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Original Research Article

Correlation of Malaria Parasite Density with Blood Cell Counts: A Study in an Endemic Region of Northern India

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Abstract

Malaria is an important public health concern especially in endemic nations like India. Hematologic changes play a major role in fatalities. The present study was conducted with an objective to assess blood cell counts in malaria and correlate them with parasite density, so as to enforce their role as indicators of disease severity. A total of 144 malaria positive cases diagnosed at JN Medical College and Hospital, Aligarh during the period from November 2014 to October 2015 were included in this study. Complete blood cell count was assessed along with peripheral blood smear examination of each patient for parasite identification and calculation of parasite density. The results were statistically analyzed using SPSS version 19. A significant decreasing trend of haemoglobin concentration, leukocyte count and platelets was noted with increasing parasite density ($p < 0.001$). Eosinophilia was also a key finding. In conclusion, a spectrum of deranged blood parameters such as a low leukocyte count and eosinophilia along with anemia and thrombocytopenia may serve as a basis for immediate and aggressive treatment approach in severely febrile patients of malaria.

Keywords: Malaria Parasite Density; Anemia; Leucopenia; Eosinophilia.

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Introduction

Hematologic changes, which are the most common complications of malaria, play a major role in fatalities. They include anemia, cytoadherence of infected red cells, leukocyte changes followed by the induction of cytokines, thrombocytopenia and coagulopathy, particularly disseminated intravascular coagulation [1]. Analysis of the hematological changes may be helpful in guiding the clinician to institute an early and specific therapeutic intervention in order to prevent major complications. These parameters may serve as a marker for disease prognosis.

Hyperparasitemia has been observed to affect many haematological parameters, while certain parameters guide us to a diagnosis of malaria in cases of low parasite density [2,3].

In the present study we have made an attempt to correlate leukocyte count and platelet count with malaria parasite density, thereby enforcing their role as indicators of disease severity and outcome.

Material & Methods

The study was conducted on patients admitted to JN Medical College and Hospital, Aligarh and presenting with chief complaints of fever with or without chills and rigors, splenomegaly and associated signs and symptoms suggesting complications of malaria such as headache, jaundice, altered sensorium etc. during the period from November 2014 to October 2015. The study was approved by the Institutional Ethics Committee. Out of a total of 808 febrile patients included in the study, 144 cases were diagnosed with malaria by a positive smear report. Patients with a history of intake of antimalarial treatment or any other established systemic infection like enteric fever, dengue fever etc. were excluded from the study. All the cases were subjected to a detailed history including demographic profile, history regarding the duration of fever, its nature and associated symptoms, treatment history (antimalarial drugs, antibiotics etc.), past history of any fever. In our study the following parameters were assessed; Clinical profile of the patients, laboratory data including a complete blood count by automated five part hematology analyzer (Lab Life H3D Premier automated hematology analyzer, RFCL) and peripheral blood smear examination of each patient for parasite identification along with calculation of parasite density along with a view for platelet count and differential count. 3ml venous blood was sampled in EDTA vacutainer which was pre-labelled along with clean glass slides with the patient's name, age/sex and ID no. Both thick and thin blood films were prepared and stained with Leishman and Giemsa stains respectively on different slides. White blood cells (WBC), red blood cells (RBC) and platelet counts were assessed using the automated analyzer along with other available laboratory data including Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW). Blood smear was also analyzed for species identification and calculation of parasite density along with a manual review of platelet count and differential leukocyte count. Parasite density was calculated by the formula given below.

$$\text{Parasites per microlitre of blood} = \frac{\text{Number of parasites} \times 8000\ [4]}{500\ \text{WBC}}$$

Parasites were recorded per 500 WBCs except in cases where parasite load was high and was as frequent as the WBCs when only 200 WBCs were counted. The parasite density was estimated using 8,000 WBC/µl of blood as a standard value. Thus, if 500 WBCs were counted and the number of parasites counted was 10, the parasite density was 160/µl of blood.

Data was analysed statistically using SPSS version 19 for comparison and correlation tests. Association between data was assessed by Pearson Correlation.

Observations

Among the total 144 cases positive for malaria included in the study 80 (55.55%) were males and 64 (44.45%) females, resulting in a male: female ratio of 1.25:1. Maximum number of cases were seen in the age group between 15-30 years (Figure 1). 100 (69.44%) cases were positive for P.vivax, 40 (27.78%) cases positive for P. falciparum and 4 (2.8%) cases were diagnosed with mixed infection (vivax and falciparum) (Figure 2). Parasite load was variable ranging from 320 parasites per microlitre of blood to 8000 parasites per microlitre of blood with a mean (±SD) of 1521.11 (±1622.34) parasites per microlitre of blood. It was observed that the age group 15 -30 years had the highest occurrence of malaria with the highest parasitemia of ≥ 2000 parasites per microlitre of blood (Table 1). There was no statistically significant difference in the occurrence of malaria in various age groups (p-value 0.28). Mean (±SD) parasite density in patients with

P.vivax malaria was 1630.41 (±1605.65) parasites/µL of blood which was lower as compared to P.falciparum malaria with a mean (±SD) parasite density of 2049.65 (±1634.71) parasites/µL of blood despite the fact that the frequency of P.vivax cases was more. (Fig. 3).

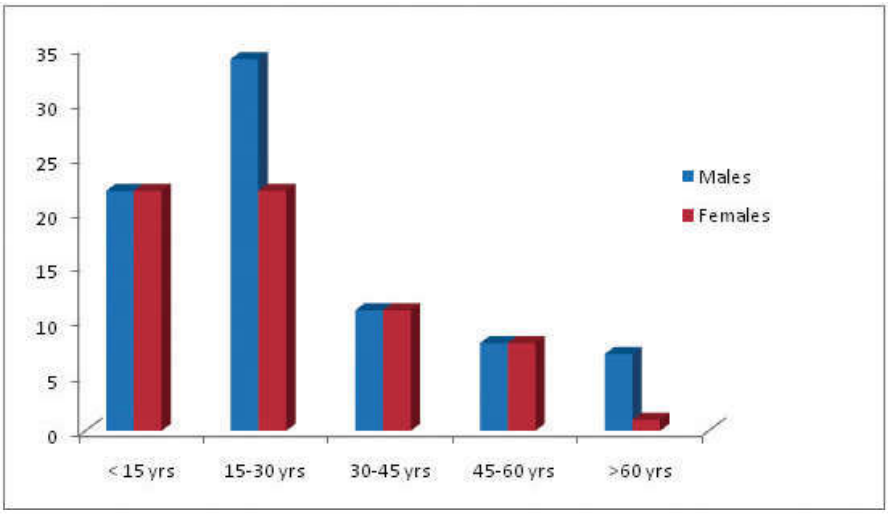

Fig. 1: Age and sex wise distribution of malaria cases

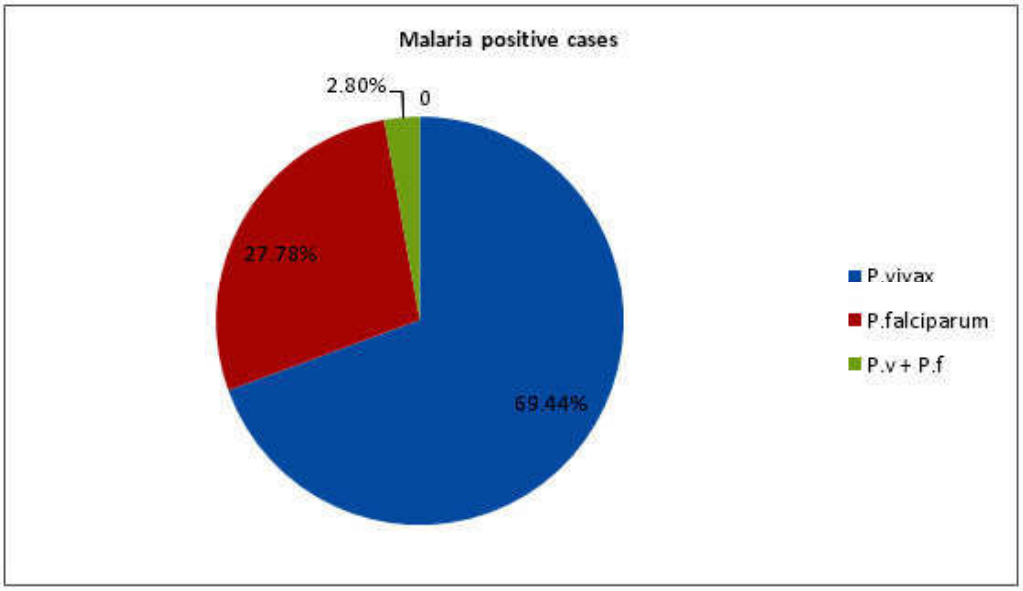

Fig. 2: Species distribution of malaria cases

Table 1: Distribution of malaria parasite density according to age and sex

Parasite Density (parasites/µL)	Age										
	<15yrs		15-30yrs		30-45yrs		45-60yrs		>60yrs		Total
Sex	M	F	M	F	M	F	M	F	M	F	
<500	5	0	5	4	1	0	2	0	0	0	17
501-1000	2	4	4	6	1	1	0	3	0	0	21
1001-1500	0	2	1	0	0	1	0	0	0	0	4
1501-2000	1	1	3	2	0	2	1	1	1	1	13
2001-4000	2	2	9	2	1	1	0	0	0	0	17

>4000	1	1	1	2	0	0	0	0	0	0	5
Total	11	10	23	16	3	5	3	4	1	1	77

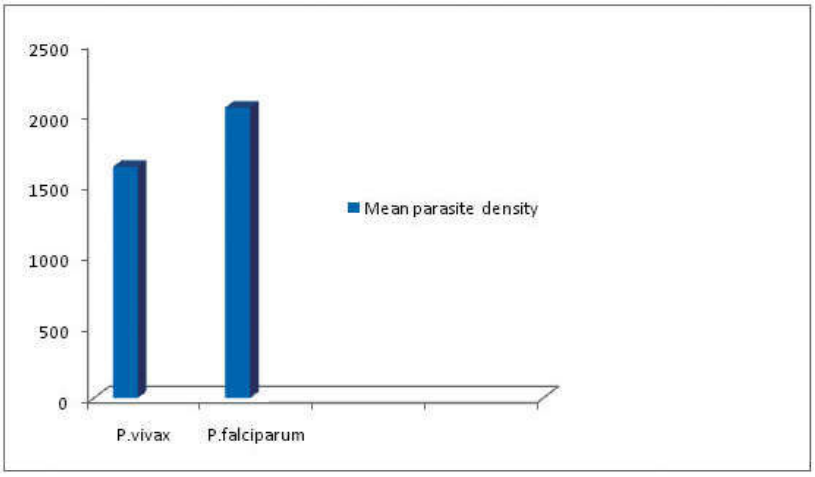

Fig. 3: Mean parasite density in different species

Variation of Blood Cell Indices in Malaria

Haemoglobin

It was observed that low haemoglobin concentration was prevalent in all age group patients of malaria. The haemoglobin ranged from 2.8 gm/dl to 15 gm/dl with a mean (±SD) of 9.16 gm/dl (±2.35). There was no statistically significant difference between age and haemoglobin concentration. Out of the total 144 malaria cases 129 (89.6%) patients had anaemia. On applying Pearson's correlation coefficient (r = -0.351) a p value <0.001 was obtained which implied a significant negative correlation between parasite density and Hb concentration (Table 2).

Table 2: Mean (± sd) of hb in relation to parasite density

Parasite density (Parasites/µl)	Mean Hb (gm/dl)
< 500	9.25 ± 2.56
500 – 1000	9.57 ± 2.30
1000 – 1500	9.11 ± 2.20
1500 – 2000	8.57 ± 2.31
2000 – 4000	7.72 ± 2.41
> 4000	7.40 ± 2.83

White Blood Cell Count

Total leukocyte count (TLC) in the malaria positive patients ranged from 2.20×103/µl to 31.60 ×103/µl with a mean (±SD) of 7.67×103/µl ± 3.78×103/µl. A normal TLC was observed in most of the patients with malaria (Table 3). A decreasing trend is noticed in leukocyte count with increasing parasite density (Table 4). Pearson's correlation coefficient (r = -0.341) with a p value <0.001 implied a significant negative correlation between the two. No statistically significant difference was observed between leukocyte count and age or sex.

Table 3: White blood cell count

Age	WBC		
	Low	Normal	High
< 15 yrs	4	26	10
15 – 30 yrs	5	52	2
30 – 45 yrs	1	20	1
45 – 60 yrs	0	15	1
> 60 yrs	0	6	1
Total	10	119	15

Normal WBC: 4-11 × 103 / µlLow: <4×103/µlHigh: >11×103/µl

Table 4: Mean (± sd) of wbc count in relation to parasite density

Parasite density (Parasites/µl)	WBC (×103/ µl)
< 500	9.01 ± 4.45
500 – 1000	8.68 ± 4.22
1000 – 1500	7.25 ± 4.01
1500 – 2000	8.30 ± 4.24
2000 – 4000	5.65 ± 3.92
> 4000	3.98 ± 4.39

Differential Leukocyte Count

Differential white cell count did not reveal any significant finding in patients of malaria except for eosinophilia. A significant positive correlation was seen with differential eosinophil count and parasite density. (r = +0.320) p- value<0.001 (Table 5).

Table 5: Mean of differential white cell count in relation to parasite density

Parasite density	N (%)	L (%)	E (%)	M (%)	B (%)
< 500	60	34	3	3	0
500 – 1000	72	22	5	2	0
1000 – 1500	78	42	8	1	0
1500 – 2000	67	60	12	2	0
2000 – 4000	58	43	10	2	0
> 4000	64	42	10	2	0

(N-Neutrophil, L-Lymphocyte, E-Eosinophil, M-Monocyte, B-Basophil)

Platelet Count

Platelet count in the present study ranged from 11 × 103/µL to 314 × 103/ µL with a mean of 119 × 103/µL. Thrombocytopenia was observed in 88 cases (61.1%) out of the total of 144 malaria (Table 6). A negative correlation was observed with parasite density in our study (r = -0.509) with a p value<0.001 which is statistically significant (Table 7).

Table 6: Platelet count

Age	Platelet count	
	Low	Normal
< 15yrs	31	9
15 – 30 yrs	35	24
30 -45 yrs	10	12
45 – 60 yrs	10	6
> 60 yrs	2	5
Total	88	56

Normal: 150-450 × 103/ µL

Table 7: Mean (± sd) of platelet count in relation to parasite density

Parasite density (Parasites/µl)	Platelet count (×103/ µL)
< 500	130.47± 66.66
500 – 1000	117.95± 65.66
1000 – 1500	121.00± 66.70
1500 – 2000	86.63 ± 65.58
2000 – 4000	66.61 ± 65.52
> 4000	40.80 ± 63.99

Red cell indices did not reveal any significant derangements except for a decreasing trend of MCH with increasing parasite density (Table 8). A significant negative correlation (r = -0.313) was observed between MCH and parasite density. (p-value<0.001).

Table 8: Mean (+ sd) of mcv, mch & mchc in relation to parasite density

Parasite density	MCV (fL)	MCH (pg)	MCHC (g/dL)
< 500	87.47±6.79	29.47±2.17	32.94±2.03
500 – 1000	81.85±6.61	29.19±2.08	31.19±2.04
1000 – 1500	87.00±6.30	30.50±2.15	32.50±2.09
1500 – 2000	83.81±6.66	28.18±2.06	31.63±2.02
2000 - 4000	84.60±6.70	27.72±2.09	31.61±1.98
> 4000	82.20±6.72	27.80±2.69	31.00±2.43

MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.

The most significantly encountered derangements in malaria infection were found to be thrombocytopenia and anaemia followed by eosinophilia. Thrombocytopenia was observed to have an odds ratio of 24.62 indicating that it was a better predictor of malaria infection than other parameters (p-value <0.001). Anaemia showed an odds ratio of 22.11 with p-value <0.001 (Table 9).

Discussion

Malaria is known to inflict changes in certain haematologial parameters as the portal of entry of the parasite is the host circulation. Malaria parasite identification request is usually accompanied with a full blood count for proper diagnosis, treatment and management of malaria infection. Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathology. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocytes. Haematological derangements play a major role in fatal complications. The extent of these alterations varies with level of malaria endemicity, background haemoglobinopathy, nutritional status, demographic factors, and malaria immunity [5]. The diagnostic value of these haematological parameters

Table 9: Variation of different haematological parameters in cases vs controls

Haematological Parameters	Cases (n = 144)	Controls (n = 50)	Sensitivity (%)	Specificity (%)	Odds ratio	p- value
Hb< 11g/dL	129	14	89.6	72	22.11	0.000
TLC< 4 × 103/µL	10	3	6.9	94	1.17	1.000
Platelet count <150×103/µL	88	3	61.1	94	24.62	0.000
MCH < 28pg	28	12	19.4	76	0.76	0.540
Eosinophil%>6	58	8	40.3	84	3.54	0.002

and their correlation with disease severity has been examined in this study.

The present study showed a male: female ratio of 1.25:1 among the malaria positive cases indicating a higher male predominance as has been reported in previous studies [6,7]. The reason for male preponderance could be due to the fact that most of the males are busy with outdoor and farming activities whereas females are usually confined within their homes and are usually better clothed. Another reason of male preponderance can be attributed to the fact that females in India prefer traditional methods of treatment for fever than visiting the hospital.

Maximum number of patients (40.98%) were in the age group of 15- 30 years in the present study, which was similar to the studies conducted elsewhere [6,7]. A higher incidence of P.vivax malaria in our study is comparable with other studies in India [7-9].

Malaria parasite density was variable with a mean (±SD) of 1521.11 (±1622.34) parasite/µL of blood. It was observed that although the frequency of P.vivaxmalaria cases was more than that of P.falciparumbut the mean parasite density was lower in the former group. This observation is in accordance with previous studies [2,3].

The haematological parameters which were significantly altered in our study were haemoglobin (Hb), Total leukocyte count (TLC), platelet count, eosinophil percentage and Mean Corpuscular Haemoglobin (MCH).

It was observed that low haemoglobin concentration was prevalent in 89.6% of malaria positive patients with no statistically significant difference with age. A significant negative correlation (r = -0.351) was observed between parasite density and Hb concentration. This similar finding was also observed in other studies [2,5-6]. Anaemia is a common complication in malaria infection especially in high endemic areas. Various factors contributing to its pathogenicity include the red blood cell being the parasite's primary target resulting in accelerated red cell destruction, bone marrow dysfunction and the level of parasitemiaalongwithred cell deformability, spleenic phagocytosis or pooling [10].

The mean red blood cell indices (MCV, MCH, MCHC, RDW) of patients with malariahave been observed to be normal in most studies [11-12]. They attributed this finding to the fact that the study group included only cases of uncomplicated malaria which is associated with milder biochemical changes, lower production of cytokines, less sequestration and less hemolysis as opposed to complicated/severe malaria.

A significant reduction in MCH was noted with increasing parasite density in this study which is in contrast to other studies which demonstrated no significant correlation of red cell indices with malarial parasitemia [11-12]. MCV, MCHC and RDW showed no significant correlation in this study.

White Blood Cell (WBC) count has been variably affected in different studies conducted on patients diagnosed with malaria. Leucopenia defined as a total WBC count < 4000/µL was observed to be associated with malaria infection in research studies in malaria endemic zones [7,11]. While a higher WBC count has been observed in malaria infected patients in other contrasting studies [13]. An overall normal mean WBC count was observed in malaria positive patients in the present study which is in accordance with other studies [7,12]. A low leukocyte count was however noted with increasing parasite density implying a significant negative correlation between the two. Although it cannot be denied that leukopenia may be a confounding factor in assessment of malaria parasite density on the basis of an assumed WBC count of 8000 cells/ µL [14]. No statistically significant difference was observed between decreased leukocyte count and age or sex.

Previous studies have reported contrasting differential white cell percentages with malarial parasitemia. Few researches have reported in their study that monocytosis was the most important leukocytic change associated with malaria infection while others have emphasized that lymphopenia was more indicative of hyperparasitemia [2,15]. Eosinophila was present in 40.27% of malaria positive patients in our study. Differential eosinophil percentage showed a consistent significant increase with increasing parasite density. This finding is in accordance with earlier research works [2,5]. They concluded that eosinophilia was also a key significant haematological indicator of malaria infection.

A decreasing trend of platelet count with increasing levels of malarial parasitemia was observed in this study which has been previously noted in other studies [6,7,11]. Thrombocytopenia was observed in 61.11% of malaria positive patients in the current study. Few researchers observed no significant correlation of malarial parasite density with platelet counts [16]. It was concluded in another study that although the mean platelet

count in parasitemic subjects was lower than non parasitemic subjects, the difference was not statistically significant [12].

The cause of low platelets in malaria can be attributed to increased peripheral destruction, excessive splenic pooling as well as platelet consumption by the process of DIC [13,15]. Thrombocytopenia can also result from immune-mediated destruction of circulating platelets. Elevated levels of specific IgG are observed in the blood of malaria infected patients which binds to platelet-bound malaria antigens possibly leading to accelerated destruction [17]. Endothelial activation has been observed to be a key element in malaria infection contributing to loss of barrier function of the endothelium and organ dysfunction. This process may lead to increased platelet consumption resulting in thrombocytopenia [18].

It has been observed in certain studies that thrombocytopenia is the most reliable haematological parameter for predicting malaria in people from endemic areas which is in concordance with our study [6,11]. Leuocopenia was observed to be the next most common predictor of malaria infection in their study. Patients with leucopenia were 2.7 times more likely to have malaria infection than those with normal leucocytes counts which is in variance with our research which shows an insignificant correlation with malaria infection although it does correlate with disease severity or increasing parasitemia.

Conclusion

To conclude it is to be noted that along with anaemia, a low leukocyte and platelet count may also guide towards a diagnosis of severe parasitemia in patients of malaria. Due to limited resources and lack of trained health personnel in developing countries where malaria is endemic, a spectrum of deranged haematological parameters such as a low leukocyte count and a low platelet count may serve as a basis for a thorough search for the parasite in cases of low parasitemia and avoid delay in effective treatment.

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