ORIGINAL ARTICLE

The Association of Malarial Parasites with Platelet Count in Our Population

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[1]Idea & Analysis of samples/results, compilation of Manuscript & statistics
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ABSTRACT

Objective: The present study was designed to assess the association of malarial parasite positive cases with platelets count in our population.

Place and Duration: This multicenter study was conducted in Islamabad, Rawalpindi, Peshawar and Dera Ismail Khan from October 2015 to September 2016.

Methodology: In this Cross Sectional Descriptive study, total of 500 malaria positive cases were included. Out of which, (100) each from Islamabad, Rawalpindi, and Deera Ismail Khan, while (200) from Peshawar. Blood samples were taken and analyzed through standard procedures. The data obtained was analyzed through SPS version 16.

Results: Out of 500 malarial positive cases, 444((88.8%) were infected with Plasmodium vivax, 45(9.0%) by Plasmodium Falciparum, with an average platelet count of 151 ×10^9/L and 143×10^9/L respectively. Cases infected with both Plasmodium Vivax and Plasmodium Falciparum came out to be 11(2.2%) with an average of 145×10^9/L platelets. During the present study, no case of Plasmodium Malariae and Plasmodium Ovale were observed. Gametocyte form in both plasmodium Vivax and Falciparum, caused most cases of thrombocytopenia while Ring form did not affect much.

Conclusion: The results of our study showed that infection with Plasmodium Vivax does not grossly affect the average platelet count, while Plasmodium Falciparum causes a mild decrease in the platelet count. Similarly, the infection with both Plasmodium Vivax and Plasmodium Falciparum (mixed type) causes mild thrombocytopenia.

Key Words: Malaria, Platelet count, Pakistani population.

Introduction

Genus Plasmodium is causative agent in mosquito-borne infectious disease of humans called Malaria , an Italian name mala aria, meaning "bad air", comes from the linkage suggested by Giovanni Maria Lancisi (1717) of malaria with the poisonous vapours of swamps. Malaria is the most significant parasitic disease of humans worldwide, and claims the lives of children more than any other infectious disease. Since 1900, malaria exposed area has been halved, yet two billion more people are presently infected. It has been reported that reduction in the incidence of malaria coincides with increased economic output. [1]

Fever, fatigue, vomiting and headaches are the common symptoms of malaria . In severe cases, it can cause yellow skin, seizures, thrombocytopenia, leucopenia and sometimes pencytopenia followed by coma or death. These symptoms start beginning ten to fifteen days after being bitten. However, in peoples who have not been appropriately treated, the disease may recur months later.

Re-infection typically causes milder symptoms, however, high levels of mortality and morbidity are caused by Plasmodium falciparum species.[1]

Pakistan with an estimated burden of 1.5 million cases annually, has been categorized by WHO in the Group 3 countries of the Eastern Mediterranean Region, along with, Sudan, Afghanistan, Djibouti, South Sudan, Somalia and Yemen. It is reported that 95% of the total regional malaria burden lies in these countries. The major contributing factors to this fatal disease are mass population movements within the country and across international borders, unpredictable transmission patterns, the low immune status of the population, climatic changes, poor socioeconomic conditions, declining health infrastructure, resource constraints, poor access to preventive and curative services, and mounting drug and insecticide resistance in parasites and vectors.[2]

Platelets as well as regulating blood homeostasis are an important component of human body defense against invading microbial pathogens and protect during malarial infection by binding Plasmodium-infected erythrocytes (IE) and killing the parasites inside. More recent studies have now revealed the platelet Plasmodial factor, platelet factor 4 (PF4) and the red cell expressed Duffy antigen molecule as a central players in the parasite killing activity of parasites. Beside this, platelets are increasingly implicated in immunological processes, including direct pathogen killing function (reviewed by Yeaman and their colleagues). Platelets share many properties with classical immune cells.[3]

Malaria infection are commonly accompanied by a thrombocytopenia or loss of platelets, the severity of which closely mirrors the increasingly parasite mass. It is now clear from studies that platelets protect the host during erythrocytic infection. Mice with pre-existing platelet deficiency are more susceptible to infections and exhibit higher loads of viable parasites. It has been noticed that purified human platelets, when added to cultured Plasmodium falciparum, inhibit parasite growth. Pre-treatment of these platelets with inhibitors (including aspirin) blocks the parasite killing effect. Platelets bind preferentially Plasmodium falciparum IE, mainly through interactions between the platelet-expressed scavenger receptor protein, CD36 and the Plasmodium falciparum erythrocyte membrane protein (PfEMP1), produced by the parasites and trafficked to the erythrocyte surface. Importantly platelet binding to IE is associated with parasite death.[3]

Acute febrile illness with thrombocytopenia in a patient increases the probability of malaria infection in endemic areas and may increase suspicion of malaria in settings where technical laboratory support is not available. Few studies have been done to assess the clinical or prognostic significance of the circulating platelet count. In a study of 215 Senegalese children[4-5] with severe malaria, those with a platelet count of less than 100,000/μL were more likely to die than those with a platelet count of greater than 100,000/μL (odds ratio [OR] = 6.31, 95% CI = 2.0–26.0). Similarly, in another study, 75 children with cerebral malaria, those with a platelet count of less than 100,000/μL were similarly at greater risk of a fatal outcome than children with higher platelet counts (OR = 9.43, 95% CI = 2.3–54.4).[3]

Methodology

This multicenter study was conducted in Islamabad, Rawalpindi, Peshawar and Dera Ismail Khan from October 2015 to September 2016. In this cross-sectional descriptive analytical study. 500 blood samples of malarial parasite positive cases. Out of these 500 cases, we collected 200 malaria positive cases from Peshawar and 100 cases each from Islamabad, Rawalpindi and Dera Ismail Khan.

Pre-structured questionnaire was developed and used as a tool for collection of data. A detail Performa consisting of Name, Age, Sex, Address, Medical History and Laboratory results was prepared. Informed consent was taken from all the individuals of the study before filling the questionnaire. Data analysis was done through SPS version 16.

Inclusion criteria:

- Malaria parasite positive cases
- All ages
- Both sexes

Exclusion criteria:

- Malaria parasite negative cases
- None of the mentioned cases
- Unwilling persons

Sampling Techniques: Probability sampling technique

Procedure for Malarial Examination Smear: Peripheral smear examination for the malarial parasite is the gold-standard in confirming the diagnosis of malaria. Both thick and thin smears prepared from the peripheral blood are used for this purpose.

A number of stains like Field's, Giemsa's, Wright's and Leishman's are suitable, however, thick films are ideally

stained by the rapid field's technique or giemsa's stain for screening of parasites.

Platelet count procedures: for platelet counts, freshly collected blood specimen to which EDTA is suitable. Platelet count was done on fully automatic hematology analyzer and rechecked through smear by light microscopy.

Results

The results of the study are tabulated in tables 1-6

Table1 shows that out of 500 cases, 444 cases were infected by plasmodium vivax and 45 cases were infected by plasmodium falciparum. We also noticed in 11 cases of mixed type infection having both plasmodium vivax & falciparum.

Table No.I: Relationship between malarial parasites and total number of malarial cases

S#	Type of plasmodium	Number of cases	Percentage
1.	Vivax	444	88.8%
2.	Falciparum	45	9.0%
3.	Mixed	11	2.2%
Total		500	100

The results tabulated at **table II** showed gender wise distribution of patients, depicting that out of 500 malaria positive patients, 338(67.6) were male while 162(32.4%) were female, showing that males are more vulnerable to malaria as compared to female.

Table No.II: Relationship between gender and total number of malarial cases

S#	Gender type	Number of cases	Percentage
1.	Male	338	67.6%
2.	Female	162	32.4%
Total		500	100

Tables III of our study showed, gender wise distribution of malaria positive cases, in which plasmodium vivax was 67.56 % in male patients while, 32.43% was in female patients. Similarly, plasmodium falciparum was 64.4% in males while 35.56 in females. The mixed infection reported was 81.8% in males and 18.2 % in females.

Table No. III: Relationship between gender and malarial parasites in total number of malarial cases

Gender	Plasmodium vivax	Plasmodium falciparum	Mixed
Male	300(67.56%)	29(64.44%)	9(81.8%)
Female	144(32.43%)	16(35.56%)	2(18.2%)

Tables IV showed the average platelet count seen in plasmodium vivax and plasmodium falciparum infection. Plasmodium vivax did not cause gross decrease in average platelet count and maintained it at the lowest normal limit of platelet count, in contrast, plasmodium falciparum & mixed infection causes decrease in average platelet count causing mild thrombocytopenia.

Table No IV: Effect of Malarial Parasites on Platelet Count (Average) in Our Case Study

Malarial Parasites	Total Number	Average Platelet Count (×10^9/L)
Plasmodium vivax	444	151×10^9/L
Plasmodium falciparum	45	143×10^9/L
MIXED	11	145×10^9/L

Table V of our study showed that mixed infection was observed in Rawalpindi region only and 1 case was reported from Islamabad region. Rings of both types of malarial parasites were common in all regions. High frequency of trophozoites is noted in all regions except Islamabad where schizonts were found more. However, most complicated

Table No.V: Prevalence of Malarial Parasites and Its Forms in Total Number of Malarial Cases (n=500)

Malarial Forms	Plasmodium Vivax				Plasmodium Falciparum				Mixed Infection			
	DIK	Pes	Ibd	Rwp	DIK	Pes	Ibd	Rwp	DIK	Pes	Ibd	Rwp
Rings	11	36	31	05	03	03	02	03	00	00	00	00
Schizonts	00	00	11	00	00	00	00	00	00	00	00	01
Trophozoites	43	135	06	40	11	12	01	00	00	00	00	00
Gametocytes	12	04	03	00	04	00	01	00	00	00	00	00
Rings and Trophozoites	00	10	02	37	00	00	00	05	00	00	10	00
Schizonts and Trophozoites	00	00	25	00	00	00	00	00	00	00	00	00
Trophozoites and Gametocytes	16	00	17	00	00	00	00	00	00	00	00	00
TOTAL	444				45				11			
Grand Total	500											

cases were reported in Dera Ismail Khan where even gametocytes of malarial parasites were observed.

(69.18%) had thrombocytopenia[7], which is an agreement to our study.

Table VI: Mild, Moderate & Sever Thrombocytopenia In Malaria Positive Cases

Conditions	Platelet range/count	Species of Malaria		
		Plasmodium vivax (n%)	Plasmodium falciperum(n%)	Mixed type(n%)
Mild thrombocytopenia	100,000-<150,000)	87(17.4%)	02(0.4%)	01(0.2%)
Moderate Thrombocytopenia	75,000-100,000	65(13.0%)	07(1.4%)	00(0.0%)
Sever thrombocytopenia	<75,000	71(14.2%)	13(2.6%)	01(0.2%)
Total		223(90.3%)	22(8.9%)	02(0.8%)
Grand Total		247(100%)		

Table IV of our study showed that out of 500 positive cases, thrombocytopenia was noted in 223(90.3%) cases of plasmodium vivax, 22(8.9%) cases of plasmodium falciparum while, 02(0.8%) cases of mixed type. Mild, moderate and sever thrombocytopenia were 17.4 %, 13.0% and 14.2% in plasmodium vivax positive cases respectively, similarly, 0.4%, 1.4% and 2.6% in plasmodium falciparum positive cases respectively, while, a total of 0.8% was noted in mixed type positive cases.

Discussion

In our populations mostly malaria is caused by Plasmodium Vivax with 444(88.8%) out of 500 cases to be infected by Plasmodium Vivax, 45(9.0%) were infected by Plasmodium Falciparum and 11(2.2%) cases by both, which can be seen at the table no. I. Controvert to our study, Shiraz*, et al(2012)* carried out a study in Hayatabad Medical Complex, Peshawar and found that out of 121 cases, 82 patients (68%) had Falciparum malaria while 39 patients (32%) had Vivax infection.[6] Moreover, infection of malaria was found to be more in male (67.6%) than females (32.4%), which is evident at table No. 2 of our study.

Thrombocytopenia has been noted commonly in all forms of malaria. Different mechanisms have been proposed as immune mediated mechanisms including immune destruction of circulating platelets, splenic pooling, and reduced platelet lifespan. Our study carried out in different populations has shown that out of 500 cases, 182 (36.4%) had normal platelet counts, 303 (60.6%) had thrombocytopenia, and 15 (3%) cases have platelet count above normal range due to some other infections, which can be seen at table No. 5 of our study. A study carried out in Pakistan by Shuaib *et al(2009)* has shown that out of 370 cases, 114 (30.81%) had normal platelet counts, and 256

Thrombocytopenia is a common feature of acute malaria and occurs in both Plasmodium falciparum and Plasmodium Vivax infections regardless of the severity of the infection. As we have seen in our study that Plasmodium Vivax does not affect average platelet count much though Plasmodium falciparum causes more decrease in platelet count but not to the great extent. Malarial cases with both types (mixed) also affect average platelet count and cause mild thrombocytopenia (Table no.4 and Table no. 5). Similar results were obtained by another study, in which it is shown that most of the malarial patients have thrombocytopenia but it is benign in nature and improves in uncomplicated cases without a need for platelet transfusions.[8]

Thrombocytopenia is rarely accompanied by clinical bleeding or biochemical evidence of DIC. Platelet count can fall to below 8,000 $\times 10\hat{}9$/L but this is uncommon, however, platelets counts rise rapidly with recovery. *Another study conducted by UM Jadhav, et al(*2004) concluded that Although the absence of thrombocytopenia is uncommon in malaria, its presence is not a distinguishing feature between the two types. Thrombocytopenia less than 20,000/ in P. Vivax malaria although statistically more common with P. falciparum malaria.[9]

Our study can note that Plasmodium falciparum causes mild thrombocytopenia, despite its severity of its infection. Similar results were found by Mahmood and Yasir (2003) that concluded an extended search for the malarial parasite in patients having thrombocytopenia on smear. Mild to moderate and moderate to severe thrombocytopenia was seen in hospitalized patients and was considered enough alert to the possibility of malarial infection, and Plasmodium falciparum was reported as the common species in these patients. [10]

Our study was also supported by Ansari *et al*(2009) who reported that high frequency of mild to moderate thrombocytopenia was noted in the Plasmodium Falciparum malaria cases. Furthermore, finding of thrombocytopenia is of diagnostic help as it raises the suspicion of malaria. [11]

General results of different regions of our study shows similar trend. Prevalence of Plasmodium Vivax infection was seen in majority and Plasmodium Falciparum was seen in minority in every region. It is supported by M.Iqbal and Juma Khan(2013), in which they found that in clinically suspected cases of malaria, the SPR was high. The high prevalence of Plasmodium vivax poses a significant health hazard, and should be of great concern for the Malaria Control Programme in Pakistan. [12]

Thrombocytopenia is reported by various researchers in all forms of malaria.[13-14] Gerardin and others [24] reported that in Senegalese children those who died were severely thrombocytopenic than others who recovered. In the cited study, children with severe malaria had a platelet count of less than 100,000/μL (N = 110) and were 6.3 (95% confidence interval [CI] 2–26) times more likely to die than those who had a platelet count of more than 100,000/μL. Similarly, in another study of much larger scale in coastal area of Kenya, Ladhani and colleagues [14] reviewed 1,016 hospitalized children that fulfilled the WHO criteria for severe malaria, and found that the median platelet count was significantly lower among these children than those with mild, uncomplicated malaria. In another study conducted in Kenya, no association has been found between the presence or severity of thrombocytopenia and a fatal outcome of the illness.

It has been reported that platelets may play a role in the pathogenesis of cerebral malaria.[15] Among children analyzed in a study, a subset of those who died were studied at autopsy and were found to have a significantly greater degree of platelet accumulation in brain microvasculature than children who died of severe malarial anemia or of non-malarial encephalopathies.[16] However, sequestration of platelets contributes to peripheral thrombocytopenia in malaria is not known with certainty.

Similarly, it has been noticed that in malaria infected individuals, not only platelets count are on decline side but also WBC count, which is evident by analysis of WBC counts in individuals presenting at Thai and Peruvian outpatient malaria clinics in 1998 and 1999, which showed that the *P*lasmodium *falciparum*–infected patients had lower WBC counts than the *P*lasmodium *vivax*–infected patients and that both of these groups of patients had lower WBC counts than the uninfected patients.[17] Similarly, in two previous studies, both in Asia [18-19], have also reported low WBC counts during *P*lasmodium *falciparum* and *P*lasmodium *vivax* infections in residents of regions in which *Plasmodium* parasites are endemic. [20]

Conclusion

We have confirmed the prominence of thrombocytopenia in malaria and is a feature common to all forms of malaria. Among malaria infected children, those with plasmodium falciparum are more likely to be thrombocytopenic than that plasmodium vivax. Infection with Plasmodium vivax does not grossly affect the average platelet count, while infection with Plasmodium Falciparum and mixed type infection causes a mild decrease in the platelet count. During the present study no case of Plasmodium Malariae and Plasmodium Ovale were observed. It is also observed in our study, that in both types of plasmodium (Vivax and Falciparum), the gametocyte form caused most cases of thrombocytopenia while Ring form did not affect much.

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