A Study of Different Hematological Parameters in Malaria

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Abstract
Aims: To study an alteration in hematological parameters in malaria.
Settings and Design: This is a prospective study conducted at Central Diagnostic Laboratory attached to Shree Krishna Hospital, Karamsad. All the smears positive for malaria cases, diagnosed at Pathology Laboratory were selected and studied for hematological changes.
Methods and Material: According to Proforma, detailed history regarding age, sex, nature and duration of illness is taken. Venous blood in EDTA vaccutainer was collected from patient for diagnosis of malaria and hematological study before starting anti-malarial treatment.
Results: Out of 51 malaria positive patients, 41(80.3%) patients had P. vivax infection while 10 (19.6%) were infected with P. falciparum. Anemia, Thrombocytopenia and leucopenia were observed in 25 (49.01%), 48 (94.11%) and 9 (17.64%) patients respectively.
Conclusions: Species variation is seen in altered hematological parameters. Anemia and thrombocytopenia were more severe with p.falciparum infection while mild to moderate leucopenia was observed in p.vivax infection and it has no relation with p.vivax infection.
Keywords: Malaria, P.falciparum, P.vivax.

Introduction
Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoan’s belonging to genus Plasmodium.¹ Malaria continues to be a major health problem in some of the most populated areas of the world. It is one of the important causes of febrile illnesses in our part of the world. One of the most prevalent human infections worldwide, malaria results in 225 million cases each year. Around 40% of the global population at risk of malaria resides in the South-East Asian Region. It is caused by protozoa parasite of the genus plasmodium which infects and destroys red blood cells. Four species of plasmodia (P.falciparum, P.malariae, P.vale and P.vivax) cause malaria in humans of which P. falciparum is the main cause of morbidity and mortality²,³. Some hematological changes are species specific. Thrombocytopenia is a common and early sign of malarial infection & it is observed in vivax and falciparum malaria in varying degrees. Most of these findings are more pronounced in P.falciparum as compared to vivax.⁴,⁵,⁶ However, there is no unanimity in the
findings with some studies showing minimal differences between the two and others showing a more pronounced hematological alteration in falciparum as compared to P.vivax. There have also been reports of leucopenia and leukocytosis.

Subjects and Methods
This is a prospective study conducted at Central Diagnostic Laboratory attached to Shree Krishna Hospital, Karamsad. All the smears positive for malaria cases diagnosed at Pathology Laboratory were selected and studied for haematological changes.

According to Proforma, detailed history regarding age, sex, nature and duration of illness is taken. Findings of clinical examination were noted. Venous blood in EDTA vacutainer was collected from patient for diagnosis of malaria and hematological study before starting anti-malarial treatment.

Once the blood sample received in the laboratory then kept them on blood mixture, then Hb, Platelet Count and Total WBC Count were done in automated KX-21 hematology analyzer. After that from same sample thin and thick smears were prepared. Both were stained with giemsa stain. Thick smears were examined for rough estimation of parasite by observing average no.of parasites per oil immersion field. Thin smears were examined for confirmation of Platelet Count and for identification of species and grading of parasitemia.

Results
The study of hematological changes in malaria is carried out at Shree Krishna Hospital, Karamsad from the period of February 2014 to February 2015 (one year). All malaria positive cases were included and Hemoglobin, Platelet count and Total WBC count of all were studied. Following observation from this study has made.

Out of 51 malaria positive patients 37 (72.45%) were male and 14 (27.45%) were female. Majority (23.52%) of patients were in the age group of 11-20 years. Fever with chills, rigors and headache were the commonest presenting complaints. 41 (80.3%) patients had P.vivax infection while 10 (19.6%) were infected with P.falciparum infection. From the 51 cases of malaria, anemia was observed in 25 patients (49.01%). Amongst them 68% were of P.vivax infection and 32% were of P.falciparum infection.

Thrombocytopenia was seen in 48 (94.11%) patients. Amongst them 90% were of P.falciparum and 95.12% were of P.vivax infection. Severe thrombocytopenia was seen in 40% of falciparum and 21.95% of P.vivax patients.

Leucopenia was seen in 9 patients (17.64%). Amongst them 10% were of P.falciparum and 19.51% were of P.vivax malarial infection. We have received only 10 repeat samples during whole study for laboratory investigation. In all repeat samples marked variation in platelet count was observed. Once the treatment was started, Platelet count was increased abruptly, but hemoglobin and total WBC Count were not much affected.

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Parameter</th>
<th>P.vivax</th>
<th>P.falciparum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean HB</td>
<td>11.38 ± 3.58</td>
<td>7.9 ± 6.0</td>
<td>*0.015</td>
</tr>
<tr>
<td>2</td>
<td>Mean Platelet</td>
<td>100.21 ± 153.56</td>
<td>112.02 ± 73.5</td>
<td>0.384</td>
</tr>
<tr>
<td>3</td>
<td>Mean WBC</td>
<td>6.4 ± 11.14</td>
<td>6.38 ± 6.70</td>
<td>0.13</td>
</tr>
</tbody>
</table>

*indicates P value <0.05 - statistically significant

- There is a statistically significant difference in Mean Hemoglobin value between P.vivax and P.falciparum. (P value <0.05).
- There is no statistically significant difference in Platelet count between P.vivax and P.falciparum in this study. (P value >0.05).
- There is no statistically significant difference in WBC Count between P.vivax and P.falciparum in this study. (P value >0.05).

Figure:1 Labeled thin blood film (A) and thick blood film (B)
Figure: 2 Mixed Infection (Trophozoites of P.vivax and P.falciparum) (Thin smear)

Figure: 3 Schizonts of P.vivax (Thin smear)

Discussion
Out of studied 51 cases, 41 (80.3%) were of P.vivax and 10 (19.6%) cases were of P.falciparum. This study includes 37 male patients (72.54%) and 14 females (27.45%). In the present study male are more commonly affected. These findings are comparable to other study conducted by Ranjini CY et al, who found that male (80.24%) was more commonly affected than female (19.75%). Age wise distribution in the present study showed that 72.52% patients were adults (11-50 years). Majority (23.52%) were in the 11-20 years age group. There were a total of 3 pediatric patients (5.88%) and 7 (13.72%) patients were above 60 years. Fever with chills, rigors and headache were the commonest presenting complaints. These findings are also comparable to study conducted by Sethi Bhavna et al, which showed 16% patient were in the 11-20 years age group and 26 (13%) of pediatric patient. From the 51 cases of malaria, anemia was observed in 25 patients (49.01%). This study showed statistically significant species variation in causing anemia (P value<0.05). In this study 80% of patients with P.falciparum and 41.45% of patients with P.vivax infection had anemia. Majority 59.02% and 42.43% of the patients had mild and moderate anemia respectively, while only 20% had severe anemia. Incidence of anemia in P.vivax infection in this study is 41.45% which is comparable with the study carried out by Rajini CY et al, which showed 40.74% in P.vivax infection. Various studies, have reported on the occurrence of anemia in increased frequency in falciparum malaria, it is also observed in this study. This study showed incidence of anemia in 89.7% of P.falciparum infection which is comparable with study conducted by Agravat and Dhruva showed 93% cases of anemia in P. falciparum infection. 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 no parasitized red blood cells occur. 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 P.vivax malarial infection.
Thrombocytopenia is a frequent finding in acute falciparum malaria and is reported in various studies. The various mechanism postulated are splenic pooling, immune mediated lysis by generation of anti-platelet antibodies, oxidative stress, causing premature platelet death and bonemarrow dyspoesis. In this study, out of 51 cases of malaria 48 cases had thrombocytopenia. This study showed statistically significant species variation in causing thrombocytopenia (P value >0.05). In this study thrombocytopenia is observed in 90% of P.falciparum and 95.11% of P.vivax infections. Majority of patients have mild to moderate thrombocytopenia, which is 33.33% and 35.23% respectively. Severe thrombocytopenia is observed in 25.49% of cases. Incidence of thrombocytopenia in P.vivax infection is 95.11% which is comparable with study carried out by S. Apte et al, which showed 85% of cases with P.vivax infection. Incidence of thrombocytopenia in P.falciparum infection is 90% in this study, which is comparable with study carried out by Horstmann et al. which showed 85% of cases with P.falciparum infection. But few studies reported slight lower incidence of thrombocytopenia like 40% and 59%. There was no significant difference in severity between species. The vivax malaria is commonly associated with mild hematological abnormalities. Although sever thrombocytopenia is commonly reported to be associated with Plasmodium falciparum infection and has been reported to occur in patients co infected with both Plasmodium falciparum and Plasmodium Vivax, its occurrence has been rarely reported in cases of Plasmodium vivax malaria. The precise mechanism behind thrombocytopenia, however, remains unclear. Both the immunological as well as the nonimmunological destruction of platelets have been implicated. Decreased thrombopoiesis has been ruled out, because platelet forming megakaryocytes in the marrow are usually normal or increased. Other postulates include peripheral destruction and the consumption of platelets. Immune complexes which are generated by the malarial antigen lead to the sequestration of the injured platelets by the macrophages in the spleen. This is supported by the finding that malaria patients have elevated levels of platelet-bound IgG. Some investigators have suggested disseminated intravascular coagulation as a major mechanism in malaria but others have found no evidence of disseminated intravascular coagulation in any of their patients, including those with severe thrombocytopenia. Thrombocytopenia is reported to be present in both P. falciparum and P.vivax infections. Many of the cases in our study are vivax malaria infection. A good tolerance of low platelet counts is well known in malaria. This could be explained by platelet activation and an enhanced agreeability. In most of the studies, thrombocytopenia was neither associated with the severity of the disease or death in malaria. It usually disappears with the treatment of the disease and requires no treatment for itself.

In this study leucopenia is observed in 9 patients out of 51. This study showed no statistically significant species variation in causing leucopenia (P value>0.05). Leucopenia is observed in 10% cases of P.falciparum and 19.51% of P.vivax infection, which is comparable with study of F.Ellis McKenzie et al ,which showed 10.7% cases of  P. falciparum and 15.2% cases of P.vivax infections. In comparative study of Ranjini CY et al. Leukopenia and neutropenia primarily reflects the state of hypersplenism. Laboratory diagnosis of malaria is based upon the demonstration of the malarial parasite on microscopy which requires technical expertise and repeated smear examinations. Hematological abnormalities are considered a hallmark of malaria and statistical analyses have shown that many of these hematological values may lead to an increased clinical suspicion for malaria, thus initiating a prompt institution of specific therapy even in the absence of a positive smear report for malaria. A variety of hematological alterations like progressively increasing anemia, thrombocytopenia, leukocytosis or leukopenia have been reported in cases of malaria. Our present results add to the strong but neglected case for improving the standard methods of
malaria microscopy.

**Conclusion**

Although these hematological alterations in malaria are not new to the subject, this data adds more detailed information to limited knowledge, especially from these highly affected rural and suburban zones. Amongst haematological parameters studied, frequency of alteration in platelet count and hemoglobin are more pronounced compared to total WBC count in patients of malaria. Species variation is seen in severity of thrombocytopenia. Majority patients have mild to moderate thrombocytopenia. Severity of thrombocytopenia is more in P.falciparum infection than P.vivax infection. Species variation is seen in severity of anemia. Anemia is more severe in patients P.falciparum infection compare to P.vivax infection. Species variation is also observed in severity of leucopenia. Mild to moderate leucopenia is more observed in P.vivax infection.

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