Int J Pharm Bio Sci 2015 Oct; 6(4): (B) 868 - 874

Research Article **Medicine**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SIGNIFICANCE OF HEMATOLOGICAL PARAMETERS AS A DIAGNOSTIC TEST FOR MALARIA IN PATIENTS WITH ACUTE FEBRILE ILLNESS

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ABSTRACT

The main objective of this study was Changes in complete blood count are among the most common complications encountered in malaria. This study evaluates those hematological changes as a diagnostic test for malaria in patients with acute febrile illness and to emphasize its usage for physician to institute specific antimalarial treatment. The present study conducted from June 2014 to June 2015. A total of 650 patients presenting with acute febrile illness at Sree Balaji medical college and hospital were evaluated. CBC and malarial parasite smear were done for each patient. 172 out of 650 patients (26.5%) were diagnosed to have malaria by positive smear. There were 121 males and 51 females with a male to female ratio of 2.3:1. There was a significant reduction in hemoglobin ($p<0.005$), platelet count ($p<0.001$) and total leukocyte count ($p<0.001$) levels malarial patients compared to those without the disease. A likelihood ratio for a positive result of platelets (6.2) and total leukocyte count (3.4) was relevant as compared to hemoglobin (1.61) and Red cell distribution width (1.79). The negative predictive values for hemoglobin (79%), total leukocyte count (86%), platelets (94%) and Red cell distribution width (93%) were significant. Red cell distribution width values were found to be higher in patients with malaria than in patients without malaria ($p<0.001$). This study showed that routinely lab findings such as hemoglobin, leukocytes, platelet counts and even red cell distribution width values can be used as a diagnostic clue in a patient with acute febrile illness, and hence giving the probability of malaria and as by prompting initiation of treatment.

KEYWORDS: **Malaria; Hematological parameters; Red cell Distribution Width.**

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INTRODUCTION

Malaria is a major health problem in India. One of the most prevalent human infections worldwide, malaria results in 225 million cases each year. In the Indian subcontinent, distribution is heterogeneous. It is caused by four species of plasmodia (P. falciparum, P. malariae, P. ovale and P. vivax) cause malaria in humans of which P. falciparum is the cause of morbidity and mortality.[1] Plasmodium vivax is the major malarial parasite in India, contributing towards the majority of cases[2.] The clinical diagnosis of malaria was confusing because of the non-specific signs and symptoms and overlap with other common febrile illnesses. This reduces the specificity of diagnosis and often promotes the haphazard use of antimalarials.Haematological abnormalities are considered a hallmark of malaria and analytical studies have shown that most of these haematological values may lead to an increased clinical suspicion for malaria, thus initiating a prompt institution of specific therapy even in the absence of a positive smear report for malaria. The haematological abnormalities that have been reported to accompany malaria include Anaemia, thrombocytopenia ,mild-to-moderate atypical lymphocytosis, and rarely disseminated intravascular coagulation (DIC) [3], leucopenia and leucocytosis[4]Haematological changes are among the most common complications encountered in malaria.[5]they are measurable indices of blood that can be used to diagnose the disease.[6, 7].These changes enable the clinician to establish an early diagnosis and hence start an early therapeutic intervention as to prevent the complications. In this study, we have analyzed these haematological changes in malarial cases and if they can guide for starting antimalarial treatment.

METHODS

This observational study was conducted from June 2014 to June 2015.

Inclusion criteria

All patients with acute febrile illness presenting at Sree Balaji Medical College and hospital, Chromepet, Chennai were evaluated.

Exclusion criteria

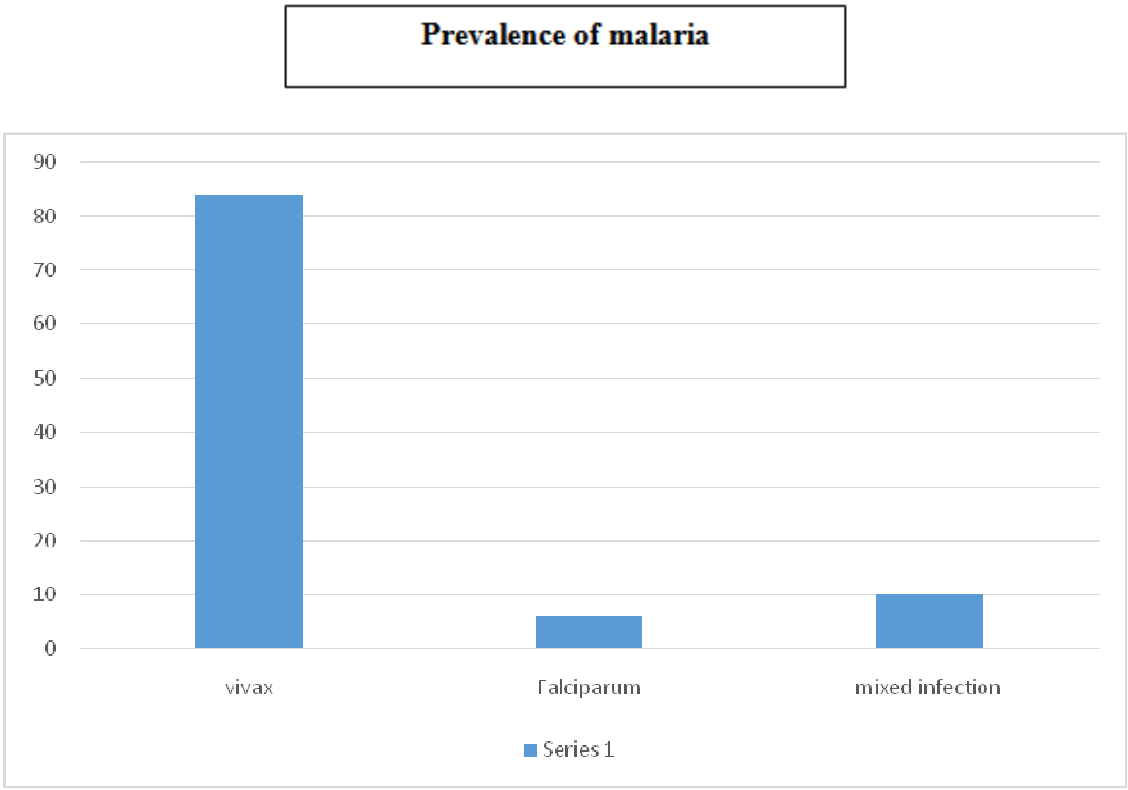
Patients with an established diagnosis of systemic infections, typhoid fever, dengue fever, and meningitis were excluded from the study. A complete blood count (CBC) and malarial parasite microscopy were performed for all patients. The peripheral smears were stained with Leishman's stain. The slides were examined for malarial parasite. The slides were initially examined by a post graduate who was blinded from the CBC results. All malaria-positive smears were reviewed by a hematologist for confirmation, identification of species and review of smear for a platelet count. Smear examination for malarial parasite was taken as the gold standard for malaria diagnosis. Statistical analysis was done using the Chi square test of significance which was applied to calculate *p* value. Sensitivity, specificity, positive and negative predictive values along with likelihood ratios (LR) for positive and negative test results were calculated for hematological parameters.

RESULTS

A total of 650 patients with acute febrile illness were included in the study. Of these, 164 were diagnosed to have malaria as confirmed by smear examination. 138 patients had *Plasmodium vivax* infection, 10 patients had *Plasmodium falciparum* and 16 had mixed infection with both *Plasmodium vivax* and *falciparum.* (Table 1).The malaria group was composed of 112 males and 52females with a male to female ratio of 2.3:1. The maximum number of cases were seen in the month of September (50 cases; 29.8%), followed by July (31 cases; 18.9%) and August (23 cases; 14.02%).

Table 1
Distribution of malaria species n=164

Plasmodium species	Frequency	Percentage (%)
Vivax	138	84
Falciparum	10	6
Mixed infection	16	10



An analysis of the hematological parameters was performed in patients with and without malaria (Table 2). The 95% confidence interval for each parameter along with *p* values is also shown in Table 2. There was a statistically significant reduction in hemoglobin ($p<0.005$), platelet count ($p<0.001$) and total leukocyte count ($p<0.001$) levels in patients with malaria compared to patients without. The percentage of neutrophils in the subjects with malaria was significantly higher ($p<0.005$) than in the non-malarial group. The monocyte count mean was reduced in malarial subjects but was found to be higher compared to the non-malarial subjects ($p<0.001$). Red cell distribution width values also were found to be higher in malarial subjects than in non-malarial subjects ($p<0.001$).

Table 2
Baseline characteristics of hematological parameters in patients with and without malaria.

Parameters	Reference range	With malaria(n=164)		Without malaria (n =486)		p-value
		Mean (+SD)	95% CI	Mean (+SD)	95% CI	
Hb(g/dl)	13-18	9.6(2.72)	9.2-10.0	11.4(2.49)	11.2-11.6	<0.005
TLC($x10^9$/L)	4-11	5(2.63)	4.6-5.4	7.2 (3.6)	6.9-7.5	<0.001
Neutrophils (%)	40-75	66(12.36)	64.1-67.8	62(16.4)	60.4-63.6	<0.005
Lymphocyte (%)	20-45	22.6(7.20)	21.5-23.6	28.4 (16.53)	27-29.8	<0.001
Monocyte (%)	2-8	2(0.24)	1.9-2.1	1 (0.23)	0.98-1.02	<0.001
Platelets	150-400	0.80(0.42)	0.74-0.86	1.22(0.66)	1.16-1.28	<0.001
RDW		17(4)	16.4-17.6	16.2(1.23)	16.1-16.3	<0.001

Hb=Hemoglobin, TLC=Total leucocyte count, RDW=Red cell distribution width SD= Standard deviation

We obtained sensitivity, specificity, predictive values and likelihood ratio for diagnosis of malaria with the hematological parameters along with 95% confidence interval (Table 3). Anemia (Hb<10gm/dl) and leucopenia showed low sensitivity and moderate specificity, although high Red cell distribution width expressed high sensitivity and poor specificity in diagnosis of malaria. The presence of low platelet count (<100,000/mm^3) was both 82% sensitive and 88% specific for the diagnosis of malaria with a likelihood ratio for a positive and negative test result of 6.35 and 0.32, respectively. The likelihood ratio for a positive test result of leucopenia was also relevant with a value of 3.4.

Table 3
Sensitivity, Specificity, Predictive values and Likelihood ratio (LR) for diagnosis of malaria with the hematological parameters.

Variables	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)
Hb<10gm/dl	36 28.7-43.3	80 75.9-82.9	34 27.7-41.4	77 (73-80)	1.42 1.01-1.85	0.74 (0.65-0.85)
TLC<5000	54 46.5-61.8	83 79.5-85.8	50 42.1-57	88 (84.4-90.5)	3.62 2.91-4.12	0.63 (0.35-0.53)
Platelets <1,00,00	82 75.1-87.4	88 85.6-91.3	68 61.3-74.5	96 93.1-97.5	6.35 5-8.1	0.32 (0.26-0.46)
RDW>15	86 79.9-90.2	50 45.2-53.7	34 29-38.3	91 87.4-93.6	1.6 1.42-1.77	0.20 (0.12-0.32)

Hb=Hemoglobin, TLC=Total leucocyte count, RDW=Red cell distribution width, PPV= Positive predictive value, NPV= Negative predictive value, LR= Likelihood ratio

Table 4
Combination of hematological parameters (Hb, TLC, Platelets, RDW) for diagnosis of malaria

Test	Test Result postive		Test result negative	
	LR positive	Post-test probability	LR negative	Post-test probability
Hb < 10 gm/dl	1.52	33	0.74	22
TLC < 5000	3.34	53	0.64	15
Platelet < 100000	6.65	66	0.32	7
RDW>15	1.70	35	0.34	8
All 4 variables together	7.31	68	0.67	20

A combination of positive tests for hematological parameters (Hb<10, TLC<5000, Platelets<100,000, RDW>15) was thought to be more useful, as all tests were done simultaneously in the auto analyzer and are available to the clinician for interpretation. The sensitivity, specificity, predictive values and likelihood ratio were obtained with the combined hematological parameters along with 95% confidence intervals for diagnosis of malaria (Table 4). The combined likelihood ratio for a positive test when all four variables were positive was 7.31, when any three were positive was 5.23 and when two were positive was 3.62.

Table 5
Posttest probabilities of variables (posttest probabilities of positive and negative test results when pre-test probability of malaria is 24%).

Combined variables Hb <10, TLC <5000, RDW >15, PI<100000	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)
All four positive Hb+TLC+Platelets+RDW	27.1%	95.3 %	70.3%	79.7%	7.31	0.67
	20.7-34.3	93.3-96.7	57.5-81.0	76-82.6	4.48-12.86	0.60-0.74
Any three positive	33.5	92.6	62.5	80.6	5.23	0.82
Platelets+TLC+RDW	26.6-41.11	90.2-94.5	57-76	79-87	3.58-7.64	0.55-0.70
Any two positive	32.7	91.9	52.7	82.4	3.62	0.83
Plateles+TLC	25.7-40.3	89.2-94.1	42.8-62.2	79.1-85.4	2.55-5.11	0.75-0.91

Pre-test (prevalence) and post-test probability are subjective probabilities of the presence of a disease before and after a diagnostic test, respectively. In the present study, pre-test probability was 24% and post-test probability for Hb<10gm/dl, TLC <5000, Platelets <100,000 and RDW >15 were 33%, 53%, 66% and 35%, respectively. A posttest probability for the four tests taken together was 68% with a likelihood ratio for a positive test of 7.2. (Table 5).

DISCUSSION

Malaria is one of the major health problems in India, because it is not just confined to the rural areas but also in urban. Accurate and swift diagnosis is critical towards the effective management of malaria. The pan-global impact of malaria has created the need to develop effective diagnostic strategies in places where malaria diagnostic expertise is often lacking.[8] In the current study, 84% cases were accounted for by *Plasmodium vivax* followed by 10% mixed infection (both P. *vivax* and P. *falciparum)* and 6% P. *falciparum.* The mean age of these patients was 29.2 years and the highest proportion of cases (46%; 76 cases) were seen in the 20-30 years age group. Almost 81% of the malaria positive cases were seen among the adults. Most of the studies have reported a high burden in males compared to females.[9]our study showed similar results with a male to female ratio of 2.3:1.[10]As observed in our study , The increase in malaria cases in the rainy (June-August) and post-rainy (September) seasons could be due to the increase in number of the female vector-Anopheles mosquito whereas biting rates and the rate of malaria parasites transmission are expected to increase in the hot humid climatic conditions and warm stagnant water.[11] The nonspecific nature of symptoms and signs in malaria impairs diagnostic specificity, and often promotes the unwanted use of antimalarials, thereby the quality of care for patients with non-malarial fevers in endemic areas were hampered[12] Laboratory diagnosis of malaria is based upon the demonstration of the malarial parasite on smear microscopy which needs expertise and repeated smear examinations. Hematological abnormalities can be considered as a hallmark of malaria and analyses have shown that many of these values may lead to a suspicion for malaria clinically, and thus initiating therapy even in the absence of a positive smear. A variety of hematological alterations like progressively increasing anemia, thrombocytopenia, leukocytosis or leukopenia have been reported in cases of

malaria.[13] Lower mean values for hemoglobin, leukocyte count and platelet count in the malaria group compared to the control group was observed in our study. This was in concordance with other studies.[14] The present study demonstrated that different hematological variables (Hb <10, TLC <5000, RDW >15, Platelets <100,000) increase the probability of malaria when their LR for a positive result was calculated and this probability increased manifold when used in combination with a LR for a positive result of 7.3 when all four tests were positive. The pre-test probability indicating the prevalence of the disease was 24% in the present study and posttest probability for Hb <10gm/dl, TLC <5000, Platelets <100,000 and RDW>15 were 33%, 53%, 66% and 35%, respectively. A posttest probability for the four tests taken together was 68% with a likelihood ratio for a positive test of 7.3.Post-test probability can be very useful to the clinician for estimating the probability of the disease in their clinical settings and guide towards decision making with regards to treatment. Anemia is known to be associated with malaria[15.]The present study demonstrates that low Hb (<10gm/dl) is a statistically significant variable (p<0.005) that increases the probability of malaria (LR+ 1.52). The pathogenesis of anemia in malaria is complex and multifactorial. Anemia in malaria is often thought to result from a combination of hemolysis red blood cells, fast removal of both parasitized and unparasitized red blood cells, ineffective erythropoiesis and anemia of chronic disease.[16] Other factors are decreased red blood cell deformability, splenic phagocytosis and/or pooling, leading to an increased rate of clearance from the circulation.[17] Leucopenia is a usual finding in malaria cases. It is mostly due to the localization of leucocytes away from the peripheral circulation, splenic sequestration[18]. Our study proved leucopenia as a statistically significant variable in malaria, the mean remained at 2.63 x 10^9 /L, in concordance with other reports.[19] Low platelet count is an important finding of malarial. In our study, thrombocytopenia (platelet<100,000/mm^3) is an important predictor of malaria, as many studies confirm [9, 14, 17, 19]. Statistically significant association of thrombocytopenia present in malaria as compared to non-malarial fevers were observed (p<0.001). A high sensitivity (82%) and specificity (88%) was seen along with an increase in probability of malaria by factor 6.3, in concurrence with observations by other authors[20] Thrombocytopenia may be caused due to peripheral destruction[7] as well as platelet consumption by the disseminated intravascular coagulation (DIC).[21] Red cell distribution width (RDW) along with mean corpuscle volume (MCV) of red cells are touted as newer parameters for malaria. RDW describes the population dispersion of red cell volume or the range of changes in size of red blood cells which mostly are enlarged after malarial invasion.[13]. In the present study RDW, values were found to be higher in the malaria group than the non-malarial cases which is similar to other study findings [13]. The presence of increased RDW has coincided with the percentage of macrocytes in one study[22]hence a combination of RDW and MCV may be more helpful. The variation in RDW is attributed to red cells infection by malaria parasites (especially *P vivax),* where the cells enlarge. This is followed by the rupture of RBC's. Since this cycle has never coinciding, parasites at more than one stage of development will usually be seen in smear, hence, RBC's of different sizes. This is not seen in *P falciparum* malaria, as they retain their original size.[13]. As the majority of our cases were of *P vivax,* this explains the increased RDW.

CONCLUSION

The hematological aspects of malaria are curious and interesting. We observed that these hematological changes such as anemia, thrombocytopenia and leucopenia showed a statistically significant correlation with malaria. So we conclude that routine laboratory findings such as hemoglobin, leukocyte and platelet counts and even red cell distribution width values associated with fever, chills and rigor can provide the clinicians diagnostic clue in a patient presenting with acute febrile illness in endemic areas, giving the chances of suspecting malaria and hence a prompt

diagnosis which helps us to initiate treatment immediately. Due to the shortage of resources and trained health personnel in malaria infested endemic areas, presumptive clinical diagnosis is the relevant and reliable option.

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