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# Hematological changes in malaria: A comparative study

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***Abstract***
***Objectives:*** *The hematological changes usually associated with malaria are well known. This study was conducted to estimate and compare the predominance & severity of hematological changes in common types of malaria.*
***Methodology:*** *This observational study included 400 suspected malarial patients attended in Out Patient Department (OPD) and In Patient Department (IPD) of NKP Salve Institute of Medical Sciences & Research Centre and Lata Mangeshkar Hospital Nagpur, during April 2009 to March 2011. The diagnosis of malaria was confirmed by thick and thin film stained with Leishman's staining for malaria parasite and Antigen test (i.e. HRP2). Complete Blood Counts (CBCs) were performed using an automated SYSMEX machine.*
***Results:*** *Among the samples of consenting participants tested, 70% of the patient had thrombocytopenia, 94% anemia, 12% lymphopenia and 17% monocytosis. The incidence of thrombocytopenia was slightly more in P. Falciparum (58.69%) than P. Vivax (30.18%) cases, p value > 0.05, whereas there was no significant difference in the incidence of anemia in two groups (34.68% vs 33.82%) with p value > 0.05. However, lymphopenia was observed in 33.33% cases of P. Vivax as compared to 11.11% in P.Falciparum cases, p value < 0.04. Eosinophilia was 12.16% and basophil count was normal in both groups.*
***Conclusions:*** *P.Falciparum as well as P.Vivax can cause significant hematological changes with high incidence of thromboctopenia, anemia, lymphopenia and monocytosis.*
***Key Words:*** *Hematology, Malaria, Plasmodium falciparum, Plasmodium vivax*

## I. Introduction

Malaria is well-known to human being since centuries; it is a disease of tropical and subtropical countries particularly Africa and Asia. In spite of advances information, malaria continues to cause significant morbidity and mortality worldwide. Malaria is one of the most prevailing human infections in the world. More than 40% of the world population reside in malaria-endemic area and it is predictable that 300-500 million cases and 1.5-2.7 million deaths occur each year.[1] Mortality rate is usually elevated (20%) in severe malaria (parasitemia >5%).[2] Hematological changes, which are the 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, atypical lymphocytosis and infrequently disseminated intravascular coagulation.[3] Leucopenia, leucocytosis, Neutopenia, Neutrophilia, Eosinophilia and monocytosis also have been reported.[2,4] The aim of this study was to assess the hematological changes which occurs in different types of malaria. In tropical countries like India, malaria remains an essential health problem. The infection rate of the world population was 250 million per year, and the mortality rate was 1-2 million per year.[1] In the present day, the most vital difficulty in the management of malaria is drug resistance of *P. falciparum* to the various anti-malarial drugs. The majorities of the shared complications commencing due to malarial consequences is from hyperparasiteamia. Mortality is very high (10- 30%) in complicated *P. falciparum* infection. Hematological changes play key role in these lethal complications.

## II. Material And Methods

The present comparative cross sectional study was conducted in central hospital laboratory of NKP SIMS & RC and LMH Hospital, Nagpur over two years period from April 2009 to March 2011.The clinically suspected cases of malaria were included in the study. The diagnosis of malaria was confirmed by thin and thick blood films stained with Leishman's stain for malaria parasite and Antigen Histadine Release Protein 2 (HRP2) test. The study was premeditated to include clinically suspected cases of malaria and patients were excluded on the basis of history and finding suggestive of Dengue, chronic liver disease, bleeding disorder, thrombocytopenia, drug intake or conditions which might have contributed in blood changes. Complete Blood Count was performed using an automated SYSMEX machine and WBC differential was also done for all patients. All malaria positive smears were studied for confirmation, identification of species and review of

smear for platelets count and other hematological changes. Data was analyzed by Epi.Info Statistical Software. *p* value of < 0.05 was taken as significant for all statistical analysis.

## III. Results

This study included 400 patients, out of which 74 (18.5%) patients were found to harbor malaria parasite by either of the techniques (*Table: I*). *P.Falciparum* malaria was commoner than *P.Vivax* having 39 cases (52.7%) versus 27 cases (36.48%) respectively, while *Mixed infection* represented only 8 cases (10.81%). Out of all the malaria positive cases, majority of cases i.e. 64 cases (86.48%) showed subnormal haemoglobin. However, significant difference in the incidence of anaemia in *P.Falciparum* 35(89.7%) and *P.Vivax* 23(85.18%) cases with p value ( > 0.05) was found, which is in contrast to the observation by Murphy GS, Oldfeild EC.[2]

**Table I: Distribution of species in Malaria Positive Cases (n=74)**

| S No | Species | Number | Percentage |
|---|---|---|---|
| 1 | **P. falciparum** | 39 | 52.71 |
| 2 | **P. vivax** | 27 | 36.48 |
| 3 | **Mixed infections** | 08 | 10.81 |
| Total | | 74 | 100 |

The total leucocytic count was normal in 60 (86%) whereas Differential leukocyte count showed normal neutrophil count in 63(85.1%), normal lymphocytes in 51(68.9%), normal monocytes in 58(78.4%), normal basophil in 73(98.99%) and normal eosinophils in 63(85.1%) patients. Monocyte as well as neutrophils were increased respectively in 14 (18.9%) and 9(12.6%) cases.However, lymphopenia was present in 18 (24.32%) cases. Commonly 53 (71.6%) had thrombocytopenia and 20 (27%) had normal platelets. Majority of the cases which showed thrombocytopenia, 31(79.48%) cases were *P.Falciparum*, 16 (59.25%) cases were *P.Vivax* and 6(75 %) cases were *Mix.infection. (Table-2).*

**Table II: Hematological profile of malaria positive cases (n=74)**

| Parameter → Species↓ | Hb% | TLC | Neu | Lymph | Mono | Eosin | Baso | Platelets |
|---|---|---|---|---|---|---|---|---|
| **P. falciparum** | 64 (84.6%) | 8 (11%) | 3 (4%) | 18 (24.3%) | 2 (3%) | 2 (2.7%) | 1 (1.23%) | 53 (71.6%) |
| **P. vivax** | 10 (13%) | 60 (80%) | 63 (84%) | 51 (68.9%) | 58 (80%) | 63 (85%) | 73 (98%) | 20 (27%) |
| **Mixed infections** | 00 (0.00%) | 6 (9%) | 8 (12%) | 5 (6.76%) | 14 (17%) | 9 (12.6%) | 00 (0.00%) | 1 (1.35%) |

The Peripheral smear examination showed 35(47.3%) of the patients were anemic with normocytic normochromic except in 29 (39.2%), where it was Normocytic hypochromic *(Table: III).* It was observed that 3 cases (4.05%) had Macrocytic Microcytic peripheral smear. Nucleated Red Blood Corpuscles (NRBCs) /100 White Blood Corpuscles (WBCs) were seen in 32(43.2%) cases. Hypersegmented nuetrophils in 2(2.7%) cases and toxic changes in 6(8.1%) cases were also seen. Atypical lymphocytes were observed in 4(5.4%) cases with their predominance in *P.Falciparum* 3(7.6%) and *P.Vivax* 1(3.7%) cases. Reticulocytes count was found to be raised in 31(41.9%) cases.

**Table III: Distribution of Peripheral smear Changes species wise in malaria Positive cases (n=74):**

| Species → Variable↓ | Pv (n=27) | Pf (n=39) | Mix (n=8) | Total (n=74) |
|---|---|---|---|---|
| Normocytic normochromic | 18 (66.70%) | 17 (43.60%) | 00 (00.00%) | 35 (47.30%) |
| Normocytic hypoochromic | 16 (59.25%) | 13 (33.33%) | 00 (00.00%) | 29 (39.20%) |
| Macrocytic microcytic | 01 (03.70%) | 02 (05.12%) | 00 (00.00%) | 03 (04.05%) |
| NRBCs* | 11(40.70%) | 20 (51.20%) | 01 (12.50%) | 32 (43.20%) |
| Reticulocytes | 10 (37.00%) | 18 (46.15%) | 03 (37.50%) | 31 (41.90%) |
| Toxic granules | 02 (07.40%) | 03 (07.69%) | 01 (12.50%) | 06 (08.10%) |
| Atypical lymphocytes | 01 (03.70%) | 03 (07.60%) | 00 (00.00%) | 04 (05.40%) |
| Hypersegmented polymorphs | 00 (00.00%) | 02 (05.12%) | 00 (00.00%) | 02 (02.70%) |

*Nucleated Red Blood Corpuscles

When hematological values were compared with malaria species, there was no significant difference in the incidence of anemia in *P.Falciparum* 35(89.7%) and *P.Vivax 23* (85.18%) cases with *p* value >0.05, but thrombocytopenia was slightly predominant in *P.Falciparum* 31(79.48%) than *P.Vivax* 16(59.25%) with *p* value >0.05 *(Table-IV).* However, there was significant difference in lymphocyte count in two groups and lymphopenia was observed in 3 (11.11%) in *P.Vivax* as compare to 13(33.33%) in *P.Falciparum* with *p* value <0.04. No difference was found in monocyte, eosinophil and basophil count in *P.Falciparum* and *P.Vivax* group. Majority of thrombocytopenia cases had not reported bleeding due to thrombocytopenia.

**Table IV: Distribution of Hematological Changes species wise in Positive cases (n=74):**

| **Species →<br>Parameter↓** | **Pv** | **Pf** | **Mixed** | **Total** |
|---|---|---|---|---|
| Number | 27 | 39 | 8 | 74 |
| Anemia | 23 (85.18%) | 35 (89.7%) | 06 (75.00%) | 64(86.48%) |
| Normal Haemoglobin % | 04 (14.80%) | 04(10.26%) | 02 (25.00%) | 10(13.5%) |
| Thrombocytopenia | 16 (59.25%) | 31(79.48%) | 6(75 %) | 53 (71.6%) |
| Normal Platelet Count | 08 (29.60%) | 10 (25.64%) | 02 (25.00%) | 20(27.00%) |
| Thrombocytosis | 01 (01.35%) | 00 (00.00%) | 00 (00.00%) | 01(01.35%) |
| Leucopenia | 03 (11.11%) | 03 (07.69%) | 02 (25.00%) | 08(10.80%) |
| Normal White Blood Cell Count | 24 (88.88%) | 34 (87.17%) | 02 (25.00%) | 60(81.10%) |
| Leucocytosis | 02 (07.40%) | 03 (07.69%) | 01 (12.50%) | 06(08.10%) |
| Neutropenia | 01 (33.33%) | 02 (66.66%) | 00 (00.00%) | 03(04.10%) |
| Normal Neutrophil | 24 (88.90%) | 35 (89.74%) | 04 (25.00%) | 63(85.16%) |
| Neutrophilia | 03 (11.11%) | 04 (10.25%) | 01 (12.50%) | 08(10.80%) |
| Lymphopenia | 03 (11.11%) | 13 (33.33%) | 02 (25.00%) | 18(24.32%) |
| Normal Lymphocyte Count | 21 (77.81%) | 33 (84.60%) | 03 (37.50%) | 51(68.96%) |
| Lymphocytosis | 01 (03.70%) | 03 (17.70%) | 01 (12.50%) | 05(06.76%) |
| Eosinopenia | 00 (00.00%) | 01 (02.56%) | 01 ((12.50%) | 02(02.70%) |
| Normal Eosinophil | 25 (85.20%) | 37 (94.90%) | 01 (12.50%) | 63(85.10%) |
| Eosinophilia | 04 (14.80%) | 04 (10.25%) | 01 (12.50%) | 09(12.16%) |
| Monocytopenia | 00 (00.00%) | 01 (02.70%) | 01 (12.50%) | 02(02.70%) |
| Normal Monocyte Count | 25 (85.20%) | 34 (94.90%) | 01 (12.50%) | 58(78.40%) |
| Monocytosis | 05 (18.50%) | 07 (17.90%) | 02 (25.00%) | 14(18.90%) |
| Basopenia | 00 (00.00%) | 00 (00.00%) | 00 (00.00%) | 00 (00.00%) |
| Normal Basophil Count | 27 (100.0%) | 37 (17.90%) | 08 (100.0%) | 73(98.99%) |
| Basophilia | 00 (00.00%) | 00 (00.00%) | 00 (00.00%) | 01(01.32%) |

Pv= Plasmodium vivax; Pf= Plasmodium falciparum; Mix= Mixed infection

## IV. Discussion

The hematological changes related with malaria infection are familiar, but precise changes may vary with category of malaria, with the background of hemoglobinopathy, nutritional status, demographic factors and malaria immunity.[5] We observed in our study several significant changes concerning with hemoglobin, platelets and white cells. Anemia was present in 86.48% and in majority of these cases was normocytic normochromic type, a finding which is parallel with the reports of Facer and Beals. [3, 6] The pathogenesis of anemia in malaria is particularly complex, multi factorial and incompletely understood. It is thought to result from a combination of hemolysis of parasitized red blood cells; accelerated removal of both parasitized and innocently un-parasitized red blood cell, depressed as well as ineffective erythropoiesis with dys-erythropoietic changes and anemia of chronic disease. [7, 8] Other factors causative to anemia in malaria include decreased red blood cell deformability, splenic phagocytosis and/or pooling, so they have an increased rate of clearance from the circulation.[9] Tumour necrosis factor alpha (TNF-α) has also been implicated and may cause ineffective erythropoiesis.[8]

Anemia develops because of direct parasitization of erythrocytes by plasmodium resulting in lysis of infected cells. Certain immunological factors also play a major role in development of anemia.[9] Nonrmocytic normochromic pattern was observed as the predominant type of anaemia and it correlate with the degree of parasitemia.[10] Reticulocytes reflects the increase erythroid activity in the marrow which is due to compensatory erythroid hyperplasia.[11]

The inconsistent degree of reduction in circulating platelet count are consistently reported in the different types of malaria.[12] Severe thrombocytopenia is quite rare in *P.Vivax* malaria[13]. In our study 71.6% of patients with malaria developed thrombocytopenia, is consistent with finding of Robinsons et al (71%)[14] and is slightly higher than that reported by other investigators Rodriguez et al(58.97%)[15] and Bashwari et al (53%)[16].

There was no significant difference in the incidence of thrombocytotopenia between *P.Falciparum* (79.5%) and *P.Vivax* (59.2%). However, percentage wise higher thrombocytopenia as observed for *P.Falciparum* in comparison to *P.Vivax* in our study is consistent with results reported by other investigators[17]. Patient who developed thrombocytopenia due to malaria seldom bleed, whatever the grade of decrease in platelets count[17].Our study also did not observe any bleeding predisposition even with platelets count of 9000/cmm.

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) Decreased thrombopoiesis, but bone marrow examination usually shows normal or increased megakaryocytes [4];

(b) Peripheral destruction, induced by *P. falciparum*, in which immune complexes generated by malarial antigens lead to sequestration of the injured platelets by macrophages in the spleen, although this mechanism has not been properly evaluated in *P. vivax* malaria[17];

(c) Some workers have suggested Disseminated Intravascular Coagulation (DIC) as a major mechanism, but others have found no evidence or have hardly ever seen DIC in any of their patients, including those with severe thrombocytopenia[18];

(d) The spleen has been implicated as a site of excess sequestration. Splenomegaly alone, however, 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 (ßTG), platelet factor 4 (PF4). Production of thromboxane A2 and prostacyclin also increased[18]. 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].

In our study thrombocytopenia was observed in 71.6% as shown in previous studies [10]. An immune mechanism contributes to destruction of platelets[19].The platelets survival is reduced in severe P. *falciparum* malaria. Enhanced splenic uptake or sequestration may contribute to thrombocytopenia. In patients with disseminated intravascular coagulation (DIC) platelets may removed from the circulation at sites of fibrin deposition. Thrombocytopenia in common findings in *P. falciparum* and *P. vivax* malaria and it does not correlate with severity, unless it is profound i.e. <20,000/cmm[7].

Contrasting to some studies which showed leukopenia a common finding in both non-immune and semi immune patients[3], we observed normal WBC count in 81.1% of the patients. Neutrophil count was normal in 85.1% of cases, a finding which differs from earlier reports of either neutropenia or neutrophilia among malaria cases[20]. Yet, lymphopenia and monocytosis was noticed in 24.32% and 18.90% patients respectively which is consistent with the previous studies [20, 21]. Normal eosinophil and basophil count was found in 82.4% and 98.99 %, which support former studies[3].

The present study showed total and differential count was normal in few number of cases. Similar views have been expressed by some authors[22]. Stages of infection detection is important and also the drugs effect since antimalarial drugs affect the leucocytes count. Because majority of cells are in expanded marginal pool, Neutrophil count usually remain normal. Neutrophils leucocytosis occurs due to associated bacterial infection[17].

Leucopenia and nuetropenia primarily reflects the state of hypersplenism. Eosinophilia observed occasionally following malarial chemotherapy and reactive Eosinophilia in weeks is in accordance with the study conducted by some authors [22] Monocytosis develops in more severe cases invariably in period of convalescences, which reflects the elevated activity of reticulo-endothelial system(RES).

## V. Conclusions

The study concludes that *P.Falciparum* as well as *P.Vivax* can cause significant hematological changes with high occurrence of thrombocytopenia, anemia and lymphopenia. 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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