Original article

Change in Hematological disturbances in Malaria in Eastern India: an observational study

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Abstract:

Objectives: We wanted to find out the change and pattern of hematological disturbances in patients suffering from malaria and whether the hematological disturbances had any correlation with morbidity in them. ***Materials and methods:*** In a retrospective, observational study, first 100 admitted malaria patients in a tertiary medical institute in Kolkata, was taken from August 2009 to July 2011 and their demographic, clinical and laboratorial records were noted and analysed. ***Results:*** Seventy-one patients (71%) had anemia, ninety patients (90%) had thrombocytopenia, twenty-six patients (26%) had leucopenia and two patients (2%) had leukocytosis. Vivax malaria patients had lower platelet count than their falciparum counterparts, whereas falciparum malaria patients had lower hemoglobin, packed cell volume and higher total leukocyte count; all these hematological disturbances were found to be correlated with higher morbidity in patients suffering from malaria. ***Conclusion:*** In patients suffering from malaria, there was high incidence of hematological disturbances which was changing pattern and also correlated with morbidity.

Key words: Anemia, leucopenia, thrombocytopenia, malaria, vivax, falciparum, morbidity

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Introduction:

Malaria is a vector-bourne disease where malaria parasites infect red blood cells. Eventually in the course of the disease, the malaria parasites lyse the red blood cells and become free to infect other red blood cells. Plasmodium vivax has affinity for younger red blood cells, whereas Plasmodium falciparum infects red blood cells of all ages. Various literatures had mentioned about hematological abnormalities present in patients suffering from malaria, namely anemia, leucopenia and thrombocytopenia of varying grade, discussed below. We had undertaken the study to know whether these abnormalities were present or changing in pattern in patients suffering from malaria in Indian subcontinent and how these abnormalities affected the course of the disease and outcome of the patients. We also wanted to know about the variations of hematological abnormalities according to species of malaria parasite involved and gender of the patients.

Methodology:

The study was retrospectively done in a tertiary medical institute in Kolkata, India during the period August 2009 to July 2011. First hundred indoor cases, where malaria was detected using microscopy and/or rapid antigen method, were included without any sampling. This hospital served patients of the city of Kolkata and the surrounding districts of West Bengal, India; zones with unstable transmission. Both adults and children of both genders from urban and rural areas, who got admitted in Medicine ward of this hospital, were taken up for our study. Outdoor patients were excluded.

The patients were observed and data was taken as cross-sectional without any follow-up. Each patient's name, age, sex, residential address, hospital serial number and type of malaria were recorded from hospital record sheet. Patient's complaints, past history, co-morbidities and clinical examination were noted from each bed-head ticket. Records were checked and values of complete hemogram, peripheral blood smear examination and reticulocyte count on admission were also noted. Complete hemogram was measured in our institute with

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Table 1.Comparison between vivax and falciparum malaria cases		**Plasmodium vivax malaria (n=57)**	**Plasmodium falciparum malaria (n=32)**	**p value**
Parameters				
Hematological parameters	Hemoglobin (gm/dl)	12.4 (1.9)	10.5 (2.6)	**0.0007**
	Packed cell volume (%)	38.6 (6.1)	32.9 (8.6)	**0.0018**
	Total Leukocyte count (per micro litre)	4700 (1808)	6100 (2882)	**0.01**
	Platelet count (per micro litre)	76000 (35683)	122500 (53135)	**<0.0001**
	Reticulocyte count (% of RBC)	1.4 (0.6)	1.35 (0.73)	0.74
	Peripheral blood smear (n/n)	47 (82.5%)	21 (65.6%)	0.12
Data are as number (%) or as median (Standard deviation); n/n = normocytic and normochromic				

SYSMEX KX-21 automated hematology analyzer. Persistence of symptoms during treatment, duration of hospitalisation and cost of treatment were also recorded.

We had made classification of anemia according to Hb (hemoglobin) level, as follows:

1. Non- anemic patients (Hb normal)
2. Mild anemia (Hb > 10 gm/dl but < normal)
3. Moderate anemia (Hb 7-10 gm/dl)
4. Severe anemia (Hb < 7 gm/dl).

We had made three sub-groups of thrombocytopenia as below.

1. Mild thrombocytopenia = platelet count 1 x 10^5 – 1.5 x 10^5 /microL
2. Moderate thrombocytopenia = platelet count 0.5 x 10^5 – 1 x 10^5 /microL
3. Severe thrombocytopenia = platelet count < 0.5 x 10^5 /microL

We had tried to find out the effect of hematological alterations in the patients of malaria on their morbidity. We had taken following parameters as morbidity was concerned:

1. Persistence of symptoms during treatment.
2. Duration of hospital stay.
3. Total cost of treatment.

The data were entered on to an Ms-Excel spreadsheet; mean/median values and standard deviations were measured. Data were analysed using GraphPad Instat software. Proportions were examined using Fisher's exact test, two sided p values were calculated. Unpaired t test was used for parametric comparisons. $p < 0.05$ was taken as the cut-off for significance. Data has been presented below as median ± standard deviation.

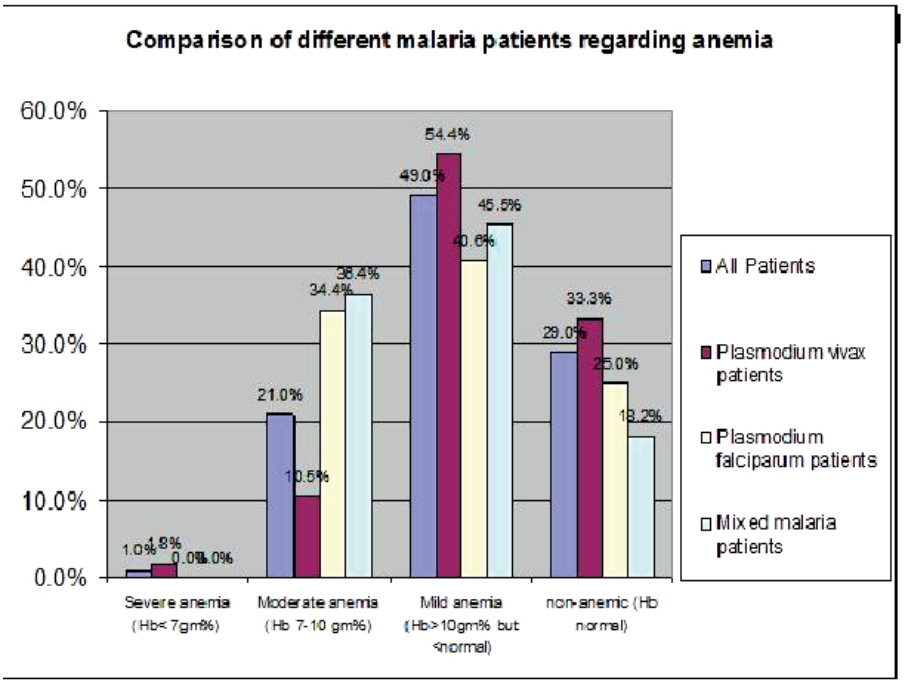
The ethics committee of our Institute had reviewed the study critically. No informed consent was obtained from the cases as the study was a retrospective and observational one and no human or animal experiment was done.

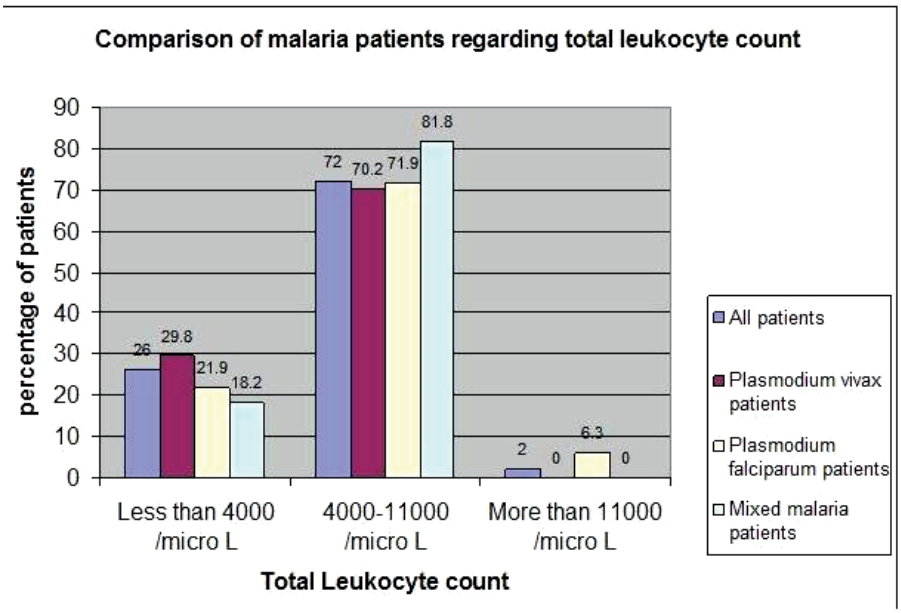
Results:

Amongst one hundred malaria patients, we had found that fifty-seven patients were suffering from Plasmodium vivax, thirty-two patients were suffering from Plasmodium falciparum and eleven patients were suffering from mixed malaria (both Plasmodium vivax and Plasmodium falciparum) infection. Sixty-six patients belonged to male gender (66%). Ages of the patients were varied from 9 years to 73 years.

Patients suffering from falciparum malaria were found to have median hemoglobin 1.9 gm/dl less than patients suffering from vivax malaria (10.5 ± 2.6 gm/dl vs. 12.4 ± 1.9 gm/dl, $p = 0.0007$); so also 5.7% lesser packed cell volume (32.9 ± 8.6 % vs. 38.6 ± 6.1 %, $p = 0.0018$). Patients suffering from falciparum malaria had median leukocyte count 1400 per micro litre higher than their vivax counterparts (6100 ± 2882 per micro litre vs. 4700 ± 1808 per micro litre, $p = 0.01$). On the contrary, median platelet count was lower in patients suffering from vivax malaria by 46500 per micro litre than their falciparum counterparts (76000 ± 35683 per micro litre vs. 122500 ± 53135 per micro litre, $p < 0.0001$). There was no significant difference in reticulocyte count between patients suffering from vivax and falciparum malaria (table 1). Forty-seven patients suffering from vivax malaria (82.5%) and twenty-one patients suffering from falciparum malaria (65.6%) had normocytic and normochromic peripheral blood smears. Rest of the patients, in their peripheral blood smears, had either microcytic and hypochromic type or a mix of the two types.

On comparison between genders, it was found that, females were suffering from lower hemoglobin level (10.5 ± 1.8 gm/dl vs. 12.7 ± 2 gm/dl, p <





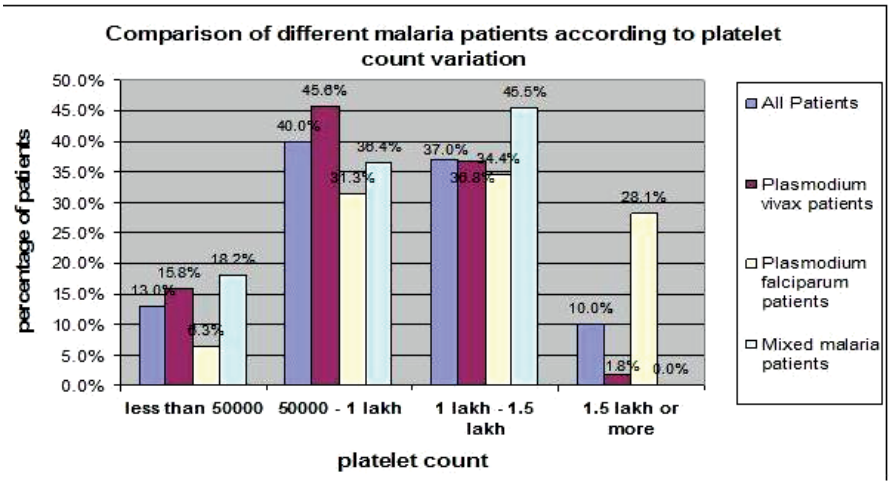


Figure 1: hematological changes in patients suffering from malaria

0.0001) and lower packed cell volume (32.7 ± 7 % vs. 38.8 ± 7.1, p = 0.0001) than their male counterparts. Total leukocyte count was not significantly different in two groups (5000 ± 2289 per micro litre vs. 5100 ± 2215 per micro litre, p = 0.83). Median platelet count was lesser in male patients of malaria by 19000 per micro litre than the female patients (82000 ± 41175 per micro litre vs. 101000 ± 49185 per micro litre, p = 058), but not quite significant. There was no significant difference in reticulocyte count in both genders (1.4 ± 0.6 per micro litre vs. 1.4 ± 0.7 per micro litre, p > 0.99). Fifty-one male (77.3%) and twenty-six female (76.5%) patients were having normocytic and normochromic type of peripheral blood smear. Rest of the patients either had hypochromic and microcytic or a mix of both types. In our study, we had found that, seventy-one patients (71%), suffering from malaria, were anaemic. When we tried to look for anaemia in patients suffering from different sub-groups of malaria, it was found that thirty-eight patients (66.7%) suffering from vivax malaria, twenty-four patients (75%) suffering from falciparum malaria and nine patients (81.8%) suffering from mixed malaria were anaemic. One patient suffering from vivax malaria was diagnosed to have severe anaemia. Her (age 17 years) peripheral blood smear had both normocytic normochromic and microcytic hy-

Table 2. Significant correlation of Hematological disturbance with morbidity			
Morbidity parameters	**Hematological parameters**	**r value**	**p value**
Persistence of symptoms during treatment	Hemoglobin	-0.1769	**0.023**
	Total Leukocyte count	0.4990	**<0.0001**
	Platelet count	-0.1774	**0.0022**
Duration of hospital stay	Hemoglobin	-0.3405	**0.0002**
	Total Leukocyte count	0.4295	**<0.0001**
Total cost of treatment	Hemoglobin	-0.2147	**0.0316**
	Total Leukocyte count	0.3850	**<0.0001**

pochromic type of red blood cells. Incidence of mild anaemia was highest amongst all subgroups as shown in figure 1.

As shown in figure 1, most of the patients suffering from different sub-groups of malaria had normal total leukocyte count (i.e., 4000 to 11000 white blood cells per micro litre of blood). Only two patients (6.3%) suffering from falciparum malaria had leukocytosis (i.e., more than 11000 white blood cells per micro litre of blood). But many patients had leucopenia (i.e., less than 4000 white blood cells per micro litre of blood). Twenty-six malaria patients (26%) had leucopenia. Seventeen patients (29.8%) suffering from vivax malaria, seven patients (21.9%) suffering from falciparum malaria and two patients (18.2%) suffering from mixed malaria had leucopenia.

We had got some surprising results regarding platelet count in patients suffering from malaria. Only ten malaria patients (10%) had normal platelet count (i.e., 1.5 lakh or more per micro litre of blood). One patient (1.8%) suffering from vivax malaria and nine patients (28.1%) suffering from falciparum malaria had normal platelet count.

Figure 1 had shown the distribution of patients suffering from different sub-groups of malaria regarding mild, moderate and severe thrombocytopenia. Amongst thirteen malaria patients (13%) suffering from severe thrombocytopenia, nine patients were suffering from vivax malaria (15.8%), two patients were suffering from falciparum malaria (6.3%) and two patients were suffering from mixed malaria (18.2%). Most of the patients suffering from vivax malaria had moderate thrombocytopenia, whereas most of the patients suffering from falciparum and mixed malaria had mild thrombocytopenia. We had correlated parameters denoting morbidity with hematological parameters and the results were shown in table 2. It was found that, lower the hemoglobin level and platelet count, higher the morbidity. But higher total leukocyte count was correlated with higher morbidity.

Discussion:

Walter RJ Taylor et al, [1] from Papua had studied changes in total leukocyte and platelet counts in patients suffering from Plasmodium vivax and Plasmodium falciparum malaria. The mean Day 0 leukocyte count (n = 152) was 6.492 (range 2.1–13.4) $\times 10^9$/L. 14 (9.2%) and 9 (5.9%) patients had leukopaenia ($<4.0 \times 10^9$/L) and leukocytosis ($>10.0 \times 10^9$/L), respectively. By Day 28, the mean leukocyte count increased significantly (P = 0.0003) from 6.37 to 7.47 $\times 10^9$/L (73 paired values) and was similar between the two groups. Ethnicity was the only leukocyte count explanatory factor and only on Day 0. The mean Day 0 platelet count (n = 151) was 113.0 (range 8.0–313.0) $\times 10^9$/L and rose significantly to 186.308 $\times 10^9$/L by Day 28 (P < 0.0001). There was a corresponding fall in patient proportions with thrombocytopenia ($<150 \times 10^9$/L): 119/151 (78.81%) vs. 16/73 (21.92%, P < 0.00001). Papuan and non Papuan mean platelet counts were similar at all time points. Only malaria species on Day 0 was a significant platelet count explanatory factor. The mean Day 0 platelet counts were significantly lower (P = 0.025) in vivax (102.022 $\times 10^9$/L) vs. falciparum (122.125 $\times 10^9$/L) patients.

UM Jadav et al, [2] from Western India had studied hematological parameters in patients suffering from malaria. Mean hemoglobin concentration was 11.6 gm/dl in subjects with Plasmodium falciparum malaria and 12.5 gm/dl in subjects with P. vivax malaria (p< 0.047) and the lowest hemoglobin concentration was 2.1gm/dl in P. falciparum infestation and 3.8gm/dl in P. vivax infection. There was no statistically significant difference noted in the mean total white cell count in subjects with P. falciparum and P. vivax malaria (6127/cumm versus 6252/cumm, p = 0.685). Statistically significant difference persisted between the platelet count (p<0.0001) of P. falciparum and P. vivax malaria.

Shuaib Ansari et al, [3] from South India had studied thrombocytopenia in patients suffering from Plasmodium falciparum malaria. Hemoglobin values were 12.7±1.4 gm% and white blood cells counts were found 12600±450/μL. Out of 370 patients, 114 (30.81%) had normal platelet counts and 256 (69.18%) had thrombocytopenia ($p<0.05$). The mild, moderate and severe thrombocytopenia were found in 39 (10.5%), 180 (48.6%) and 37 (10%) respectively ($p<0.05$) (Table-1). The mean platelet count was 170,000±56,500/μL (range 18000-380.0000/μL).

Arboleda M et al, [4] from Colombia had reported that out of 359 patients with vivax malaria, 43 patients (12%) were suffering from severe anemia and 13 patients (3.6%) were suffering from severe thrombocytopenia.

Tekeste Z et al, [5] from Ethiopia had reported that, 18 patients (8.57%), out of 210 patients suffering from falciparum malaria, had severe anemia.

McDevitt MA et al, [6] from USA had discussed role of inflammatory cytokines in regard to anemia of malaria infection.

Kasturi Haldar et al, [7] and Kanjaksha Ghosh et al, [8] had discussed about multi-factorial causation of anemia in malaria patients.

Gonzalez B et al, [9] from Venezuela had evaluated 59 individuals of both sexes, infected with Plasmodium vivax and 30 controls. They had got significant differences by gender for hemoglobin, erythrocytes and hematocrit ($p < 0.01$ in all three analyses). They had got the mean total leukocyte count on the day of diagnosis to be within reference range. Thrombocytopenia on the day of diagnosis was also observed by them in the study.

Ramirez-Olivencia G et al, [10] from Spain had analysed imported malaria in adults and found that at the time of diagnosis, 30.4% of patients were asymptomatic and 28.1% of asymptomatic patients had anemia, 19.8% had thrombocytopenia and 14% had leucopenia.

Taylor CA et al, [11] from United Kingdom had done a retrospective study of malaria in pediatric oncology patients in Senegal. They had found that there was no association with severe anemia, leucopenia, neutropenia, or lymphopenia ($P=1.00$, $P=0.28$, $P=0.53$, and $P=0.22$, respectively) with mortality.

Robert N Maina et al, [12] from Keyna had found platelets, lymphocytes, eosinophils, red blood cell count and hemoglobin to be significantly lower in malaria infected children than their non-infected counterparts.

Nassem S et al, [13] from India had reported one case of malaria with leukopenia in a renal transplant patient.

Naha K et al, [14] from South-Western India had done a retrospective analysis regarding complications found in patients suffering from Plasmodium vivax malaria. Medical records of 213 individuals, who satisfied the inclusion criteria, were reviewed. Anaemia was found in 65 (30.5%), leucopenia in 38 (17.8%) and thrombocytopenia in 184 (86.4%) patients.

John G. Kelton et al, [15] from Canada had studied 28 patients with malaria and found association of elevated platelet associated IgG with thrombocytopenia, indicating immune-mediated thrombocytopenia in malaria.

Anju Aggarwal et al, [16], Kakar A et al, [17] and Makkar RP et al, [18] from India had reported case of severe thrombocytopenia in patients suffering from Plasmodium vivax malaria.

It was evident from the above mentioned literatures that hematological disturbances, specially anemia, thrombocytopenia and leucopenia were having high incidence in malaria patients throughout the world. We had found similar results in our study. Further research would be needed to find exact causes of those abnormalities in patients infected with malaria parasite. Some of the studies, [6, 7, 8, 15] had aimed for the same and had indicated immune mechanism, [8, 15] and role of inflammatory markers, [6, 7, 8] in causation of hematological disturbances in patients suffering from malaria. In our study, most of the patients were anemic. Thrombocytopenia was more marked in vivax malaria infection. No other study had found any correlation of hematological disturbances with morbidity; even one study, [11] had found no correlation of hematological disturbances with mortality in patients with malaria. In contrast, we had found lower hemoglobin; lower platelet count and higher total leukocyte count on admission to be positively correlated factor of morbidity like, persistence of symptoms during treatment, duration of hospital stay and cost of treatment (table 2).

To conclude, lower hemoglobin and hematocrit values and higher total leukocyte count were found in patients suffering from falciparum malaria in comparison to patients suffering from vivax malaria. Lower platelet count was found in patients suffering from vivax malaria in comparison to patients suffering from falciparum malaria. These

hematological abnormalities affected outcome of malaria patients and prolonged their morbidity. Hematological abnormalities in malaria infection were prevalent all over world where malaria was reported. So early detection of type of malarial infection and hematological abnormalities could predict about morbidities and alert the physician about management and outcome.

Conflict of interest: None

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