JMSCR Vol||05||Issue||08||Page 26473-26477||August 2017

www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i8.81

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Vivax-induced thrombocytopenia may be associated with lymphopenia and mild anaemia. Correlation of RBC parameters with WBC and platelet counts – A report of 13 patients with vivax malaria

Authors
Akansha Singh[1], Ashok Kumar Kapoor[2], Vidya Bhushan[3], Manish Singh[4]
[1]Senior Technician, Department of Haematology, RML Mehrotra Pathology Pvt. Ltd. Lucknow, Uttar Pradesh, India
[2]Pathologist, Dept of Pathology, RML Mehrotra Pvt Ltd., Nirala Nagar, Lucknow, Uttar Pradesh, India
[3]Professor medical statistics, Department of community Medicine, King George Medical University, Shahmina Road, Lucknow, Uttar Pradesh, India
[4]Pathologist, Dept of Pathology, RML Mehrotra Pvt Ltd. Nirala Nagar, Lucknow, Uttar Pradesh, India
Corresponding Author
Dr Ashok Kumar Kapoor
RML Mehrotra Pathology Pvt Ltd, Nirala Nagar, Lucknow-226020, Uttar Pradesh, INDIA
Email: *drashokkapoor2016@gmail.com*

ABSTRACT
Present study relates to the results of haematological investigations of 13 patients with vivax malaria. One of the 13 patients had moderate anaemia. Eight other patients had mild anaemia. Four other patients had normal haemoglobin (Hb) concentration. Leucopenia was detected in two patients. Three patients had lymphopenia. Two other patients developed mild eosinophilia. Thrombocytopenia was detected in 12 of 13 patients (92%). Three patients had severe thrombocytopenia (platelets <0.5 lac/mm^3). Six patients had moderate thrombocytopenia (platelets 0.5 lac to <1 lac/mm^3). Three other patients had mild thrombocytopenia (platelets 1 lac to <1.5 lac/mm^3). Thrombocytopenia might have developed subsequent to bone marrow suppression of megakaryocytes or due to platelet sequestration by splenic macrophages. In patients, results of product moment correlation (r) revealed direct correlation between Hct and neutrophil count (r = 0.932) and between MCHC Vs neutrophil count (r = 0.467). In addition, inverse relation was detected between MCV vs WBC (r = - 0.523) and between MCV vs neutrophil count (r = - 0.382). These observations suggested that Hct, MCHC and MCV all might affect neutrophil count in malaria.
Keywords: *Plasmodium - induced reduction of platelets.*

INTRODUCTION

Results of previous studies on malaria in this region revealed detection of *Plasmodium falciparum*[1,2] alone in years 1995 to 1997. Present study was carried out later after 2 decades which revealed detection of vivax malaria in many subjects with fever. However, few cases had mixed infection with *P. falciparum* and *P. vivax*. Our region has low *Plasmodium* endemicity areas. The disease appears to infect mainly the older children and adults of different age-groups.

Results of another study revealed thrombocyt-openia (platelets <1 lac/mm^3) in 42 of 100 patients with *P. vivax* monoinfection[3]. Another study

reported thrombocytopenia in 61.5% children and bleeding manifestations in 10.8% cases[4]. In addition, severe thrombocytopenia (platelets <2 × 10^4/mm^3) may be a common manifestation of vivax monoinfection[5]. Role of platelet phagocytosis has been suggested in vivax malaria[6]. Higher mean platelet volume (MPV) was found in another study where MPV was higher in thrombocytopenia patients when compared with non-thrombocytopenic patients and a negative correlation was found between platelet count and MPV[6].

Present study relates to haematological alterations in patients with vivax malaria. Another purpose of this study was to correlate the red blood cell findings with results of WBC and platelet counts.

MATERIALS AND METHODS

During 1 year period, extending from 1st January 2016 to 31st December 2016, 264 fever cases were screened for malaria and haematological investigations were done. On the basis of microscopic examination of thin and thick blood smears, 8 subjects were *P. vivax* positive (MP +ve). Five other subjects were *P. vivax* antigen positive. Thus, 13 subjects had *P. vivax* mono infection. These 13 subjects were labelled as MP +ve patients. Two other subjects had mixed vivax and falciparum infection. Other 249 subjects were negative for malarial parasite (MP –ve). These subjects acted as disease controls (cause of fever was not investigated further in these subjects).

Inclusion criteria: Only those subjects were included as patients where the diagnosis was confirmed either by microscopic smear examination or by *Plasmodium* antigen detection test.

Exclusion criteria: *P. vivax* antigen positivity was detected in another subject which was later excluded from this study because complete blood cell counts could not be done in this subject.

About 2 ml of venous blood was collected from each subject in a EDTA vial and also separately for preparation of thin and thick smears. Smears were stained using autostainer (Aerospray Pro-slide stainer cytocentrifuge, pH 6.8). Stain was sprayed onto slides in a cytocentrifuge. Leish-man-stained blood smears were microscopically examined. *Plasmodium* antigen was detected by rapid chromatographic immunoassay using on site pf/pan antigen test. It was purchased from Biotrol laboratories Pvt. Ltd. New Delhi-110016. It detected malaria pf (HRP II) and Pv (pLDH) antigens in human whole blood. Haematological parameters were recorded using Beckman counter 750. Following parameters were studied, e.g. Hb, RBC, Hct, MCV, MCH, MCHC, TLC, DLC and platelets.

Statistical Analysis: Data was analysed using statistical package for social science for windows release (17.0). Bivariate analysis was done for negative and positive correlation (r) between variables. Correlation coefficient (r) between two groups was calculated using Karl Pearson's product method. P value of <0.05 was considered to be significant. The values were represented in number, % and mean + SD.

RESULTS

Age of the patients (n = 13) ranged from 4 to 65 (median 23) years. Male female ratio was 2.2:1. One of the patients had moderate anaemia (Hb 10.0 gm/dl). Eight other patients had mild anaemia (table 1). Table 2 shows results of WBC and platelet counts in vivax malaria patients. Two patients had leucopenia. Three patients had lymphopenia. Two other patients developed mild eosinophilia. Thromobocytopenia was detected in 12 of 13 patients (92%). Three patients had severe thrombocytopenia (<0.5 lac platelets/mm^3). Six other patients had moderate thrombocytopenia (platelets 0.5 lac to <1 lac/mm^3). Three other patients had mild thrombocytopenia (platelets 1 lac to <1.5 lac/mm3). Pancytopenia was detected in 2 patients. Later, the MP +ve patients were treated with chloroquine phosphate and Primaquine phosphate.

Lower mean + SD values of following parameters were detected in patients (n = 13) when compared with the values of MP –ve controls (n = 249). Parameters with lower values were Hb, RBC, Hct, WBC, neutrophils, lymphocytes and platelets

(table 3). Table 4 shows results of correlations of RBC parameters with WBC and platelets. Absolute neutrophil counts showed direct correlation with Hct and MCHC and mild inverse relation with MCV. Inverse correlation was observed between absolute monocyte counts and MCV. Inverse correlation was detected between WBC Vs MCV. In addition, direct correlation was detected between WBC Vs MCHC.

Table 1 - RBC Parameters in Vivax Malaria patients.

Lab ID	Age Years	Hb gm/dl	RBC million/mm^3	HCT %	MCV fl	MCH pg	MCHC gm/dl	RDW-CV %	Name
111150	23	11.6	3.86	36.2	73.7	23.2	31.5	14.6	Mild microcytic anaemia
113492	65	14.3	4.76	40.2	90.9	29.5	32.4	12.6	Normal
113432	18	11.0	3.66	33.0	104. 8	33.2	31.7	14.6	Mild macrocytic anaemia
112513	47	12.9	4.3	36.1	88.7	28.5	32.1	11.8	Mild normocytic anaemia
110832	27	12.0	4.0	36.0	84.2	25.2	30.8	14.6	Mild normocytic anaemia
210215	56	10.9	3.63	30.2	86.6	28.8	33.2	14.8	Mild normocytic anaemia
110808	18	9.8	3.26	28.2	87.9	28.2	32.0	11.5	Mild normocytic anaemia
112645	33	9.3	3.1	28.2	92.8	29.1	31.3	11.0	Mild normocytic anaemia
104315	4	10.1	3.36	30.2	74.8	24.9	33.2	16.4	Normal
102151	9	10.4	3.46	30.1	71.2	23.7	33.3	16.0	Normal
260092	9	8.4	2. 8	25. 0	70.2	25.0	35.6	14.8	Mild microcytic anaemia
255665	6	11. 7	3.9	33.0	75.7	26.1	32.6	16.0	Normal
111073	59	10.0	3.33	30.0	91.4	28.8	31.5	14.6	Moderate normocytic anaemia
	Mean ± SD	10.95±1.52	3.64±0.5231	32.0±4.20	84.0±10.29	27.24±2.83	32.4±1.24	14.1±1.79	

Table 2 - WBC and platelet parameters in Vivax Malaria patients.

Lab ID	TLC Cells/mm^3	NE# Cells/mm^3	LY# Cells/mm^3	MO# Cells/mm^3	EO# Cells/mm^3	PLT Cells/mm^3	Name
111150	8450	7000	1000	200	250	94000	Thrombocytopenia (++)
113492	8200	6000	1100	200	300	50000	Thrombocytopenia (++)
113432	3600	2100	1100	200	200	60000	Leucopenia (+) and Thrombocytopenia (++)
112513	5200	4200	500	300	200	33000	Thrombocytopenia (+++) Lymphopenia
110832	2600	2000	400	100	100	46000	Leucopenia (++), Lymphopenia and Thrombocytopenia (+++)
210215	4300	3000	800	400	100	89000	Thrombocytopenia (++), Lymphopenia
110808	4500	2300	1600	400	600	<10000	Eosinophilia (+) and Thrombocytopenia (+++)
112645	6900	4700	1700	300	200	165000	Normal
104315	9800	4700	3800	1200	100	104000	Thrombocytopenia (+)
102151	5000	2300	2100	500	100	72000	Thrombocytopenia (++)
260092	8700	7000	1100	300	300	65000	Thrombocytopenia (++)
255665	7100	2800	2600	1200	500	117000	Thrombocytopenia (+) and Eosinophilia (+)
111073	4700	2300	1700	500	200	106000	Thrombocytopenia (+)
Mean ± SD	6080±22.41	3876±18.60	1500±9.30	446.15±355	242±155.25	77769±40735.23	

Abbreviations: + mild, ++ moderate, +++ severe

Table 3 : shows mean ± SD of blood cell indices in different groups.

Blood cell indices	MP +ve Patients (n = 13)	Subjects with mixed infection (n = 2) (separate values are given)	MP –ve Controls (n = 249)	t value
Hb gm/dl	10.95 ± 1.52	8.1, 11.2	12.65 ± 2.90	3.71***
RBC million/mm^3	3.64 ± 0.5231	3.26, 4.83	4.12 ± 1.10	2.98**
Hct %	32.0 ± 4.20	24.2, 33.2	37.47 ± 8.62	4.25***
MCV fl	84.0 ± 10.29	83.1, 80.5	87.75 ± 18.50	0.31 NS
MCH pg	27.24 ± 2.83	26.8, 29.9	28.49 ± 6.30	1.42 NS
MCHC gm/dl	32.4 ± 1.24	32.3, 37.2	32.42 ± 5.06	No difference
RDW %	14.1 ± 1.79	12.7, 16.0	14.72 ± 3.70	1.12 NS
WBC ul	6080 ± 22.41	3600, 14000	7457.42 ± 5350	4.06***
NE # cells/mm^3	3876 ± 18.60	2000, 8000	4967.5 ± 592.5	28.79***
Lympho # cells/mm^3	1500 ± 9.30	1300, 1900	1749.5 ± 322.5	12.11***
Mono # cells/mm^3	446.15 ± 355	100, 200	548.5 ± 375.0	0.85NS
Eo # cells/mm^3	242 ± 155.25	100, 100	191.3 ± 60.0	1.17NS
PLT cells/mm^3	77769 ± 40735.23	31000, 40000	175337.3 ± 135250	6.89***

Abbreviations: NS not significant statistically (p >0.05), *** very highly significant (p <0.001)

Table 4 shows correlation of RBC parameters with WBC and platelets in MP+ patients.

RBC parameter	WBC Cells/mm^3	Neutrophils Cells/mm^3	Lymphocytes Cells/mm^3	Monocytes Cells/mm^3	Eosinophils Cells/mm^3	Platelets Cells/mm^3
Hb gm/dl	- 0.082	0.031	- 0.33	- 0.17	- 0.029	- 0.33
RBC million/mm^3	- 0.083	0.032	- 0.33	- 0.17	- 0.029	- 0.33
Hct %	- 0.051	0.932***	- 0.34	- 0.24	- 0.097	- 0.266
MCV fl	- 0.523**	- 0.382*	- 0.361	- 0.41*	- 0.013	- 0.104
MCH pg	- 0.399	- 0.318	- 0.267	- 0.27	0.089	- 0.089
MCHC gm/dl	0.466**	0.467**	0.224	0.268	0.036	- 0.067
RDW %	0.144	- 0.125	0.444	0.528	- 0.319	0.195

Product moment correlation coefficient was calculated to examine the relationship of RBC, WBC and platelets. The correlations are shown with positive and negative signs measuring direct and inverse relationships. The correlations above 0.38 were found to be statistically significant ($p < 0.05$) and are marked as *. The correlations which are not significant (whether + or –ve) can not be given importance. * $p < 0.05$ statistically significant, ** $p < 0.01$ highly significant, *** $p < 0.001$ very highly significant.

DISCUSSION

Most important feature of this study was the detection of *Plasmodium vivax* instead of *Plasmodium falciparum* in most of the cases. Earlier, most of the patients in this region had *P. falciparum* monoinfection[1,2]. However, in the present study, only 2 cases had mixed infection. Factors responsible for this change were not clear.

Another important feature of this study was the detection of thrombocytopenia in most of the patients with vivax malaria. Pathogenesis of thrombocytopenia in malaria requires further investigations. However, several mechanisms may be involved. First, thrombocytopenia might have developed following suppression of megakaryocytes in bone marrow. Second, cross-reactive autoantibodies might have developed against platelets resulting in accelerated platelet destruction. Third, platelet sequestration by splenic macrophages might have contributed to thrombocytopenia. Fourth, Quinine or drug immune complex may adhere to megakaryocytes or platelets, resulting in activation of complement and lysis through the action of membrane attack complex (innocent bystander effect[7]). In addition, platelet parasitization may also contribute to thrombocytopenia in acute vivax malaria[8].

Another important feature of the current study was the detection of anaemia in 9 patients. In another study, vivax-infected erythrocytes appeared to inhibit *in vitro* expansion of CD 34^+ haemato-poietic stem cells leading to ineffective erythropoiesis and anaemia[9]. *Plasmodium* - infected erythrocytes may drive T-cells to proliferate and secrete cytokines, e.g. both INF γ and TNF α may induce dyserythropoietic anaemia[10]. In addition, the mechanisms involved in loss of bystander red cells are not clear[11].

In patients, Hct was directly correlated with neutrophil count. In addition, MCHC was also directly correlated with WBC and neutrophil count. These observations suggested that lower Hct and MCHC might be associated with poor neutrophil-mediated phagocytosis against malaria. However, inverse relationship was detected between MCV vs WBC and between MCV Vs neutrophils and between MCV vs monocytes. Later observations suggested that higher MCV might be associated with reduced neutrophil and monocyte counts resulting in suppression of non-specific immunity against malaria.

Another important feature of this study was the detection of lymphopenia in 3 of 13 patients (23%). Similar finding has been reported earlier in vivax malaria[12]. Lymphopenia in patients might have suppressed adoptive immunity against *P. vivax*.

Earlier, *P. falciparum* was endemic in our population[1,2]. Recurrent infection by this parasite might have resulted in immunity against this parasite. However, many difficulties surround the attainment of sufficient level of immunity to eradicate malaria[13]. Subsequent introduction of a related species (*P. vivax*) in susceptible non-immune population might have resulted in evolution of intracellular hepatic forms of vivax malaria[14]. Further, *Plasmodium* parasite and its products may be engulfed by osteoclasts, eliciting a strong cytokine-mediated inflammatory response[15]. Persistent release of inflammatory cytokines may affect maturation of haemic cells in bone marrow.

CONCLUSION

Thrombocytopenia and anaemia were detected in most of the patients with vivax malaria. Few patients also developed lymphopenia, suggesting suppression of specific immunity. Absolute neutrophil count directly correlated with Hct and HCHC. Conversely, WBC and neutrophil counts inversely correlated with MCV.

Ethical consideration: Written consent was taken from all the individuals included in this study.

Financial or Other Competing Interests: None

REFERENCES

1. Nirmala, Kumar Archana, Kapoor AK, Lal B, Dutta GP, Swaroop A. *Plasmodium falciparum*: Drug-resistant malaria complicating leukemias and lymphomas in children. Experimental parasitology 1999; 93: 33-37.
2. Kapoor AK, Nirmala, Lal B, Dutta GP. Detection of raised serum ferritin levels in patients with Falciparum malaria. J Nepal Gunj Medical College 2001; 1: 21-23.
3. Muley Arti, Lakhani J, Bhirud S, Patel A. Thrombocytopenia in *Plasmodium vivax* malaria : How significant ? J Tropical Med 2014; 2014, 1-4.
4. Kochar DK, Tanwar GS, Khatri et al. Clinical features in children hospitalized with malaria : a study from Bikaner, Northwest India. Amer J Trop Medicine and Hygiene 2010; 83: 981-989.
5. Kochar DK, Das A, Kochar A, Middha S, Acharya J et al. Thrombocytopenia in *Plasmodium falciparum, Plasmodium vivax* and mixed infection malaria : a study from Bikaner (Northwestern India). Platelets 2010; 21: 623-627.
6. Coelha HCC, Lopes SCP, Pimentel JPD, Nogueira PA, Costa FTM, Siqueira AM, Melo GC, Monteiro WM, Malheiro A, Lacerda VG. Thrombocytopenia in *Plasmodium vivax* malaria is related to platelet phagocytosis. PLOS ONE 2013; 8(5): e 63410 doi:101371/Journal. Pone. 0063410.
7. Kishiyama JL, Tevrizian AT, Avila PC. Drug Allergy. Chapter 30, In Parslow TG, Stites DP, Terr AI, Imboden JB (editors). Medical Immunology 10th edition. New York : Lange medical books / McGraw-Hill publishing division : 2001, 394-400.
8. Bithel TC, Miscellaneous forms of thrombocytopenia, Chapter52. In Lee GR, Bithel TC, Foerster J, Athens JW, Lukens JN (editors), Wintrobe's clinical Hematology, Lea & Febiger, Philadelphia (publisher) : 1993, 1363-73.
9. Panichakul T, Payuhakrit W, Panburama P, Wongborisuth C, Hongeng S, Undoms-angpetch. Suppression of erythroid development *in vitro* by *Plasmodium vivax.* Malaria Journal 2012; 11:175, 3-22.
10. Miller LH, Good MF, Milon G. Malaria pathogenesis. Science1994;264:1878-1883
11. Castro-Gomes T, Mourao LC, Melo GC, Monteiro WM, Lacerda MVG, Braga FM. Potential immune mechanisms associated with anaemia in *Plasmodium vivax* malaria : a puzzling Question. Infection and immunity 2014; 82: 3990-4000.
12. Abro AH, Ustadi AM, Younis NJ, Abdou AS, Hamed DA, Saleh AA. Malaria and hematological changes Pakistan J of Medical Sciences 2008; 24, 287-291.
13. Anderson RM, May RM. Vaccination and herd immunity. Nature 1885;318:323-329.
14. Plorde JJ, White NJ. Malaria, Chapter 154, In Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fauci AS (editors). Harrison's principles of internal medicine 11th edition, New York : 1987, p 778-84.
15. Lee MSJ, Maruyama K, Fujita Y, Konishi A, Lelliott PM, Itaguki S, Horli T, Lin J, Khan SM, Kuroda E, Akira S, Ishii KJ, Coban C. *Plasmodium* products persist in the bone marrow and promote chronic bone loss. Science Immunology 2017, 2:eaam 8093, p1-11.