ABSTRACT: Malaria continues to be a great health problem in the most populated areas of the world and continue to cause significant morbidity and mortality. Hematological changes usually associated with malaria are well known and this study was conducted to compare the hematological profile of Plasmodium vivax and Plasmodium falciparum. METHODOLOGY: This is the cross-sectional study conducted in Sarojini Naidu Medical College, Agra. It included all the cases presented with fever. Diagnosis of malaria was confirmed by the thick and thin blood film stained with Leishman’s stain for malarial parasite and M. P. Elisa. RESULTS: The difference in the mean platelet count according to the severity of infection was highly statistically significant. According to ANOVA test both for P. vivax and P. falciparum, platelet count show decreasing trend according to the severity of infection. Difference in the mean hemoglobin level, mean platelet counts TLC min and TLC max of P. vivax and P. falciparum cases was also statistically significant. CONCLUSION: The low level of platelet count can be used as a predictor of severity of infection and thus prediction of hematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications.

KEYWORDS Hematological profile, Plasmodium falciparum, Plasmodium vivax malaria, TLC min, TLC max.

INTRODUCTION: Malaria is well known to human being since centuries. It is a disease of tropical and sub-tropical countries particularly Africa and Asia. It is caused by protozoan plasmodium transmitted by female anopheles which typically bite between dusk and down.

Inspite of advance information malaria continue to cause significant mortality and morbidity worldwide. Malaria is one of the most prevailing human infection in the world more than 40% of the world population reside in Malarial endemic area and it is predictable that 300-500 million cases and 1.5-2.7 million death occur each year.1 mortality rate is usually elevated (20%) in severe malaria cases (Parasitemia more than the 5%).2 Hematological changes which are most common complications play a significant role in these serious complications.

The hematological abnormalities that have been reported to consistently companion which comprise anemia, thrombocytopenia infrequently D.I.C.,3 leukopenia, leukocytosis, neutropenia, eosinophilia, and monocytosis also has been reported.3,4 The aim of our study was to compare the hematological changes which occur in P. vivax and P. falciparum malaria.

METHODOLOGY: The present comparative cross-sectional study was conducted in Sarojini Naidu Medical College, Agra. The confirmed cases of malaria taken from October 2012-October 2013.
The diagnosis of malaria was confirmed thick and thin blood film stained with Leishman's stain for malarial parasite and antigen histidine release protein-2 (HRP-2 test). Complete blood count was performed using an automated SYSMEX machine and WBC differential counts were also done for all the patients. The study was pre-mediated to include clinically suspected cases of malaria and patient were excluded on the basis of history and finding suggestive of dengue, chronic liver disease, bleeding disorder, thrombocytopenia, drug intake or condition which might have contributed in blood changes.

All malaria positive smears were study for confirmation and identification of species and review of smear for platelet counts and other hematological changes. Data was analyze by Epi.info statistical software where p-value of less than 0.05 was taken as significant for all statistical analysis. Beside it history taking, clinical examination, routine laboratory work, thick blood films were prepared and examine for defining the species involved. Minimum of 200 fields (oil emersion) were assessed to label a negative smear.

**RESULTS:** The study included 200 patients out of which 130(65%) suffering from P. vivax and 70(35%) from P. falciparum. Platelet count <10000/mm³ was observed in 4.61% in P. vivax cases and 2.85% P. falciparum included in it. Platelet count 10000-50000/mm³ was observed in 28.38% of P. vivax cases and 27.14% of P. falciparum cases. The platelet counts 50000-150000/mm³ found in 10% of P. vivax cases and 17.85% in P. falciparum cases.

| Platelet count | Group A(N=130) | Group B(N=70) |
|---------------|----------------|---------------|
|               | Male | Female | Male | Female | Male | Female |
| <10000        | 6    | 4.61   | 2    | 2.85   | 0    | 0       |
| 10000-50000   | 29   | 22.31  | 29   | 41.43  | 9    | 12.86   |
| 50000-150000  | 13   | 10.00  | 25   | 35.71  | 0    | 0       |
| >150000       | 19   | 14.62  | 5    | 7.14   | 5    | 7.14    |
| Total         | 67   | 51.54  | 56   | 40.00  | 14   | 20.00   |

**Table 1: Platelet Counts (/mm³)**

| Platelet count | Male | Female | Total |
|---------------|------|--------|-------|
|               | Group A | Group B | Group A | Group B | Group A | Group B |
| Mean          | 78340  | 96235  | 68345  | 44567  | 73342  | 70401  |
| SD            | 2.98   | 3.76   | 2.78   | 2.49   | 2.88   | 3.12   |
| t-value       | 2.4499 | -5.4641| 2.3444 |        |        |        |
| p-value       | 0.0006*| <0.0001*| 0.0671*|        |        |        |

**Table 2: Comparison of Both the Groups on the Basis of Platelet Count (mm³)**

*The two samples are significantly different. Above table shows the comparison of platelet counts in both P. vivax and P. falciparum group that indicate the above sample are significantly different.
**Table 3: TLC Min/mm$^3$ (TLC at the Time of Admission)**

TLC min $<4000$/mm$^3$ found in 10% of P. vivax cases and 10.71% of P. falciparum cases. TLC min $>11000$/mm$^3$ was observed in 2.31% in P. vivax cases and 3.57% in P. falciparum cases. Most of cases included in study have normal TLC at the time of admission.

| TLC(min/mm$^3$) | Male | Female | Total |
|-----------------|------|--------|-------|
|                 | Group A | Group B | Group A | Group B | Group A | Group B |
| Mean            | 7865   | 5698   | 6684   | 5298   | 7274   | 5498   |
| SD              | 3.94   | 2.00   | 2.55   | 1.89   | 3.24   | 1.94   |
| t-value         | 2.4339 | -5.4441| 2.0564 |
| p-value         | 0.0007*| <0.0001*| 0.0461*|

*The two samples are significantly different. Above table shows the comparison of TLC min in both P. vivax and P. falciparum group that indicate the above sample are significantly different.

**Table 4: Comparison of both the groups on the basis of TLC (Min)/cmm$^3$**

TLC max $<4000$/mm$^3$ found in 3.08% in P. vivax cases and 4.28% in P. falciparum cases. TLC max $>11000$/mm$^3$ was observed in 8.84% in P. vivax cases and 2.85% in P. falciparum. All the P. falciparum cases included in study has normal TLC at the time of discharge and 38.07% of the P. vivax cases have normal TLC at the time of discharge.

**Table 5: TLC Max/mm$^3$ (TLC at the time of Discharge)**

**Table 6: Comparison of both the groups on the basis of TLC (max)**
*The two samples are significantly different. Above table shows the comparison of TLC max in both P. vivax and P. falciparum group that indicate the above sample are significantly different. Hemoglobin <6gm% was found in 8.07% of P. vivax cases and 15.7% in P. falciparum. 34.23% of P. vivax cases and 30.71% of P. falciparum cases has hemoglobin in the range of 6-12gm%.

|               | Group A(N=130) |            | Group B(N=70) |            |
|---------------|---------------|------------|---------------|------------|
|               | Male          | Female     | Male          | Female     |
|               | No. | %     | No. | %     | No. | %     | No. | %     |
| ≤6            | 08 | 6.15  | 13 | 10.00 | 22 | 31.43 | 0 | 0     |
| 6.1 to 12     | 43 | 33.08 | 46 | 35.38 | 34 | 48.57 | 9 | 12.86 |
| >12           | 16 | 12.31 | 04 | 3.08  | 0  | 0     | 5 | 7.14  |
| Total         | 67 | 51.54 | 63 | 48.46 | 56 | 80.00 | 14| 20.00 |

Table 7: HB (gm. %)

| HB          | Male       | Female     | Total     |
|-------------|------------|------------|-----------|
|             | Group A    | Group B    | Group A   | Group B   |
| Mean        | 7.8        | 9.0        | 7.0       | 8.0       |
| SD          | 2.94       | 2.95       | 2.55      | 1.49      |
| t-value     | 3.4339     | -9.4441    | 2.0944    |
| p-value     | 0.0008*    | <0.0001*   | 0.0381*   |

Table 8: Comparison of both the groups on the basis of HB

*The two samples are significantly different. Above table shows the comparison of hemoglobin in both P. vivax and P. falciparum group that indicate the above sample are significantly different.

| Symptoms                          | Plasmodium Vivax (130 Cases) | Plasmodium Falciparum (70 Cases) |
|-----------------------------------|-----------------------------|----------------------------------|
|                                   | Male (67 Cases) | Female (63 Cases) | Male (56 Cases) | Female (14 Cases) |
| 1. Fever                          | No. | %     | No. | %     | No. | %     | No. | %     |
| 2. Nausea/Vomiting                | 67  | 100   | 63  | 100   | 56  | 100   | 14  | 100   |
| 3. Jaundice                       | 32  | 24.62 | 41  | 31.54 | 0   | 0     | 5   | 7.14  |
| 4. Bleeding/Petechiae             | 27  | 20.77 | 27  | 20.77 | 11  | 15.71 | 0   | 0     |
| 5. Convulsion                     | 25  | 19.23 | 27  | 20.77 | 6   | 8.57  | 0   | 0     |
| 6. Headache                       | 24  | 8.46  | 26  | 20    | 23  | 32.86 | 9   | 12.86 |
| 7. Decreased Urine Output         | 7   | 5.39  | 18  | 13.85 | 11  | 15.71 | 0   | 0     |
| Signs                              |               |           |           |           |
| 1. Pallor                         | 19  | 14.62 | 32  | 24.61 | 34  | 48.57 | 14  | 20.00 |
| 2. Icterus                        | 14  | 10.77 | 19  | 14.61 | 8   | 11.43 | 9   | 12.86 |
| 3. Hepatomegaly                   | 10  | 7.69  | 13  | 10    | 45  | 64.29 | 0   | 0     |
| 4. Splenomegaly                   | 13  | 10    | 21  | 16.15 | 45  | 64.29 | 9   | 12.86 |

Comparison between Symptoms and Signs of Plasmodium Vivax and Plasmodium Falciparum
DISCUSSION: The hematological changes related with malaria infection are familiar but precise changes may vary in between P. vivax and P. falciparum with the background of hemoglobinopathy, nutritional status, demographic factors and malaria immunity.\textsuperscript{5} We observed in our study several significant changes concerning with hemoglobin, platelets and TLC. Anemia was present in 84.61% of P. vivax cases and 92.86% patients suffering from P. falciparum infection. Majority of these cases was normocytic normochromic type, a finding which is parallel with reports of facer and beals.\textsuperscript{3,6} many studies show that anemia in malaria infection is mostly of normocytic normochromic.

Pathogenesis of anemia in malaria is particularly complex multifactorial and incompletely understood it is thought to result from a combination of hemolysis of parasitized RBCs accelerated removal of both parasitized and innocently un parasitized RBCs depressed as well as ineffective erythropoiesis with dyserythropoietic changes and anemia of chronic disease.\textsuperscript{7,8} or other factors causative to anemia in malaria include decreased RBC deformability, splenic phagocytosis and/or pooling so they have an increase rate of clearance from circulation.\textsuperscript{9} TNF alpha has also been implicated and may cause ineffective erythropoiesis.\textsuperscript{8}

Anemia develop because of direct parasitization of RBC by plasmodium resulting in lysis of infected cells certain immunological factors also play a major role in development of anemia.\textsuperscript{10} Normocytic normochromic pattern was observed as pre-dominant type of anemia and it correlate with the degree of parasitemia. Reticulocytes reflects the increase activity in marrow which is due to compensatory erythroid hyperplasia.\textsuperscript{11}

The inconsistent degree of reduction in circulating platelet count are consistently reported in the different types of malaria.\textsuperscript{12} Severe thrombocytopenia is quite rare in P. vivax malaria.\textsuperscript{13} In our study 2.3% of P. vivax patients suffer from severe thrombocytopenia (<10000/cmm) while 2.85% of P. falciparum cases have severe thrombocytopenia. 25.38% of P. vivax cases have platelet count in range of 10000-50000/cmm and 27.14% of falciparum cases developed thrombocytopenia in this range.

Thrombocytopenia is consistent with finding of Robinsons et al (71%).\textsuperscript{14} and is slightly higher than that reported by other investigators Rodriguez et al (58.97%).\textsuperscript{15} and Bashwari et al (53%).\textsuperscript{16} However, percentage wise higher thrombocytopenia as observed for P. vivax in comparison to P. falciparum in thrombocytopenia <10000/cmm in our study is consistent with results reported by other investigators.

In our study 20% of P. vivax patients who developed bleeding and 16.43% of P. falciparum cases developed bleeding due to malaria seldom whatever the grade of decrease in platelets count.

The ultimate mechanism of thrombocytopenia in malaria has been not described but researchers have recommended the following mechanism which might be a causative factor for thrombocytopenia in P. falciparum and P. vivax infection:

a) Decreases thrombopoiesis but bone marrow examination usually shows normal or increase megakaryocytes.\textsuperscript{4}

b) Peripheral destruction induced by P. falciparum in which immune complexes generated by malarial antigen lead to sequestration of injured platelet by macrophages in spleen. Although this mechanism has not been properly evaluated in P. vivax malaria.\textsuperscript{17}

c) Some workers have suggested D.I.C. has a major mechanism but other have found no evidence or have hardly ever seen D.I.C in any of their patients including those with severe thrombocytopenia.\textsuperscript{18}
d) Spleen has been implicated as a site of excess sequestration however it cannot be the mechanism as most patients who develop thrombocytopenia do so early in the course of the infection before splenic enlargement has developed.

e) In acute malaria infection platelets are found to be hypersensitive and there is increased concentrations of platelet-specific proteins such as beta thromboglobulin (BTG), platelet factor 4 (PF4). Production of thromboxane A2 and prostacyclin also increased. It has also been postulated that these hypersensitive (hyperactive) platelets will enhance haemostatic responses, and may be this is why bleeding episodes are rare in acute malarial infections, despite the significant thrombocytopenia.16 

The immune mechanism contributes to destruction of platelets,19 the platelet survival is reduced in P. falciparum malaria. Enhance splenic uptake or sequestration may contribute to thrombocytopenia. In patient with DIC platelet may remove from the circulation at the site of fibrin deposition. Thrombocytopenia is common finding in P. falciparum and P. vivax but it does not correlate with severity, unless it is profound i.e. <20000/cmm7 but in our study platelet counts <10000/cmm is more common in P. vivax malaria i.e. 2.3%.

Contrasting to some studies which showed leucopenia is a common finding in both non-immune and semi immune patients.3 Stages of infection detection is important and also the drug effect since anti-malarial drugs effect the leukocytes count because majority of cells are in expanded marginal pool. Neutrophil counts usually remain normal neutrophilia associated with bacterial infection.

CONCLUSION: The study concludes that P. falciparum as well as P. vivax can cause significant hematological changes with high occurrence of thrombocytopenia, anemia and TLC min and TLC max.

The blood changes are so distinguishing that the diagnosis of malaria should be considered in the existence of above findings.17

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