Original Article

Parasitemia and Hematological Alterations in Malaria: A Study from the Highly Affected Zones

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ABSTRACT

Background& Objectives: In spite of intensive worldwide efforts to reduce its transmission, malaria remains the most serious and widespread protozoal infection of humans. This study has been performed to evaluate various hematological alterations in patients infected with malaria and to add more detailed information, especially from these highly affected zones.

Materials and Methods: A two-year, hospital-based study was conducted and hematologic profiles of 200 persons infected with malaria (*Plasmodium vivax or P. falciparum*) infection were examined and the results were compared with standard normal values.

Results: One-hundred-ninety-six patients were infected with *P. vivax,* three with *P. falciparum*, and one with both. Patients with parasitemia tended to have significantly lower platelets, hemoglobin, white blood cell and red blood cell counts, blood indices and hematocrit than normal. Thrombocytopenia is identified as a key indicator of malaria in these febrile patients.

Conclusion: Anemia and thrombocytopenia are the classical changes. Changes in the white blood cells are less dramatic, may vary due to variable size and type of cases, variability of the species, and geographical differences.

Keywords: Malaria, Parasitemia, Anemia, Leukopenia, Thrombocytopenia

Introduction

Malaria continues to be a great health problem in some of the most populated areas of the world. It is caused by protozoan *Plasmodium*, transmitted by female anopheles mosquitoes, which typically bite between dusk and dawn. Clinical presentation of malaria caused by various species (*P. vivax, P. falciparum, P. malariae, P. ovale*) resembles each other. Clinical features include fever, chills, sweating, headache, vomiting, diarrhea, abdominal pain and distension, cough, splenomegaly and, hepatomegaly. Malaria is not a uniform disease; it encompasses many manifestations and its impact on epidemiological setting.

Malaria is the world's most important parasitic infection which poses major health challenges. As per world malaria report 2009, half of the world's population is at risk of malaria and an estimated 243 million cases led to nearly 8,63,000 deaths in 2008 (1). In India, the Haryana State has a malaria annual incidence rate between 1 and 100 cases per 1000 population. In the year 2008, total reported cases of *P. vivax* were 34,317 (till November) and the most affected regions being Bhiwani, Hissar, Jind, Kaithal and Fatehabad while total cases of *P. falciparum* were 1294 and the most affected regions were Mewat, Karnal, Sonepat, Fatehabad, Yamunanagar and Sirsa (2). This institute is draining especially the rural and suburban population, from Fatehabad, Sirsa, Hissar and Jind; the most affected zones of malaria.

Hematological alterations associated with malaria are well recognized, but specific changes may vary with level of malaria endemicity, background, hemoglobinopathy, nutritional status, demographic factors, and malaria immunity (3).

This study aimed to evaluate and determine the frequency of various hematological alterations in patients infected with malaria and to add more detailed information, especially from these highly affected zones. The level of parasitemia was also assessed and correlated with these hematolologic changes.

Materials and Methods

This study was conducted in the Departments of Pathology and Microbiology at Maharaja Agrasen Medical College, Agroha, catering to the needs of rural and semi-urban population from highly affected malaria zones of Haryana, India in 2008 and 2009 prospectively as well as retrospectively. All clinical records of the patients were assessed. The patients who were clinically suspicious of malaria and were confirmed by peripheral blood film (PBF) examination or by card test or both and those who were slide/card test positive but not clinically suspected of malaria were included in the study. Besides history taking, clinical examination, and routine laboratory work, thick and thin blood films were prepared and examined for defining the species involved. The thin and the thick smears were made on different slides and stained with Leishman and Giemsa stains respectively. Minimum of 200 fields (oil immersion) were assessed to label a negative smear. The percentage and grading of parasitemia was done after counting schizonts, ring and amoeboid forms in oil immersion on thin smears (4). The rapid diagnostic test (RDT) used was an Antigen (pLDH) based card test; Morepen Pf/Pv pan Ag cards, Morepen Laboratories Ltd., New Delhi, India having a good sensitivity.

In addition, blood film (red and white blood cells, and platelets) examination and hematological profile by five-part cell counter was done in all patients. Anemia and thrombocytopenia were labeled when hemoglobin (Hb) was < 11.0 g% and platelet counts were <1.5 lakhs/mm3, respectively (5). Reticulocyte count was done from smear stained with new methylene blue. Erythrocyte sedimentation rate (ESR) samples were collected in sodium citrated anticoagulant tubes and done by Westergrens method. The results were compared with normal standards (6).

Patients with clinical history and/or finding suggestive of chronic liver disease, bleeding disorder, thrombocytopenia, drug intake or conditions which might have contributed in blood changes were excluded from the study.

Results

Epidemiological and clinical features

Study included total of 200 patients, 119 (59.5%) were male, 81 (40.5%) were female, with age ranging 10 months to 71 years and mean age of 33 yr. Maximum number (21%) of cases belonged to 21-30 yr age group (Fig. 1). One hundred fifty nine presented with classical symptoms in form of fever, rigors, chills, sweating, splenomegaly, while 39 cases were not suspected to have malaria. Four cases positive by smear (thick) examination were found negative by card test. The rapid test picked 19 (9.5%) more cases that were missed by blood film examination. Ninety eight percent were infected by *P. vivax* while 1.5% was infected with *P. falciparum* and 0.5 % had mixed infection. Multiple stages were most commonly seen followed by schizonts, trophozoites (amoeboid) and ring stages alone. Maximum number of cases presented in months of August and September.
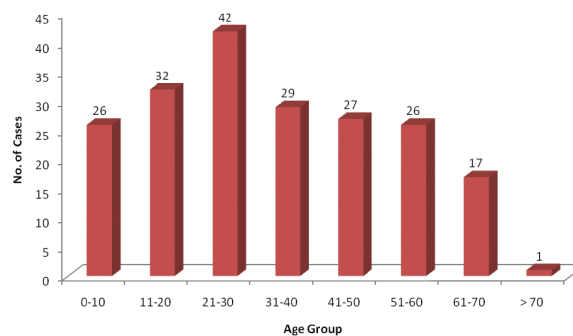

Fig. 1- Age-wise distribution of cases (n=200)

Hematological

The hemoglobin was reduced in 65.5% cases ranging 2.5-17.0 g%, with mean of 8.2g%. The total red blood cell (RBC) count ranged 1.7-5.7x1012/L with mean of 3.9x1012/L. Packed cell volume (PCV) was reduced in 69.5% and ranged 21.8 - 53 % with mean of 29.5%. Blood indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were reduced in 29, 30.5 and 20.5% ranging 59.0-101.5 fl, 15.5-33 pg and 29.5-35.0 g/ dl with mean of 77.8, 28.4 and 31.2 respectively. Blood picture was normocytic normochromic in 50.5% with varied picture in others. Reticulocyte count was high in 27.5% cases only (Table 1&2).

Table 1 - Hematological profile in malaria cases (n= 200)

Hematological parameters	Normal No. (%)	Low No. (%)	High No. (%)	Reference range
Hemoglobin g/dl	64(32)	131(65.5)	5(2.5)	13 ± 2 g/dl
Total WBC × 10^9 / l	152(76)	39(19.5)	9(4.5)	$7 \pm 3 \times 10^9$ / l
Platelet count × 10^9 / l	73(36.5)	124(62)	3(1.5)	$150 - 400 \times 10^9$ / l
Packed Cell Volume	56(28)	139(69.5)	5(2.5)	$45 \pm 5\%$
MCV	123(60.5)	58(29)	19(9.5)	85 ± 9 fl
MCH	125(62.5)	61(30.5)	14(7)	29.5 ± 2.5 pg
MCHC	148(74)	41(20.5)	11(5.5)	33 ± 2 g/dl
ESR	90(45)	None	110(55)	Up to 16 ± 2 mm in 1st hr
Reticulocyte count	142(71)	3(1.5)	55(27.5)	$0.2 – 2.0$ %

WBC = White blood cells, MCV= Mean corpuscular volume, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, ESR= Erythrocyte Sedimentation Rate, No.= Number of cases.

Table 2- Type of blood picture in malaria cases

Type of blood picture	No. of cases (%)
Normocytic normochromic	101(50.5)
Normocytic hypochromic	16(8)
Microcytic hypochromic	31(15.5)
Dimorphic (N/N to microcytic)	4(2)
Dimorphic (N/N to microcytic hypochromic)	15(7.5)
Dimorphic (microcytic -macrocytic)	18(9)
Dimorphic (normocytic to macrocytic)	15(7.5)
Total	200

N/N= normocytic normochromic

Total white blood cell (WBC) count was normal in majority of cases (76%) and leucopenia was noticed in 19.5% with total leukocyte count (TLC) varying from 1.5x109/L to 17x109/L and mean of 6.1 x109/L. The neutrophil count was normal in 79% cases, low in 12.5% cases, and ranged from 24% to 93% of the TLC.

Lymphocyte count was normal in 60.5% , low in 31%, ranging 5% to 67% of TLC, monocytosis, eosinophilia and basophilia were observed in 12.5%, 16% and 4.5% respectively and were constituting 1.0 to 11%, 0.5% to 13%, 0 to 2% of the TLC respectively (Table 1&3).

Table 3. White blood cell changes in malaria cases (n= 200)

White blood cells count	Low No. (%)	Mean absolute count (low)*	Normal Range No.(%)	Mean absolute count (NR)	High No. (%)	Mean absolute count (high)	Reference range
Neutrophils	25(12.5)	1.5×10^9/L	158(79)	4.7×10^9/L	17(8.5)	9.2×10^9/L	$2\text{-}7 \times 10^9$/L (40-80%)
Lymphocytes	62(31)	0.9×10^9/L	121(60.5)	1.9×10^9/L	17(8.5)	4.0×10^9/L	$1\text{-}3 \times 10^9$/L (20-40%)
Monocytes	11(5.5)	0.2×10^9/L	164(82)	0.5×10^9/L	25(12.5)	1.2×10^9/L	$0.2\text{-}1 \times 10^9$/L (2-10%)
Eosinophils	13(6.5)	0.03×10^9/L	155(77.5)	0.15×10^9/L	32(16)	0.55×10^9/L	$0.02\text{-}0.5 \times 10^9$/L (1-6%)
Basophils	_	0.05×10^9/L	191(95.5)	0.07×10^9/L	9(4.5)	0.14×10^9/L	$0.02\text{-}0.1 \times 10^9$/L (<1-2%)
Band forms	_	_	169(84.5)	0.3×10^9/L	31(15.5)	0.75×10^9/L	$0\text{-}0.70 \times 10^9$/l (0-3%)
Atypical lymphocytes	_	_	_	_	39(19.5)	_	_

*mean of absolute cell counts in individuals with lower counts than the reference levels
No.- number of cases, NR- normal range

The platelet count was low in 62% cases and ranged from 10.1 to 530×10⁹/ l with mean of 90.3×10⁹/l. Eleven (5.5%) cases revealed severe thrombocytopenia out of which one had bleeding manifestation. The mean platelet volume was normal in 85% and high in 15% cases (Tables 1& 4).

The parasitic forms determining acute infection were found in 181 cases on PBF examination while 19 cases labeled negative on PBF were found positive by rapid card test. More than 5% (severe) parasitemia was observed in 3 cases, one each with mixed, *vivax* and falciparum infection. Most commonly, parasitemia was of 'low' grade (0.05%) or 'very low' (0.005-0.05%) grade, with 250 to 25000 parasites /µl. It correlated well with clinical presentation, grade of anemia, thrombocytopenia and leucopenia (Table 5).

Table 4. Grading of Thrombocytopenia (n=124)

Grade of Thrombocytopenia	Platelet count x 10⁹/L	No. of cases (%)
Mild	100-150	64(51.61)
Moderate	20-100	49(39.52)
Severe	<20	11(8.87)
Total	-	124*

*Out of 200 cases, 124 had low platelet count

Table 5. Grading of parasitemia on thin smears

Parasites/HPF	Grading	Parasitemia (%)	Parasite/µl	No. of cases (%)
>10/HPF	6+ (severe)	>5	>250000	3(1.5)
5-10/HPF	5+(high)	2.5-5	125000-250000	7(3.5)
1-5/HPF	4+(medium)	0.5-2.5	25000-125000	40(20)
1-10/10HPF	3+(low)	0.05-0.5	2500-25000	64(32)
1-10/100HPF	2+(very low)	0.005-0.05	250-2500	58(29)
1/100HPF-1/200HPF	1+(rare)	0.0025-0.005	125-250	5(2.5)
No parasite in 200HPF	Negative	<0.0025	<125	23(11.5)*
Total cases	-	-	-	200

*19 cases picked by card test, 4 by thick smears

Discussion

Malaria causes high incidence of morbidity and mortality in people living in the highly affected zones of Haryana. *Plasmodium vivax* is the widest spread species found in Haryana as well as India (2, 7). The present study shows that *P. vivax* infection is much more common than *P. falciparum*/mixed infection in these zones of Haryana, in contrast to lots of studies done in the world (8,9).

The disease was more common in months of August-September (31%) followed by March-April (21%) as was noticed by Farogh *et al.* (8). There was slight male preponderance (59.5%) as compared to females (40.5%), and the age ranged from 10 months to 71 years with peak incidence between 21 to 30 years similar to previous study (9). The patients presented with in 2 to 9 days of classical symptoms in the form of fever (79.5%) with rigors (45%) or chills (31%) and/or sweating (55%), and splenomegaly (39%) (8). Rapid

diagnostic test picked 19 (9.5%) more cases of malaria that were missed on PBF examination, probably since it picks asexual forms also or due to poor quality smears/subjective errors, while four (2%) cases positive by smear (thick) examination were found negative by card test because of decreased sensitivity of RDT at 100 parasites/µl parasitemia and good sensitivity (5-10 parasites/µl) of smear examination in hands of skilled pathologist (4,7).

Hematological abnormalities are considered a hallmark of malaria, and the one that have been reported to invariably accompany infection with malaria include anemia, thrombocytopenia, splenomegaly, mild to moderate atypical lymphocytosis and rarely disseminated intravascular coagulation (DIC) (10).

The findings in our study have shown that in acute malaria, anemia (Hb <11g/dl) and thrombocytopenia (<1.5 lakhs/mm3) are common presentations, presenting in 65.5 and 62% cases, respectively which was in concordance with other studies (5, 9). Severe anemia (Hb< 7g/dL), however, was seen in lesser patients (10.5%), especially infants and children. The pathogenesis of anemia in malaria is extremely complex, multifactorial 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 unparasitized red blood cells, depressed as well as ineffective erythropoiesis with dyserythropoietic changes, and anemia of chronic disease (10, 11). Other factors include decreased red blood cell deformability, splenic phagocytosis and/or pooling (12), so they have an increased rate of clearance from the circulation. Studies in experimental murine malaria have shown that the progressive defective erythropoietic activity of the bone marrow was the result of depletion of colony-forming unit-pluripotent stem cells, although the mechanism of this stem cell depletion is unknown (10,13). Tumour necrosis factor alpha (TNF-α) has been implicated, and

may cause ineffective erythropoiesis (10, 11). It should be borne in mind also that red cell morphology in malaria patients may be influenced by their nutritional status i.e., patients could be iron deficient, folic acid or vitamin B12 deficient or they may have a concurrent thalassemia, which aggravates the severity of the anemia, as was the case in some of the patients in this study. Anemia was normocytic-normochromic in the majority (50.5%) of cases, which is concordant with other reports (9, 10).

However, microcytic hypochromic picture was seen in 31 (15.5%) cases (8, 9). In these latter cases, there was documented concurrent iron deficiency and/or a thalassemia, and 45% of them were children. It is known that in heavily endemic malaria areas, it is almost inevitable that malarial infection will be associated with anemia, although malaria may not be the prime cause of it (10). Blood indices were normal in majority and reduced in few of the cases, PCV was reduced in 69.5%, and ESR was high in 55%, while reticulocyte was high in 27.5% cases, mostly going with the level and type of anemia, as was observed by Agravat and Dhruva (5).

Although, some controversies appear to exist, there have been reports of leucopenia as well as leukocytosis (10, 14). Other hematological reactions to malaria that have been reported include neutropenia, eosinophilia, neutrophilia and monocytosis (5, 15, 16). In our study, the majority of patients (76%) had a normal total WBC count with leucopenia in only 19.5 %, similar to the study by Bashawri *et al.* and Abro *et al.*, (9,17) whereas according to other studies leukopenia appears to be a common finding (10). The differential white cell counts showed a normal neutrophil count in the majority (79%) of cases (9), which differs from other studies, which reported either neutropenia or neutrophilia among malaria cases (15).

Lymphopenia and the presence of reactive lymphocytes are well reported in literature (9). Although monocytosis has been reported to

occur (15, 16), the present study showed that the majority (82%) of malaria patients had a normal monocyte count. Besides, there was no significant increase in eosinophils, which agrees with previous reports (10). Another finding, the presence of bands, seen in 15.5% patients, even as little as 3%, was stressed in previous report (9).

Thrombocytopenia, which occurred in more than half (62%) of the patients, was a characteristic finding. It is so characteristic of malaria that in some places, it is used as an indicator of acute vivax malaria in patients presenting with pyrexia of unknown origin (PUO) (10, 18). Severe thrombocytopenia was seen in 5.5% cases (Table 4) and the one with lowest level had mixed infection and bleeding manifestation. It has also been observed that there is an inverse relationship between platelet count and parasite level. The mean platelet volume (MPV) is often raised confirming the presence of giant platelets on stained blood films. In our study, the MPV was found to be high in about 15% of the patients whenever reported. It has also been reported that thrombocytopenia occurs early in illness and resolves within a few days of treatment. The precise mechanism behind thrombocytopenia, however, remains unclear. Decreased thrombopoiesis can be excluded because platelet-forming megakaryocytes in the marrow are usually normal or increased. Immune-mediated destruction of circulating platelets has been postulated, and it has been found that malaria patients have elevated levels of platelet- bound IgG (10, 19).

Some workers have suggested DIC as a major mechanism, but others have found no evidence of DIC in any of their patients, including those with severe thrombocytopenia (19).

Another proposed mechanism is that of platelets engulfing malaria parasites, and in the process becoming damaged and thus being removed from circulation (10). This has not been confirmed. Hypersensitive platelets have been found in acute malaria infection as well as additional changes in platelet function. These include raised concentrations of platelet specific proteins such as beta thromboglobulin (β-TG), platelet factor 4 (PF4), and other changes such as enhanced production of thromboxane A2 and prostacyclin, but spontaneous platelet aggregation did not occur in these studies. It has also been postulated that these hypersensitive (hyperactive) platelets will enhance hemostatic responses, and maybe this is why bleeding episodes are rare in acute malaria infection, despite the thrombocytopenia (19).

The level of parasitemia commonly found was 0.05%-0.5%, i.e 2500-25000/μl (32%) followed by 0.005-0.05%,i.e 250-2500/μl (29%). Severe parasitemia was noticed in two cases of falciparum infection, one with mixed infection and one with *vivax* infection, although it has not been reported commonly in vivax (20).

Around half of the cases (44.5%) picked thin smear examination, revealed more than one stage of parasite (including gametocytes) in the blood film, while others revealed schizonts (18%), amoeboid (16%) and ring forms (10%) alone. Two cases infected by *P. vivax* had two rings and one case had three rings (7). It correlated well with clinical presentation, grade of anemia, thrombocytopenia and leucopenia in most of the cases (20).

One must always keep malaria at the top in the differential diagnosis of an acutely febrile patient from a malaria endemic zone with anemia and/ or thrombocytopenia, and a keen examination of thick and thin smears should always be carried out in these cases to look for parasitemia.

Limitations: Comparison between the profiles of *P. vivax* and *P. falciparum* cases could not be accertained due to only 3 cases of *falciparum* infection. Hematological values may vary a lot due to age and sex variability. Anemia is prevalent in malaria negative healthy appearing cases in these zones. Subjective errors and manual errors cannot be excluded.

Conclusion

Although these hematological alterations in malaria are not new to the subject, this data adds more detailed information to the limited knowledge, especially from these highly affected rural and suburban zones. Anemia and thrombocytopenia are the classical changes. Changes in the white blood cell are less dramatic, may vary due to variable size and type of cases, variability of the species, geographical differences, and there has been conflicting reports regarding these changes. It would be interesting and beneficial to study and compare the different reports discussing the hematological findings in patients living in endemic areas.

Acknowledgement

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