

Descriptive Study of the Hematological Parameters with Special Reference to the Total Leucocyte and Platelet Count in Cases of Malaria in all age Groups

P. Fotedar¹, S. S. Rairikar^{2*}, A. J. Vankudre³ and S. V. Mahajan⁴

¹Senior Resident, Department of Pathology, Dr. Vasant Rao Pawar Medical College, Nashik; fotedar_p@yahoo.com

²Associate Professor, Department of Pathology, Dr. Vasant Rao Pawar Medical College, Nashik; shubhrai46@gmail.com

³Assistant Professor Department of Community Medicine, Dr. Vasant Rao Pawar Medical College, Nashik; easyashok@gmail.com

⁴Professor and Head, Department of Pathology, Dr. Vasant Rao Pawar Medical College, Nashik; drmahajansuresh@yahoo.co.in

Abstract

Introduction & Aim: To study variation of haematological parameters in different species of malaria with special reference to the total leucocyte and platelet count. **Methods:** This study was conducted in a Tertiary Health Care Centre, from June 2010 to June 2012. All positive cases of malaria proved on peripheral blood smear in both IPD and OPD patients were included in our study and all cases negative for malaria were excluded. **Results:** During this study, 119 positive cases of malaria were found in all OPD and IPD patients. In these, 74 cases (62.2%) were of Plasmodium vivax, 35 cases (29.4%) were mixed infections and 10 cases (8.4%) were of Plasmodium falciparum. Amongst the positive cases the ring forms of Plasmodium vivax, mixed infection & Plasmodium falciparum were 56(47.1%), 35(29.4%) and 4(3.4%) respectively. Out of the 109 pure vivax and mixed infection cases, the number of cases showing amoeboid forms were 14(11.8%). Cases of immature schizont were 75(63%) and mature schizont were 17(14.3%). The gametocytes of Plasmodium vivax, mixed infections & Plasmodium falciparum in our study were 5(17.9%), 2(7.1%) and 21(75%) respectively. Mean & Median platelet count in our study was 1,05,075/cumm & 90,000/cumm respectively. The mean platelet count was found significantly lower in cases of P. vivax having gametocyte compared to P. falciparum with gametocyte. Higher parasitic index was associated with lower platelet count.

Keywords: Haematological Parameters, Malaria, Parasitemia.

1. Introduction

Malaria is a mosquito borne disease caused by a eukaryotic protist of the genus Plasmodium. Plasmodium vivax is the most frequent and widely distributed cause of the recurring (tertian) malaria worldwide. Malaria¹ is one of the most important parasitic disease in human with transmission in 103 countries, affecting more than one billion people and causing between one to three million deaths each year. Most deaths² occur because of Plasmodium falciparum infection¹, which causes life threatening cerebral,

respiratory, renal, hepatic, hemodynamic, and hematologic dysfunction in 1% of cases¹. In contrast, most Plasmodium vivax infections are relatively milder and run a benign course. Falciparum malaria¹ presents with protean manifestations and is associated with a variety of complications and has a high mortality. The global case fatality rate of falciparum infection is around 2 million deaths per year³. A hematological change like progressively increasing anaemia, thrombocytopenia and rarely disseminated intravascular coagulopathy has been reported in Plasmodium falciparum malaria².

*Author for correspondence

Malaria infection has been increasing over recent years due to a combination of factors including:

1. Increasing resistance of malarial parasites to chemotherapy.
2. Increasing resistance of the *Anopheles* mosquito vector to insecticides.
3. Ecologic and climate changes.
4. Increased international travel to malaria endemic areas⁴.

Hematological changes are considered a hallmark of malaria and reported to be most pronounced in *Plasmodium falciparum* infection, probably as a result of higher level of parasitaemia found in these patients⁵. The cause of thrombocytopenia is poorly understood, although increased platelet destruction is significant and platelet lifespan is reduced during malaria. It is often associated with palpable splenomegaly and circulating immune complexes. Thrombocytopenia⁶ alone was said to be a predictor for malaria and with combination of anaemia it was considered as the next best parameter. The inflammatory responses to *Plasmodium falciparum* infection in malaria results in decrease in the peripheral platelet counts which may be implicated in development of disseminated intravascular coagulation⁷. Thrombopoietin (TPO)⁸, expressed constitutively by the liver is the most important lineage specific growth factor for megakaryothrombocytopoiesis. White Blood Cell (WBC) counts during malaria are generally characterized as being low to normal, a phenomenon that is widely thought to reflect localization of leukocytes away from the peripheral circulation, to the spleen and other marginal pools, rather than actual depletion or stasis⁹.

2. Materials and Methods

After the approval from the Ethics Committee, this descriptive study was carried out in our tertiary care hospital affiliated to a teaching institute. The period of the study was from June 2010 to June 2012. The smear was reported positive for malarial parasite on peripheral blood smear when ring forms, schizonts or the sexual forms of any of the species of malaria was seen. They were further classified according to their characteristics, morphologic features on microscopy.

A total of 119 cases were found to be fulfilling the above criteria. All positive cases of malaria proved on

peripheral blood smear in both IPD and OPD patients were included in our study. Under all aseptic precautions, 2 ml of venous blood sample was collected from the ante-cubital vein, a drop was placed on the clean glass slide for the preparation of the smear and the rest was transferred into a properly labeled purple topped bulb (EDTA bulb). The sample was shaken properly and then was run on the automated analyzers ERMA or BECKMAN COULTER. Peripheral smear was made and slides were stained using Field's stain.

The stained smear was thoroughly examined for the malarial parasite in the area where the smear was evenly spread and the RBC's were just touching each other.

If the malarial parasite was present in the smear then it was further evaluated for the specific species and the specific form or stage of the parasite. A thorough search was made for the presence of the sexual forms of the parasite. Parasitic index was given on the smear. The level of parasitemia was expressed either as a percentage of parasitized erythrocytes. The smear was considered negative if no parasite was found in 200 oil immersion fields.

Rapid Diagnostic Tests (RDT) were performed only on clinical advice along with peripheral smear examination. Only smear positive patients were included in the study. Smear negative patients but RDT positive patients were not included.

Full blood counts were done on the automated analyzers differential WBC count was also done on the same smear by counting 100 WBC's patients with decreased platelet counts (thrombocytopenia) were re-evaluated by manual method. According to severity, patients with thrombocytopenia have been divided into three main groups:

Mild thrombocytopenia-having platelets counts between $50-150 \times 10^3$ cells/ μ l.

Moderate thrombocytopenia-having platelet count between $20-50 \times 10^3$ cells/ μ l.

Severe thrombocytopenia-having platelets count less than 20×10^3 cells/ μ l^{10,11,12}.

3. Results

The mean age of our study population was 27.62 ± 14.72 years. In the study 81 cases (68.1%) were males and 38 cases (31.9%) were females. Distribution of various hematological parameters are as follows. (Table 1)

As shown in Figure 1, maximum cases belonged to *P. vivax* species (62%).

Table 2 shows that the maximum ring forms were seen in *P. vivax* (56).

Out of the 109 pure vivax and mixed infection cases the most predominant morphological form of *P. vivax* was immature schizont 75 cases (63%). (Table 3)

Table 4 shows maximum gametocytes were seen in *P. falciparum*, 21(75%) compared with *P. vivax*.

Of the 119 cases studied maximum (82) patient showed platelet count between 50,001–150000 while only 21 patients had normal platelet count. (Table 5)

As per Table 6, there is a significant low platelet count in *P. vivax* species compared to *P. falciparum* ($P < 0.05$).

Table 1. Various haematological parameters in study group

| Sr.No | Haematological parameters | Mean ± SD |
|-------|-----------------------------|------------------------|
| 1 | Hemoglobin (Hb) | 10.23 ± 3.01 gm/dl |
| 2 | Total leucocyte count (TLC) | 6768 ± 4676/cumm |
| 3 | Neutrophil count | 57.48 ± 14.64%, |
| 4 | Lymphocyte count | 39.21 ± 13.43%, |
| 5 | Eosinophil count | 0.80 ± 1.36% |
| 6 | Monocyte count | 2.66 ± 2.85% |
| 7 | Platelet count | 1,05,075 ± 64,977/cumm |

Table 2. Distribution of species of malaria as per ring forms

| Species of malaria | Frequency (%) |
|--|---------------|
| Ring forms of <i>P. vivax</i> | 56 (47.1%) |
| Ring forms of both <i>P. vivax</i> and <i>falciparum</i> | 35 (29.4%) |
| Ring forms of <i>P. falciparum</i> | 4 (3.4%) |

Table 3. Frequency of various forms of *P. vivax*

| Form of Vivax Species | Frequency (%) |
|-----------------------|---------------|
| Amoeboid form | 14 (11.8%) |
| Immature schizont | 75 (63.0%) |
| Mature schizont | 17 (14.3%) |

Table 6. Association of platelet count and the gametocytes of *P. vivax* and *falciparum* in cases of malaria

| | Platelet Count | | | P Value |
|-----------------------------------|----------------|----------------|--------------|---------|
| | n | Mean ± S.D | Min–Max | |
| Gametocyte of <i>P.vivax</i> | 5 | 67804 ± 33003 | 33000–112000 | 0.037 |
| Gametocyte of <i>P.falciparum</i> | 21 | 125005 ± 54851 | 54000–234000 | |

4. Discussion

The present study was done with the aim to find out the hematological changes that occur in malaria infection, i.e. changes in hemoglobin and total leucocyte count, with special reference to platelet count. Also we tried to find out whether there was any significance of the platelet count abnormalities with the different species of malaria, different morphological forms of malaria and gametocytes of malaria. Further association of Total Leucocyte Count with platelet count was made.

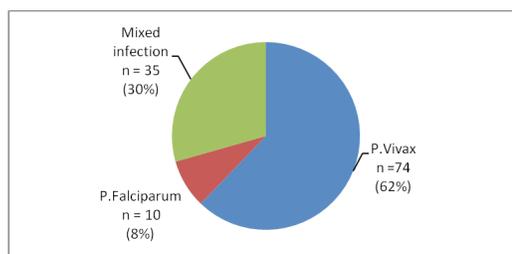


Figure 1. Graph showing distribution of malarial cases as per species (N = 119).

Table 4. Distribution of gametocyte as per malarial species

| Malarial species | Frequency (%) |
|-------------------------------------|---------------|
| Gametocyte of <i>P. vivax</i> | 5 (17.9%) |
| Gametocytes of mixed infection | 2 (7.1%) |
| Gametocytes of <i>P. falciparum</i> | 21 (75%) |

Table 5. Distribution of cases according to the severity of thrombocytopenia

| Platelet count (Cumm) | Frequency (n=119) | % |
|-----------------------|-------------------|-------|
| < 20,000 | 1 | 0.8% |
| 20,000–50,000 | 15 | 12.6% |
| 50,001–1,50,000 | 82 | 68.9% |
| >1,50,000 | 21 | 17.6% |

4.1 Species Wise Distribution of Malarial Parasite

In our study, out of the 119 malaria positive cases, 74 cases (62.2%) were of *P. vivax*. 10 cases (8.4%) were of *P. falciparum* and the mixed infection constituted 35 cases (29.4%). This was in concordance with the findings of Erhart LM et al.¹³ and Jadhav UM et al.³, where *vivax* was seen in 59% and 62% cases respectively. However, Rathod DA et al.², Rasheed A et al.¹⁴ and Abro et al.¹⁵ found out that *falciparum* was the most common species in their studies.

P. malariae and *ovale* was not found in our study.

4.2 Anaemia in Malaria

Of the 119 cases evaluated, the mean hemoglobin was 10.23 ± 3.01 gm/dl. The incidence of anaemia in our study was 61.3%. Anemia was also found in studies done by Menendez C et al.¹⁶, Abdalla S et al.¹⁷, Sitalakshmi S et al.¹⁸

Rasheed A et al.¹⁴, who found out 50% patients infected with *P. falciparum* and mixed infections to be anaemic while 29% patients infected with *P. vivax* had anaemia. In the study done by UM Jadhav et al.³, they found the mean hemoglobin as 11.6 gm/dl in patients infected with *P. falciparum* and 12.5 gm/dl in patients infected with *P. vivax*, the mean hemoglobin was higher as compared in our study. It is a known fact that malaria is the most common cause of hemolytic anaemia¹⁹. Hemolysis in malaria has been attributed to direct damage of the red cell by the parasite, autoimmune destruction, hypersplenism, splenic pitting with formation of microspherocytes, and loss of negative charge from the red cell. Surface secondary to usurpation of the red cell's metabolic functions by the parasite²⁰. *P. vivax* invades only young red cells, while *P. falciparum* invades both young and old cells. Thus, anaemia tends to be more severe in the latter form of malaria¹⁹.

4.3 Parasite Morphology

Ring forms of *P. vivax* were seen in 56 cases (47.1%), ring forms of mixed Plasmodium infection were seen in 35 cases (29.4%) and ring forms of only *P. falciparum* were in 4 cases (3.4%). Amoeboid form of *P. vivax* was seen 14 cases (11.8%), Immature schizonts were present in 75 cases (63%) and mature schizont were present in 17 cases (14.3%). Gametocytes of *P. vivax* were seen in 5 cases (6.8%) and of *falciparum* were seen in 21 cases

(17.6%). However only 2 cases (1.7%) were found where gametocytes of both *P. vivax* and *falciparum* were seen.

In the present study there was a statistically significant finding between the different platelet counts in patients showing gametocytes of *P. vivax* and the gametocytes of *P. falciparum*. Though the majority of our cases came in the mild thrombocytopenia category, it was found that the infection with gametocytes of *P. vivax* were associated with lower platelet count as compared to *P. falciparum*. The mean platelet count in gametocytes of *P. vivax* was 69571 ± 27269 /cumm and the mean platelet count in the gametocytes of *P. falciparum* was 119355 ± 55206 /cumm, with a P value of 0.004. This was in concordance with the study done by F E McKenzie et al²¹ which showed that *P. vivax* patients with gametocytes had lower platelet count than in the *P. vivax* patients without gametocytemia, whereas *P. falciparum* patients with gametocytemia had similar platelet counts than patients without gametocytemia²¹. In our cases studied we found no significant correlation between thrombocytopenia and the two species of malaria, the various morphological forms of *P. vivax* (i.e. amoeboid, ring, mature and immature schizonts), were not associated with any particular platelet category.

4.4 Level of Parasitemia

The mean parasitic index in our study was 1.92 ± 2.12 % with a median of

1.0%. As already mentioned that there was a negative relationship between the parasitic index and the platelet count. The mean parasitic index in the severe thrombocytopenia category was 6.0 ± 0.0 %, the mean parasitic index in the moderate thrombocytopenia category was 3.13 ± 0.87 %, and the mean parasitic index in the mild thrombocytopenia category was 1.75 ± 1.73 % which is statistically significant. There were 9 cases with a parasitic index of > 4% and having *P. vivax* and mixed infection. Hence high parasitic index is associated with lower platelet count.

4.5 Evaluation of Platelet Parameters

In our study of 119 cases we came across 98 cases (82.4%) who have shown various degree of thrombocytopenia ranging from mild to severe in intensity. This is comparable to the results of the studies done by Rasheed et al¹⁴, Sharma et al.²², Um Jadhav et al.³, Abro et al.¹⁵, Sheraz Jamal Khan et al.⁶, Faseela et al.²³, Shaikh QH et al.²⁴, which

showed thrombocytopenia of 80%, 90%, 79.4%, 87.27%, 58%, 82.7%, 80.6% respectively. Thrombocytopenia is a common feature of acute malaria and occurs in both *P. falciparum* and *P. vivax* infections regardless of the severity of infection. The absence of normal quantity of platelets on a peripheral blood smear in a case of fever is often a clue for the diagnosis of malaria³.

4.6 Evaluation of Total Leucocyte Count

We got 15 cases (12.6%) whose Total Leucocyte Count was below 4000/cumm i.e. patients had leucopenia. Amongst these maximum cases were associated with *P. vivax* and mixed infection, only one case was there with the total count of 2500/cumm associated with *P. falciparum* infection. Hence there were 10 cases (13.5%) of *P. vivax* which showed leucopenia, 1 case (10%) of *P. falciparum* and 4 cases (11.4%) of mixed infection which showed low total leucocyte counts. Rasheed et al¹⁴ showed similar findings in their study, leucopenia was present in 22.1%, 20.9% and 18.4% patients in *P. vivax*, *P. falciparum* and mixed infection¹⁴. There were 9 cases (7.5%) which showed leucocytosis. Leucocytosis i.e. Total Leucocyte Count of >11,000/cumm was seen in 9 cases with infection by *P. vivax* and mixed infection. With no cases of pure *P. falciparum* showing leucocytosis. 6 cases (8.1%) *P. vivax* showed leucocytosis and 3 cases. 8.5% of mixed infection showed leucocytosis. The comparison between leucocyte count and the species of malaria did not reveal any statistically significant findings also there was no statistically significant evidence of neutrophilia or lymphocytosis in our cases, the eosinophils and the monocytes were also within normal range.

5. Conclusion

Thrombocytopenia was associated more with the gametocytes of *P. vivax* than with gametocytes of *falciparum*.

Lower platelet counts were associated with higher parasitic index.

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