and increased (>17.5mg/dL). For females, Hb was categorized as low (<12.0 mg/dL), normal (12.0-15.5mg/dL), and increased (>15.5mg/dL). Low TLC was taken as $<4\times10^3/mm^3$, normal as $4\text{-}11\times10^3$, and increased as $>11\times10^3$. Low platelet count was taken as $<1.5\times10^5/mm^3$, normal as $1.5\text{-}4\times10^5$, and increased as $>4\times10^5$.The inclusion criteria for cases was malaria positivity, and that for controls was presenting with flu/fever in the malarial season, but with a negative malaria test. The exclusion criterion was patients who had diseases other than malaria. As a first step of questionnaire development, literature on the topic was reviewed in detail. Subsequently, questions were designed and reviewed by an expert in medical research and a statistician. Later, the data collection tool was pre-tested through 50 patients other than those taken in the sample. Corrections were made to the first version of the questionnaire and the new version was again reviewed by the experts before being used for data collection in the main study. Data was double entered and analyzed using Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, USA). Descriptive statistics, including frequencies, means, and standard deviations (SD) were calculated. To interpret the data, p value <0.05 was taken as level of statistical significance. Chi-squared was used to assess the significance of difference in age, gender, platelets, Hb, and blood group. Ethical considerations such as confidentiality, participants' right to refuse, and informed consent were noted.

Results

Of the 446 individuals included in the study, 244 were malarial patients while 222 where non-malarial patients. The mean age of participants was 31.6 (±17.4) years, with 56.8% males and 43.2% females. Male malarial cases comprised 61.3% of all the males, while female malarial cases comprised 41.1%. The association of gender with malaria was significant ($p<0.001$) Malarial females were more prone to developing low Hb and platelet counts than were males, whereas the TLCs remained normal in the majority of the two populations (Table 1). In the malarial cases as well as controls, the proportion of blood group O was highest, about one-third of the total population ($p = 0.229$) (Figure 1). Within the malarial cases, the proportion of blood groups was A=25%, B=30.2%, and AB=11.4%, with controls showing a relatively similar percentage; thus, this was not statistically significant ($p = 0.246$) (Table 2).

Table 1. Association of gender with malaria, Hb, TLC, and platelet count

Variables		Sex (case-control malaria)			
		Male		Female	
		Count	%	Count	%
Hb level	Low	117	72.2%	63	77.8%
	Normal	44	27.2%	16	19.8%
	Above normal	1	0.6%	2	2.5%
Platelets	Low	130	80.2%	58	71.6%
	Normal	30	18.5%	21	25.9%
	Above normal	2	1.2%	2	2.5%
TLC	Low	37	22.8%	19	23.5%
	Normal	121	74.7%	58	71.6%
	Above normal	4	2.5%	4	4.9%

Hb: hemoglobin; TLC: total leukocyte count

Table 2. Association of blood group with malaria, Hb, TLC, and platelet count.

Variables		Blood group							
		A		B		O		AB	
		Count	%	Count	%	Count	%	Count	%
Hb level	Low	51	71.8%	50	67.6%	60	81.1%	19	86.4%
	Normal	20	28.2%	22	29.7%	14	18.9%	2	9.1%
	Above normal	0	0%	2	2.7%	0	0%	1	4.5%
Platelets	Low	58	81.7%	58	77.3%	55	74.3%	16	72.7%
	Normal	12	16.9%	15	20.0%	18	24.3%	6	27.3%
	Above normal	1	1.4%	2	2.7%	1	1.4%	0	0%
TLC	Low	18	25.4%	20	26.7%	12	16.2%	6	27.3%
	Normal	52	73.2%	50	66.7%	61	82.4%	15	68.2%
	Above normal	1	1.4%	5	6.7%	1	1.4%	1	4.5%

Hb: hemoglobin; TLC: total leukocyte count.

In malarial cases, Hb, platelet count, and TLC were significantly reduced ($p < 0.01$), while any change in the TLC was not significant (Table 3). Of 303 anemic patients, 59.4% were malaria cases while 40.6% were controls. A total of 206 patients were thrombocytopenic, of which 91.7% were malarial cases and 8.3% were controls. The mean Hb value among malarial cases was 11.5mg/dL, while non-malarial cases had a mean HB level of 12.5mg/dL ($p <0.01$). When Hb levels between blood groups were compared, no significant association was found ($p = 0.79$). The percentage of anemics with malaria in blood groups A, B, O, and AB was 71.8%, 67.6%, 81.1%, and 86.4%, respectively.

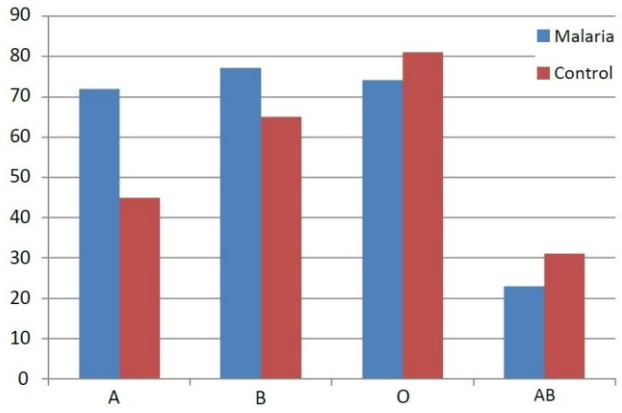
The mean platelet count in malarial cases was 117,000, while in non-malarial cases, it was 245,000 ($p < 0.01$). When the degree of thrombocytopenia was compared among the blood groups, no significant association was derived ($p = 0.52$). The percentage of thrombocytopenic patients with malaria within blood groups A, B, O, and AB was 81.7%, 77.3%, 74.3%, and 72.7%, respectively. The mean TLC in malarial cases was 6,065, while in non-malarial cases, it was 7,745 ($p = 0.072$). There was no association of TLC with blood group ($p = 0.679$).

Table 3. Association of malarial cases with Hb, TLC, and platelet count.

Variables		Case-control			
		Malaria		Control	
		Count	%	Count	%
Hb level	Low	180	59.4	123	40.6
	Normal	60	38.2	97	61.8
	Above normal	3	60.0	2	40.0
Platelets	Low	189	91.7	17	8.3
	Normal	51	21.1	191	78.9
	Above normal	4	22.2	14	77.8
TLC	Low	56	72.7	21	27.3
	Normal	180	50.8	174	49.2
	Above normal	8	22.9	27	77.1

Hb: hemoglobin; TLC: total leukocyte count.

Figure 1. Distribution of blood groups among malarial cases and controls



Discussion

Malaria causes a statistically significant drop in hemoglobin and platelet levels in all ABO phenotypes compared to the normal population. However, different ABO blood phenotypes show no statistically significant difference when compared to each other. Blood group frequency corresponds to the ABO phenotypic distribution found in the region [10,11], the most common being O, followed by B, A, and AB. In the present study, significant association between blood group and malaria was not found (> 0.05). No blood group was associated with an increased prevalence of malaria when compared to the non-malarial population. The prevalence largely corresponds to the ABO phenotypic distribution in Pakistan and suggests the

association of malaria with demographic distribution of blood groups in the region. Studies in Sindh and Baluchistan showed similar ABO phenotype [10,11]. These findings are similar to what was reported by Montoya *et al*., who reported that no association could be established between the presence of ABO antigens and malaria by *P. falciparum* or *P. vivax* in four different ethnic groups in Colombia [12]. In another Amazonian area, Beiguelman *et al*. also failed to find such association [13]. Thakur and Verma [14] concluded in their study that ABO blood groups did not show differential susceptibility to malaria. The study showed that in their selected population, malaria did not pose a selective pressure on the ABO phenotype since the patients with malaria had similar blood groups compared to the control population. This also suggests that strains of malaria in Pakistan are less severe when compared to other regions such as the sub-Sahara Africa and Sri Lanka, where malaria is chloroquine resistant and leads to severe hematological and clinical outcomes, thus increasing the chance of death. However, there is a need to evaluate the strains of malaria in other parts of the country.

Platelet count was significantly reduced in malaria cases of *P. vivax* and *P. falciparum* when compared to the control group ($p < 0.05$); thus, thrombocytopenia was a significant finding. Nearly 75% malarial patients had thrombocytopenia (platelet count of less than 150 ×103/uL); these finding are supported by Malik *et al.* [15] and Memon *et al*. [16], who reported that 70% of malarial cases had thrombocytopenia. It is a general consensus that thrombocytopenia is very common in malaria [17,18]. The degree of thrombocytopenia has been considered a criterion of disease severity by David *et al*. in the United Kingdom [19]. According to our study, no significant association was found between blood group and thrombocytopenia, and all ABO blood groups showed similar falls in platelet levels when compared, suggesting that thrombocytopenia is unaffected by the ABO blood group phenotype. A study from Colombia showed similar results [20]. This shows that malaria should always be on the list of differentials diagnosis in patients with thrombocytopenia. Studies have also shown the incidence of a less severe anemia of *P. falciparum* malaria in blood group O [21,22]. However, in this study, the majority of cases were of *P. vivax*, which seemed to show no difference in the severity of anemia in ABO blood groups. Others studies have also shown no association [20,23].

Malarial cases in our study showed a significant change in their TLCs. Many recent studies also show leucocytosis among the malaria patients. Ansar *et al.* [24] reported no such association in his study; however, Adedapo *et al*. reported leucocytosis in about 9.5% of the patients with malaria [25]. This study suggests that hematological finding do not vary between different ABO phenotypes in malaria. Anemia and thrombocytopenia, which are critical in disease severity, are unaffected by blood groups. The interplay between malaria parasites and blood group antigens remains a fascinating subject with the potential to contribute to the development of new interventions to reduce the global burden of malaria. This study was the first of its kind in Pakistan, and further studies need to be conducted with improved methodologies and increased sample sizes to validate the present study. This study attempted to find an association of ABO blood group with severity of malaria using hematological finding alone due to the lack of resources and funding. Further studies need to be conducted, combining the clinical and hematological aspects of malaria, to assess ABO blood group association with malaria.

Conclusions

The study suggests that malaria does not pose a selective pressure on the ABO phenotype since the patients with malaria had similar blood groups compared to the control population. However, the lack of power in sample size is a significant limitation of this study, proposing a need for further evaluation to establish a reliable conclusion. Efforts should be made to diagnose and treat the disease promptly before severe symptoms develop.

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