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Evaluation and comparison of hematological parameters between Vivax and Falciparum malaria

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ABSTRACT

Background: Malaria is a widely prevalent parasitic disease caused by the Plasmodium species. The parasite mainly infects the erythrocytes and causes various hematological abnormalities like anemia, thrombocytopenia and disseminated intravascular coagulation (DIC). Aim: To evaluate and compare the hematological parameters between vivax and falciparum malaria. Settings and design: A cross sectional, prospective study in the hospital laboratory. Materials and methods: Blood samples from 889 febrile patients were tested for malaria by the rapid malaria antigen detection test and peripheral blood smear study. The hematological investigations included study of platelet counts, white and red blood cell counts, hematocrit, hemoglobin and red blood cell indices using an automated cell counter. Results: Out of the 889 patients screened, 81 patients tested positive for malaria. Fifty four patients (66.67%) had P.*vivax* infection, while 22 (27.16%) were infected with P.*falciparum* and five (6.17%) had mixed infection. Thrombocytopenia was seen in 95.06% patients. All five patients who had mixed infection, 96.29% of vivax malarial patients and 90.90 % of patients with P.*falciparum* had thrombocytopenia. Anemia was seen in 34 cases (41.97%). Conclusion: Though commonly reported in falciparum malaria, thrombocytopenia and anemia occur in P.*vivax* infection also.Significant changes in P. *falciparum* infection were relative increase in neutrophil differential count, fall in lymphocyte differential count and hematocrit when compared to vivax malarial infection. Finding of thrombocytopenia may help in increased rate of detection of malaria.

KEY WORDS: **Malaria, thrombocytopenia, anemia, hematocrit, peripheral smear**

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INTRODUCTION

Malaria is one of the most widespread of parasitic diseases. Worldwide, around 247 million cases occur annually with an estimated 881,000 deaths[1]. About 70% of reported cases in the South East Asian region are from India[2]. The commonly infecting species in India are Plasmodium vivax and P. falciparum. The hematological changes in malaria include anemia, thrombocytopenia and disseminated intravascular coagulation (DIC) [3,4]. An understanding of these hematological changes will help in diagnosis and treatment and may also serve to predict and prevent various complications. Hence this study was carried out to evaluate the hematological parameters and compare the results between vivax and falciparum malaria.

MATERIALS AND METHODS

Blood samples from 889 patients between the ages of two and seventy years of either sex, with history of fever (< seven days) were tested for malaria[5]. The investigations were performed on the request of the physicians based on clinical suspicion of malaria. Patients with a history of fever of more than seven days duration and those who tested negative with the card test for malaria were excluded from the study. The study was done over a period of one year from June 2009 to August 2010. Two ml of blood was collected in EDTA containing vacutainer tubes and tested for malaria in the department of microbiology by the SD Bioline One step Malaria antigen Rapid test (Standard Diagnostics, Republic of Korea)[6]. The test device is coated with monoclonal antibodies to histidine rich protein-2 (HRP-2) antigen specific for P. falciparum and polyclonal antibodies specific to Lactate dehydrogenase (pLDH) pan specific to all Plasmodium species. Five µl of blood was taken with a disposable specimen loop provided with the kit and placed in the sample well. Four drops of the assay diluent were added into the diluent well and results interpreted after 20-30 minutes. The test was considered valid only if the control color band appeared. The presence of color line at Pf region along with the control line was considered as test positive for P. falciparum and appearance of color line at the Pan region was considered as positive of other Plasmodium species. Presence of three color bands was taken as mixed infection. All the positive samples were further confirmed by microscopic examination of peripheral blood smear study using venous blood, collected in EDTA tube. The species of Plasmodium was diagnosed by microscopy of Leishman stained thin blood films. Peripheral blood smear positive for malarial parasite was taken as a gold standard for the diagnosis of malaria[7]. Hematological investigations included study of complete blood picture: white and red blood cell counts, hematocrit, hemoglobin, platelet count and red blood cell indices. The complete blood counts were done with the automated five part differential counter (Coulter A^CT^{TM} 5 Diff Beckman Coulter, Inc., Brea, California). Cut off value for anemia was taken as Hemoglobin (Hb) less than 10 gm/dl[5] and severe malarial anemia was defined as Hb less than 5 gm/dl[8]. Total leukocyte count less than 4000 x 10^3 /µl and platelet count of less than 150 x 10^3 /µl were used to define leukopenia and thrombocytopenia respectively[5]. Thrombocytopenia was regarded mild if platelet counts were between 100 - 150 x 10^3/µl, moderate when counts were between 50 - 100 x 10^3/µl and severe if less than 50 x 10^3 /µl [9] .Data from age and sex matched negative controls was also included in the study. Platelet counts of patients on the second day of admission and on discharge were also taken. (Table 1)

Table 1
Comparison of demographic and hematological indices between Vivax and Falciparum malaria

S.no	Parameter	P.vivax	P.falciparum	P value	Negative controls
1	Average age (years)	24.81+/- 11.49	29.64 +/- 14.11	-	26.56 +/- 12.42
2.	Sex: No of males No of females	 45 9	 19 3	-	 65 16
3	Mean RBC (x 10^6 /µl)	4.21 +/- 0.89	3.77 +/- 1.07	0.27	5.07 +/- 0.87
4	Mean WBC (x 10^3 / µl)	6187+/- 3131	6531+/- 2277	0.97	8906 +/- 3092
5	Mean platelet count (cells/ µl) on admission	74926+/- 59262	104777+/- 105533	0.492	266580+/- 84670
6	Mean platelet count (cells/ µl) on second day of admission	71545 +/- 56462	86963+/-84872	0.32	264356+/- 75643
7	Mean platelet count (cells/ µl) at discharge	256000+/- 125000	185000+/- 10423	0.42	286214+/- 83216
8	Mean Hb (g/dl)	11.58 +/- 2.69	9.99 +/- 3.47	0.064	14.01 +/- 2.45
9	Mean Hematocrit (%)	35.51 +/- 7.36	30.09 +/- 9.58	0.023	38.76 +/- 5.94
10	Mean MCV (fL)	84.32 +/- 7.71	80.58 +/-12.66	0.119	77.17 +/- 9.46
11	Mean MCH (pg)	27.98 +/- 3.87	27.99 +/- 6.25	0.989	27.74 +/- 3.44
12	Mean MCHC (g/dl)	33.01 +/- 2.38	32.57 +/- 2.54	0.483	35.96 +/- 2.37
13	Mean Neutrophil DC (%)	50.56 +/- 19.59	61.82 +/- 17.71	0.019*	61.80+/- 13.18
14	Mean Lymphocyte DC (%)	37.94 +/- 18.59	27.50 +/- 15.87	0.024*	29.38 +/- 11.69
15	Mean Monocyte DC (%)	8.19 +/- 4.74	7.77 +/- 4.6	0.73	5.02 +/- 7.16
16	Mean Eosinophil DC (%)	2.46 +/- 3.92	2.45 +/- 2.42	0.57	4.52 +/- 3.65

indicates P value <0.05—statistically significant; DC = differential count

Statistical Analysis

Statistical analysis was done by using SPSS version 17. Independent samples't' test was used as test of significance for comparison between the means of vivax and falciparum group after performing the Levene's test for equivalence of variance. $P<0.05$ was considered statistically significant.

RESULTS

Out of 889 patients tested for malarial parasite, 81 were found to be positive. 65 were male patients (80.24%) and 16 were females (19.75%). Majority (49.38%) were in the 21-30 years age group. There were a total of 13 pediatric cases (16.04%). Fever with chills, rigors and headache were the commonest presenting complaints. Hepatosplenomegaly was present in 45 patients (55.55%).Three patients developed cerebral malaria and one patient developed multiple organ dysfunction. None of the patients had bleeding manifestations. No death was reported.

Fifty four patients (66.67%) had P.vivax infection while 22 were infected with P.falciparum and five (6.17%) had mixed infection. No cases of P.malariae or P.ovale were detected.

The total RBC count ranged between 2.08-6.08 x 10^6/µl (normal range: 4.5 - 6.5 x 10^6/µl). Of the 81 confirmed cases of malaria, anemia was observed in 34 patients, (41.97%) with 40.74% of vivax patients (mean Hb - 11.57 g/dl) and 50% of patients with falciparum (mean Hb - 9.99 g/dl) having low hemoglobin levels. There was a significant difference in the mean hematocrit of the two groups (Table 1). A fall in hematocrit was observed in 49.38% of patients and it was more profound among the P.falciparum infected group (63.63%) compared to the vivax affected patients (38.88%). All five subjects who had mixed infection had decreased hematocrit. Blood indices like MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin) and MCHC (Mean Corpuscular Hemoglobin Concentration) did not show statistically significant changes.

The mean total count of leukocytes in this study was 6541x 10^3/µl. The total white blood cell (WBC) count in the normal range of 4000-11000 x 10^3/µl was observed in 79% of the patients. All five patients in the mixed infection group had normal total WBC counts. Normal neutrophil differential count (range: 50-75%) was seen in 35.18% of vivax patients and 54.54% of falciparum affected group. Neutrophilia was relatively higher in P.falciparum patients (27.27%) compared to P.vivax patients (18.51%). Reduced neutrophil differential count of <50% was seen in 46.29% and 18.18% of P.vivax and P.falciparum infection respectively. Reduced lymphocyte differential count (<30%) was noted in 63.63% of P.falciparum infection compared to 33.33% of P.vivax affected patients.

Thrombocytopenia was seen in 95.06% patients when compared to the age and sex matched negative controls. While 90.90% of patients with P.falciparum had thrombocytopenia, low platelet count was also observed in 96.29% of vivax malarial patients and in all five patients who had mixed infection. Severe thrombocytopenia was seen in 60% of falciparum infected cases compared to 40.38% among vivax patients.

DISCUSSION

Malaria is an endemic infection in India with seasonal increase in incidence during the rainy season. In this study, maximum numbers of cases (37) were seen between July and August 2009. This may be due to increased rainfall, optimum temperature and humidity favoring the breeding of mosquitoes during this time. As the malarial parasites mainly infect erythrocytes, anemia is frequently observed in malaria. It arises from the combination of hemolysis of the infected red blood cells (RBCs) by the parasite and suppression of erythropoiesis. The mechanisms of red cell destruction include reticuloendothelial hyperplasia, reduced

deformability of infected red cells, membrane changes and immune mechanisms[10]. Our study showed no significant species variation in causing anemia as 50% of patients with P.falciparum and 40.74% of patients with P.vivax had anemia. While majority (61.76%) of the patients had mild anemia, 23.52% had moderate and only 14.70% had severe anemia (Table 2). Various studies have reported on the occurrence of anemia in increased frequency in falciparum malaria. A study by Agravat and Dhruva[11]showed 93% cases of anemia during P. falciparum infection. A study carried out in Indore by Jain et al[12],also showed 56.06% P.falciparum and 31.8% P.vivax infection induced anemia respectively. This could be attributed to the differential preference of the malarial parasites to erythrocytes of different ages. While P.vivax infects reticulocytes or young RBCs, P.falciparum can attack erythrocytes of all ages. Also, in falciparum malaria, destruction of both parasitized and nonparasitized red cells occurs. Lower peripheral parasitemia, increased activation of host inflammatory immune response and increased deformability of infected erythrocytes leading to reduced cytoadherence in microvasculature are the other factors cited for the benign pathology in vivax malarial infection[13].

Thrombocytopenia is a frequent finding in acute falciparum malaria and is reported in various studies[14]. The various mechanisms postulated are splenic pooling, immune mediated lysis by generation of anti platelet antibodies, oxidative stress[15], causing premature platelet death and bone marrow dyspoeisis. In this study, there was no statistically significant difference among the two species, namely vivax and falciparum in causing thrombocytopenia which was seen in 95.06% of our patients. All five patients who had mixed infection had thrombocytopenia (Table 3).

Very few studies have reported on the occurrence of thrombocytopenia in vivax malaria[16]. Our study showed a higher incidence of thrombocytopenia in vivax patients (96.29%) than the falciparum group (90.90%). Kumar and Shashirekha[17] had also reported of 27 cases of acute vivax malaria out of which 24 cases had thrombocytopenia even before anemia and splenomegaly could set in.

The severity of thrombocytopenia is more in falciparum malaria compared to vivax malaria. In our study also, more than half of the patients having falciparum infection had severe thrombocytopenia when compared to only 38.88% of vivax patients. A study by Francischetti et al[18] had observed that thrombocytopenia in vivax malaria might be immune mediated destruction of platelets, occurring in the absence of blood coagulation activation. In contrast, thrombocytopenia in falciparum malaria is most often accompanied by activation of the coagulation cascade along with immune-mediated lysis and peripheral destruction.

Table 2
Grading and comparison of anemia between vivax and falciparum malaria

Infection type	Mild (Hb 8-10.9 g/dl)	Moderate (Hb 5-7.9 g/dl)	Severe (Hb<5 g/dl)	Normal (Hb>10 g/dl)
Plasmodium vivax (n=54)	17 (31.48%)	3 (5.55%)	2 (3.7%)	32 (59.25%)
Plasmodium falciparum (n=22)	4(18.18%)	4 (18.18%)	3 (13.63%)	11 (50%)
Mixed infection (n=5)	-	-	1 (20%)	4 (80%)

Table 3
Grading and comparison of thrombocytopenia between vivax and falciparum malaria

Infection type	**Mild** (platelet count 100-150 x10^3 /µl)	**Moderate** (platelet count 50 –100 x10^3 /µl)	**Severe** (platelet count < 50 x10^3 /µl)	**Normal** (platelet count >150 x10^3 /µl)
Plasmodium vivax (n=54)	9 (16.66%)	22 (40.74%)	21(38.88%)	2 (3.70%)
Plasmodium falciparum (n=22)	3 (13.63%)	5 (22.72%)	12 (54.54%)	2 (9.09%)
Mixed infection (n=5)	2 (40%)	1 (20%)	2 (40%)	-

Peripheral blood smear examination is considered as the gold standard for diagnosis of malarial infection and is the widely prevalent method for detection of malaria in developing countries like India.Though it is confirmatory, peripheral smear study is time consuming, requires technical expertise and has a variable sensitivity ranging from 25-75%, whereby diagnosis becomes difficult in cases of low parasitemia. In such cases, the finding of thrombocytopenia has diagnostic significance and can serve as a valuable clue for more diligent screening for presence of malarial parasites[19].

In tropical countries, dengue fever constitutes one of the main differential diagnosis for patients presenting with fever and low platelet counts. The bleeding diathesis seen in dengue hemorrhagic fever is due to interplay of thrombocytopenia, coagulopathy and vasculopathy. Thrombocytopenia in dengue fever occurs due to direct dengue virus infection of the hematopoietic progenitor cells, platelet dysfunction, oxidative stress, hemophagocytosis and immune mediated injury of platelets[20].Though most of these factors operate in the genesis of thrombocytopenia in malaria also, bleeding manifestations are rarely reported in malaria. Probably, plasma leakage resulting in the leak of clotting factor proteins contributes more to the coagulopathy and subsequent bleeding in dengue fever than just thrombocytopenia.

Sixteen patients in our study were transfused with platelets, of which nine had vivax, four had falciparum infection and three had mixed infection. In this study, the fall in platelet counts was not associated with any clinical

complication like bleeding diathesis or disseminated intravascular coagulation. Repeat platelet values of the in patients showed that the mean platelet counts fell on day two and again came back to normal levels at the end of treatment indicating that these changes were transient. The therapeutic implication of this finding is that platelet transfusion is unwarranted in malarial patients unlike in dengue fever, where it is essential and life saving.

Eosinophilia is usually present in parasitic infestations. In this study, the mean eosinophil and basophil counts were within normal limits though a relative monocytosis (normal 1-6%) was observed in both groups which were not statistically significant (Table1). Similar findings on differential counts were observed in a study done by Khaleda Taha[21]. Camacho et al[22] had observed that in patients with falciparum malaria, eosinophil counts were not elevated at the time of presentation though peak eosinophilia was observed at the end of first week of infection following antimalarial chemotherapy. It has been hypothesized that a variation in TH_1 and TH_2 cytokine response occurs during the course of the illness. The acute phase of plasmodial infection is characterized by a predominant TH_1 mediated cytokine release, namely IL-1, TNFα. Release of TH_2 related cytokines like IL-4, IL-5 at the end of first week is related to eosinophilia and good hematological recovery.

Neutrophilia accompanied by reduced lymphocytes in patients usually leads to the suspicion of acute bacterial infection among clinicians. Our study shows that a similar blood picture can occur in malarial infection also. Statistically significant difference was observed in the neutrophil differential count and lymphocyte differential counts of the two groups. These variations could be attributed to the sequestration of lymphocytes in spleen and also release of cytokines.

CONCLUSION

Among the hematological parameters studied, thrombocytopenia was the predominant hematological abnormality seen in malarial infection followed by anemia. P.vivax infection is usually considered to have a benign course when compared to falciparum malaria. This study shows that no species variation exists between vivax and falciparum infection in causing these hematological complications, as they occur in similar frequency. Also, we found that the incidence of thrombocytopenia was higher among P.vivax infected patients, though its severity was more in P.falciparum infected patients.Variation in the activation of cytokines and coagulation cascade might explain the difference in the severity and hematological alterations occurring between vivax and falciparum malaria. The transient nature of thrombocytopenia could probably explain the lack of bleeding manifestations and hence platelet transfusion is unwarranted in the case of malaria.

The other hematological changes which were significant include reduced hematocrit, increased polymorphonuclear leukocyte differential count and reduced lymphocyte differential count which were observed in falciparum infected patients compared to vivax infected patients. While thrombocytopenia is a reliable indicator of malarial infection and help in increased detection of malarial parasite during peripheral blood smear study, abnormalities in leukocyte counts including elevated neutrophils also help in a diagnosis of malaria.

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