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

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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/mm³). Six patients had moderate thrombocytopenia (platelets 0.5 lac to <1 lac/mm³). Three other patients had mild thrombocytopenia (platelets 1 lac to <1.5 lac/mm³). 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 to 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 thrombocytopenia (platelets <1 lac/mm³) in 42 of 100 patients with P. vivax monoinfection. Another study
reported thrombocytopenia in 61.5% children and bleeding manifestations in 10.8% cases. In addition, severe thrombocytopenia (platelets <2 × 10^4/mm^3) may be a common manifestation of vivax mono-infection. Role of platelet phagocytosis has been suggested in vivax malaria. 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.

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 an EDTA vial and also separately for preparation of thin and thick smears. Smears were stained using autostainer (Aerospray Pro-slide staincytocentrifuge, pH 6.8). Stain was sprayed onto slides in a cytocentrifuge. Leishman-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. Thrombocytopenia 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/mm^3). 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.
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/mm³ | HCT % | MCV fl | MCH pg | MCHC gm/dl | RDW-CV % | Name                  |
|--------|-----------|----------|-----------------|-------|---------|---------|-------------|-----------|-----------------------|
| 11150  | 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    | 28.6    | 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/mm³ | NE# Cells/mm³ | LY# Cells/mm³ | MO# Cells/mm³ | EO# Cells/mm³ | PLT Cells/mm³ | Name       |
|--------|---------------|---------------|---------------|---------------|---------------|---------------|------------|
| 11150  | 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 (+) |
| 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, 445.15±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/mm³    | 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±2.72                | 32.2, 37.2                                                  | 32.4±5.06                | No difference |
| RDW %              | 14.1±1.79                | 12.7, 16.0                                                 | 14.72±3.70               | 1.12 NS |
| WBC ul             | 6000±22.41               | 6000, 14000                                                | 7457.42±3550             | 3.06*** |
| NE # cells/mm³     | 3876±18.60               | 2000, 8000                                                 | 4967.5±592.5             | 28.79*** |
| Lympho # cells/mm³ | 1500±9.30                | 1300, 1900                                                 | 1749.5±322.5             | 12.11*** |
| Mono # cells/mm³   | 446.15±355               | 100, 200                                                   | 546.5±735.0              | 0.85NS |
| Eo # cells/mm³     | 242±155.25               | 100, 100                                                   | 191.3±60.0               | 1.17NS |
| PLT cells/mm³      | 77769±40735.23           | 31000, 40000                                              | 175337.3±135230          | 6.89*** |

Abbreviations: NS not significant statistically (p>0.05), *** very highly significant (p<0.001)
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+ haematopoietic 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 \(\gamma\) and TNF \(\alpha\) 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.

| 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 gm/dl                                    | -0.390           | -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 * \(p < 0.01\) highly significant, ** \(p < 0.001\) very highly significant.

Akansha Singh et al JMSCR Volume 05 Issue 08 August 2017
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

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