Original Article

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Parasite and the Circulating Pool- Characterisation of Leukocyte Number and Morphology in Malaria

Pathology Section

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ABSTRACT

Introduction: Haematological changes are the most common complications encountered in malaria. There is significant correlation between several of the haematological parameters and the clinical profile, prognosis and mortality in malaria. White cell counts and differentials are among the most basic and primary investigations done in a patient presenting with fever of short duration.

Aim: This study analyzes the numerical and morphological changes in White Blood Cells (WBCs) in peripheral blood in patients with acute malaria in endemic region in an effort to get a picture of specific changes that could be identified by basic investigations.

Materials and Methods: This study was conducted in tertiary care hospital in a region endemic for malaria. EDTA anticoagulated venous blood samples from 600 patients diagnosed with vivax and falciparum malaria was analysed in Coulter counter LH 500 for the white cell count and differentials. Morphological changes were looked for in Leishman stained peripheral blood smear. Comparison with age matched healthy controls was done by ANOVA with Bonferroni test wherever applicable.

Results: Patients with malaria showed significant leucopenia, neutrophilia, lymphocytopenia, monocytosis and eosinopenia. Lymphocytopenia was more severe in the falciparum group as compared to the vivax group. A higher White Cell Count (WCC) was seen in patients with higher haemoglobin levels in vivax group. The total leukocyte count showed a negative correlation with neutrophil count in falciparum malaria and a strong positive correlation with neutrophil count in vivax malaria. Band neutrophils were seen in 10% of the patients with falciparum and 1.1% of patients with vivax malaria. Atypical plasmacytoid lymphocytes were the only notable morphological finding.

Conclusion: Changes in leukocyte number and morphology in the peripheral blood are common. A combination of monocytosis and eosinopenia in a patient presenting with fever should alert the observer to the presence of malaria and should prompt a repeat blood smear examination in case of initial negative results for the parasite.

Keywords: Atypical lymphocytes, Eosinopenia, Leukocytes, Malaria, Monocytosis

INTRODUCTION

Malaria is one of the most important infectious diseases which contributes significantly to morbidity and mortality. World over, malaria is endemic in 91 countries and over 40% of the world population is at risk of developing malaria, with an infection rate of 250 million per year and the mortality rate of 1-2 million per year [1,2]. Haematological changes are the most common complications encountered in malaria and play a major role in the fatality. Focus of current research is on the parasite host interactions as well as mechanisms detailing the pathogenesis of complications [3-5].

There is significant correlation between leukocyte parameters in malaria, the clinical severity of the disease and mortality. Analysis on the leukocyte parameters have brought forward varied combination of findings. A normal White Cell Count (WCC) with increase in the band forms, leukocytosis, leukopenia, lymphopenia and monocytosis have been documented in endemic zones of Thailand and UAE [6,7]. As opposed to adults, in children of endemic zones a significantly positive correlation between leukocyte number and the parasite load with poorer clinical outcomes in those harbouring a larger number of parasites was noted [5]. In non immune patients who presented with imported disease in malaria free zones lymphopenia, leucocytosis, leucopenia and thrombocytopenia have been recorded [8].

The outcomes of the different studies done so far are not only different but at times are contrasting. Though endemic for malaria, there is little data available on the leukocyte characteristics in malaria in the Indian population and fewer ones which compare them to healthy controls drawn from the local semi-immune population [9,10].

AIM

The objective of the current study was to Analyze the numerical changes in the white cells occurring in the peripheral blood in patients with malaria with aim to identify those parameters which could be obtained in a cost effective manner so as to make it applicable in clinical scenario.

MATERIALS AND METHODS

The study was commenced after obtaining approval from the institutional ethics committee. Majority of the patients were from Mangaluru city which is situated in endemic zones of coastal Karnataka in southern India. The study was conducted over a period of three years from June 2012 to June 2015. Patients were included after obtaining informed consent. Patients on anti-malarial medications prior to obtaining the samples were excluded from the study. EDTA anticoagulated venous blood samples were obtained from newly diagnosed smear positive patients. The haematological parameters were determined using Beckman Coulter analyser LH-500. A manual differential count was performed in all the samples which showed morphological flag of LIC (Large Immature Cell) [11].

The parasite load was determined as follows-

- + = 1–10 parasites per 100 oil-immersion thick film fields,
- ++ = 11–100 parasites per 100 oil-immersion thick film fields,
- +++ = 1–10 parasites per single oil-immersion thick film field,
- ++++ = more than 10 parasites per single oil-immersion thick film field [11].

The name, age, sex, chief complaints and the duration of symptoms were recorded.

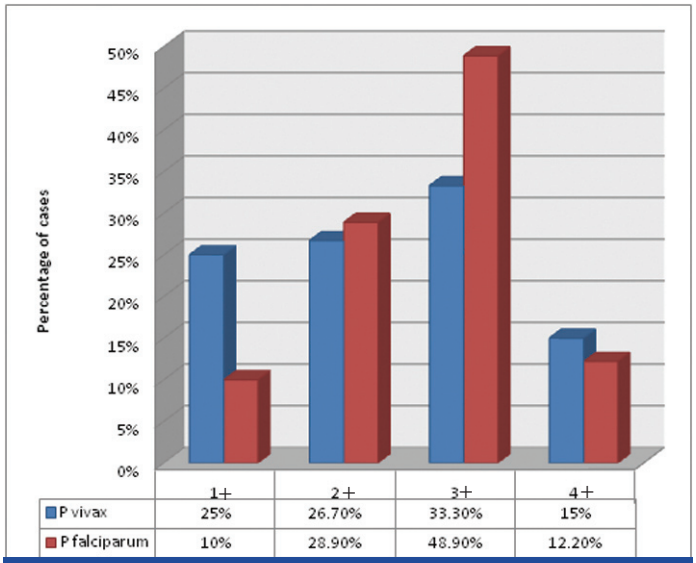
The control samples were taken from healthy adults. ANOVA was used to compare parameters between the cases and the controls. Patients of malaria were further categorized by species into those with *P vivax* and *P falciparum* infection and were compared with the controls and with each other.

RESULTS

After exclusion, a total of 600 patients were included in the study along with 313 controls. Three hundred sixty patients were positive for *P vivax* and 240 were positive for *P falciparum*. Men comprised 70% the total number of cases.

Most common symptom at presentation was fever followed by headache, vomiting, pain abdomen. A little over 3% of the cases had no fever at presentation and 76.5% of them presented with vomiting. Parasite load ranged from + to ++++ [Table/Fig-1].

As compared to the control population the WCC was lower in patients with malaria. The difference between the vivax and the other two groups was highly significant. There was no significant difference in the WCC between the control and the falciparum group. Also there was no significant correlation between the WCC counts and the parasite load in patients in either of these groups. Additionally a higher WCC was seen in patients with higher haemoglobin levels in vivax group [Table/Fig-2,3].



[Table/Fig-1]: Distribution of cases by parasite load.
1+,2+,3+,4+- represents parasite load

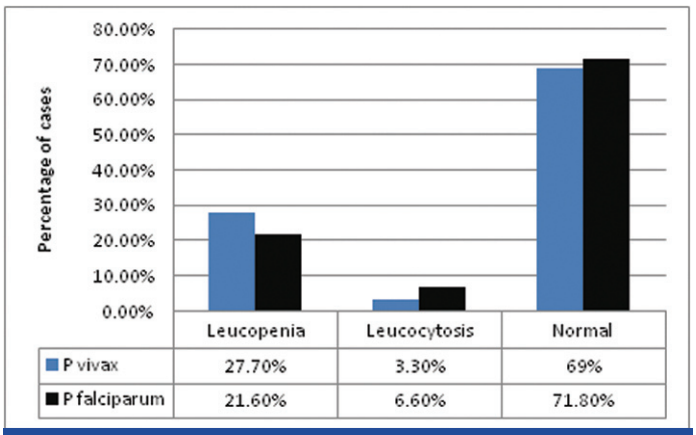
The neutrophil counts in malaria were higher as compared to controls. The difference between the control and vivax groups when compared with the falciparum group was highly significant [Table/Fig-4]. Also, patients with higher haemoglobin levels showed higher counts in malaria group. Surprisingly the total leukocyte count showed a negative correlation with neutrophil count in falciparum malaria and a strong positive correlation with neutrophil count in vivax malaria.

Lymphocytopenia was striking in patients with malaria with counts being significantly lower in falciparum as compared to vivax malaria. Lymphocytopenia was more common in patients with higher haemoglobin level and WCC in vivax malaria. Atypical lymphocytes in malaria were large with abundant dark blue cytoplasm and eccentrically placed nucleus of plasmacytoid morphology [Table/Fig-5].

Significant monocytosis was noted in the malaria group as compared to the control population. The degree of monocytosis in falciparum was significantly higher in comparison to controls and the vivax group. A higher monocyte count was associated with a lower WCC in falciparum malaria. Also, monocyte counts were significantly higher in patients who had higher haemoglobin levels as compared to patients with lower haemoglobin levels in malaria.

Eosinophils and basophils were significantly less in patients with vivax and falciparum malaria though the counts between the two groups were not different [Table/Fig-6].

A little over 5% of the vivax and 18.3% of the falciparum patients showed atypical lymphocytes in their peripheral blood smear. Band



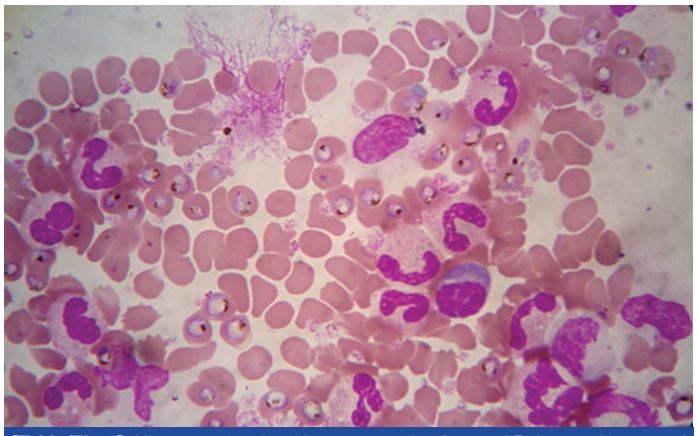
[Table/Fig-2]: Distribution of cases by WBC counts.

		N	Mean	Std. Deviation (SD)	95% Confidence Interval for Mean		ANOVA F value	p-value
					Lower Bound	Upper Bound		
Total Wbc Count (/µL)	*P vivax*	360	5507.78	2377.985	5261.30	5754.25	14.852	p<0.001 HS
	P falciparum	240	6539.17	4688.280	5943.01	7135.32		
	Control	313	6638.02	1449.179	6476.85	6799.19		
	Total	913	6166.37	2998.109	5971.64	6361.11		
Neutrophil(%)	*P vivax*	360	60.34	13.201	58.97	61.71	17.011	p<0.001 HS
	P falciparum	240	63.96	12.335	62.39	65.53		
	Control	313	58.27	7.917	57.39	59.16		
	Total	913	60.58	11.609	59.83	61.34		
Lymphocyte (%)	*P vivax*	360	30.83	13.433	29.43	32.22	41.619	p<0.001 HS
	P falciparum	240	25.25	10.979	23.85	26.65		
	Control	313	33.96	8.029	33.07	34.85		
	Total	913	30.43	11.665	29.68	31.19		
Monocyte(%)	*P vivax*	360	7.02	3.495	6.66	7.38	200.046	p<0.001 HS
	P falciparum	240	7.98	3.073	7.59	8.37		
	Control	313	3.58	1.276	3.44	3.72		
	Total	913	6.09	3.360	5.87	6.31		

[Table/Fig-3]: ANOVA for total leukocyte, neutrophils, lymphocytes and monocytes. HS-Highly significant.

			p	
Total WBC Count (/µL)	*P vivax*	*P falciparum*	.000	HS
		Control	.000	HS
	P falciparum	Control	1.000	HS
Neutrophil (%)	*P vivax*	*P falciparum*	.000	HS
		Control	.046	HS
	P falciparum	Control	.000	HS
Lymphocyte (%)	*P vivax*	*P falciparum*	.000	HS
		Control	.001	HS
	P falciparum	Control	.000	HS
Monocyte (%)	*P vivax*	*P falciparum*	.000	HS
		Control	.000	HS
	P falciparum	Control	.000	HS

[Table/Fig-4]: Bonferroni test for comparison between individual group.
HS – highly significant, sig- Significant, NS- Not significant



[Table/Fig-5]: Hyperparasitemia with numerous ring forms of P.vivax and increased number of neutrophils with a single atypical lymphocyte. Neutrophilia in malaria is an indication of poorer prognosis. Original magnification 400x. Giemsa.

Multiple Comparisons				
			p	
Eosinophil(%)	*P vivax*	*P falciparum*	.165	
		Control	.000	HS
	P falciparum	Control	.000	HS
Basophil(%)	*P vivax*	*P falciparum*	.957	
		Control	.000	HS
	P falciparum	Control	.000	HS
Band Forms(%)	*P vivax*	*P falciparum*	.000	HS
		Control	.372	
	P falciparum	Control	.000	HS
Atypical Lymphocyte(%)	*P vivax*	*P falciparum*	.000	HS
		Control	.042	sig
	P falciparum	Control	.000	HS

[Table/Fig-6]: Comparision of eosinophil, basophils, band forms and atypical lymphocyte counts.
HS- Highly significant. Sig- Significant

forms were seen in 10% and 1.1% of patients with falciparum and vivax malaria respectively. The band forms in all the cases comprised less than 5% of the total leukocyte count.

DISCUSSION

The incidence of leukopenia in this study is higher than all the other studies done in the endemic areas and the incidence of leukocytosis was comparable to the rest of the groups. When compared to the local control population. However, the degree of leukopenia was highly significant only in the vivax group. This finding is similar to the study done by Koltas et al., who studied haematological parameters in 90 *P. vivax* cases and found significantly lower leukocyte count as compared to controls in the endemic zones of Turkey [12] [Table/Fig-7].

Author (year)	Groups	Normal WBC counts	Leuko-penia	Leuko-cytosis	Zones
Present study (Year)	Average of both groups	70.4%	24.6%	5%	Endemic
	P falciparum	71.8%	21.6%	6.6%	
	P vivax	69%	27.7%	3.3%	
Taylor et al., 2008 [13]	Both groups	85%	9%	6%	Endemic & non endemic
Abro et al., 2008 [7]	Both groups	86%	11%	3%	Endemic
Bashwari et al., 2002 [6]	Both groups	78.3%	13.3%	7.2%	Non endemic
Reily et al., 1971 [3]	Both groups	63.5%	31.2%	5.3%	Non endemic
Ladhani et al., 2002 [5]	Falciparum	69.7%	10.2%	20.1%	Endemic
Richards et al., 1998 [8]	Falciparum	91%	7%	2%	Non endemic

[Table/Fig-7]: Observations of present study in comparison with similar studies.

Comparisons between the results of malaria versus non malarial control patients in endemic regions of Nigeria yielded significantly higher White Cell Count (WCC) and increase in absolute lymphocytes and monocytes counts. in the malaria infected patients as opposed to lower WCC, monocytes, lymphocytes and neutrophils encountered in Thailand. Additionally the Neutrophil-Lymphocyte Ratio (NLR), and Monocyte-Lymphocyte Ratio (MLR) were found to be higher in patients in comparison to non-malaria patients [14,15].

The variations in the leukocyte numbers is said to be dependent on many factors including the acuteness of infection, the parasitemia, the severity of the disease the state of host immunity to malaria (endemicity) and concurrent infections. Since circulating pools of white cells represent only a small proportion of the total body leukocyte pool it is thought that changes in the peripheral blood in malaria often represent redistribution of these cells between different vascular and tissue compartments. Leukocytosis when present is considered a marker of poorer prognosis with respect to both morbidity and mortality especially in children with falciparum malaria [5,16]. Taylor et al., who followed up patients with malaria, reported normalization of counts in 28 days in most of the individuals [13].

NEUTROPHIL

In this study there was a significant increase in the neutrophil percentage in the malaria patients and more so in those with falciparum infection. Khaled et al., noted in their study an increase in the neutrophil percentage in the falciparum and the mixed infection group but not in the vivax group. Like WCC, a higher neutrophil count is also associated with poorer clinical outcome in falciparum malaria [5,17].

Presence of bands in the peripheral smear of the cases may be a representation of early release of neutrophil precursors from the bone marrow in response to the infection. This feature was exclusively confined to the infected patients [18,19]. The degree of lymphocytopenia was more in falciparum than in vivax group in comparison to controls [Table/Fig-8]. This finding is to similar to that of other researchers [9,20]. Lymphocyte counts have been known to normalize within 2 weeks of initiating therapy [19,20]. Studies of lymphocyte subsets have found that the major changes in lymphocyte numbers are in the T-cell compartment and the changes are reversed after treatment [21,22].

Two main theories have been proposed for this

First, there is disease induced relocation of T lymphocytes to the site of inflammation in the periphery followed by rapid re-emergence

Author (year)	Groups	Lymphocytopenia	Zones
Present study	Average of both groups	23.9%	Endemic
	P falciparum	30%	
	P vivax	17.8%	
Abro et al., 2008 [7]	*P vivax*	36%	Endemic
	P falciparum	15%	
Bashwari et al., 2002 [6]	Both groups	42.9%	Non endemic
Richards et al., 1998 [8]	Falciparum	63%	Non endemic

[Table/Fig-8]: Comparison of lymphocytopenia in present study in comparison with various series.

and hence repopulation of blood lymphocytes following therapy [23,24]. Second, there is apoptosis of the T cells brought about by increased levels of FasL in the serum secondary to inflammation induced by the parasite. This theory was supported by the fact that there was a parallel rise and fall in the serum FasL corresponding to that of the T cells counts though a direct relationship between the two is not yet been proved [25]. Lymphocytosis on the other hand is distinctly uncommon and is thought to be a predictor of poor prognosis [5].

The presence of reactive lymphocytes in the circulation in patients with malaria has been noted in other studies as well. These cells increase in the circulation by day 3 or 4 and then decline. It is believed that they may represent activated T cells responding to malaria antigens or mitogens, or they may result from reactivation of certain latent viruses such as EBV or CMV [26,27].

Monocyte

Monocytosis was a very highly significant finding in the present study. As in the present study researchers have found higher differential monocyte count in P. falciparum as compared to controls and the vivax group [9].

Monocytosis represents the reticulo-endothelial hyperplasia brought about by the parasite which is clinically evident in form of hepato-spleenomegaly and a brisk monocyte response and is associated with better prognosis [3,5,6].

Eosinophils

Eosinopenia is a common observation in several studies [28,29]. Abdalla et al., observed eosinopenia in Gambian children with acute malaria. These changes were mostly reversed by days 3 to 7 after starting treatment. Mild rebound eosinophilia was seen after starting treatment [16].

Eosinopenia persisting for around five days was observed in adults which was followed by rebound eosinophilia reaching a peak about one month after the initial diagnosis of malaria. The mechanisms by which peripheral blood eosinopenia occurs in acute malaria are not known. It is suggested that the initial eosinopenia is due to the Th1 response and the rebound eosinophilia that occurs following recovery is due to the Th2 response that eventually takes over as a protective response against over-activity of the pro-inflammatory Th1 response. Eosinopenia is not a manifestation of haematopoietic suppression as evidenced by a study in children which showed an increased number of eosinophils and precursor in the bone marrow Experimental in vitro work has shown that eosinophil products inhibit the growth of P. Falciparum [28-30].

LIMITATION

We have not followed up patients during and in the post treatment period to determine the time required for complete haematological recovery. It is also necessary to determine if any parameter by itself or in combination could be used reliably to predict the severity of the disease in these patients. Further research in this aspect is needed to establish its relevance in the clinical setting.

CONCLUSION

A number of important findings occur in leukocyte number and morphology in the peripheral blood. Many of these changes are hypothesized to be due to redistribution of the white cells into the circulating pool which is sampled during the peripheral blood examination. A combination of absolute monocytosis and a low eosinophil count can point towards malaria in a patient with acute febrile illness.

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