Study on Plasmodium vivax density and haematological profile of Malaria patients from North India- A Hospital based prospective study.

Abstract:

Introduction: Malaria, the vector borne disease still remains one of the most deadly infections for many continents.

Aim: This hospital based prospective study was conducted to correlate the Plasmodium vivax parasitic load with the haematogical parameters of malaria patients.

Material and Methods: A total of 200 patients of Acute Undifferentiated fever (AUF)were enrolled and screened for Malaria by microscopy of Peripheral blood smear(PBS) and Rapid malarial antigen test (RMAT). The parasitic load of the Plasmodium vivax infection was classified into low, moderate and high parasitic count and was further correlated with the haematological parameters.

Results: A total of 150 cases were diagnosed as Malaria positive. Of these 139 (92.7%), 10 (6.7%) and 1 were classified as due to Plasmodium vivax, undifferentiated and Plasmodium falciparum infections respectively. The parasitic load of Plasmodium vivax was found to be low, moderate and high in 66 (47.5%), 67 (48.5%) and 6 (4%) cases respectively. It was observed that low and moderate parasitemia were associated with moderate anaemia and thrombocytopenia which was statistically significant ($p<0.05$)

Conclusion: The correlation between the haematological parameters with the parasitic load, in patients with P. vivax malaria, may aid the clinicians to determine the severity of the illness.

1. Introduction

Malaria is still the leading cause of death in tropical countries like Africa and India. In 2017, there was an estimated 219 million cases of Malaria in 87 countries, with a mortality rate of 4,35, 000 cases.[1]Plasmodium vivax and Plasmodium falciparum are the predominant species causing Malaria as reported from India. Plasmodium vivax alone accounts for 60-65% of the cases in India and more than 80% of the cases are in Delhi.[2]

Severe malaria is defined based on the clinical symptoms and signs, the infecting species of the malarial parasite, the haematological abnormalities, parasitic load and various end organ dysfunctions.

Most common complications observed in malaria is in the Haematological profile, as these changes involve the red blood cells, leukocytes and thrombocytes.[3] According to WHO, the severity of malaria is defined by the haematological parameters like haemoglobin concentration <5g/dl and <7 g/dl or hematocrit of 15% and 20% in children younger than 12 years of age and in adults respectively along with a parasite count of >10000/µl. Though the severity of anaemia, thrombocytopenia and leukocytosis or leukopenia in malaria has been well studied for Plasmodium falciparum, however the extent of these alterations in Plasmodium vivax malaria is less well known.

In this prospective hospital-based study, the correlation between the load of parasitemia by microscopy ie Peripheral blood smear (PBS) and the haematological profile of patients with malaria was done. In addition, age wise changes in the haematological values in relation to the parasitic load has also been analysed.

2. Materials and Methods

2.1 Study design

The present study was a hospital based prospective study, conducted at a 2700 bedded tertiary care hospital in New Delhi, India. This tertiary care hospital caters to patients from whole of the northern zone of India, with a daily OPD visit of 8000-10000 patients per day. This area experiences monsoon from July to September, which is the mosquito breeding season and so there is a spike in the mosquito borne illnesses like Malaria, Dengue and Chikungunya. Hence this study was conducted over a period of 4 months (from July 2017 to October 2017), when maximum number of malaria cases visit the hospital.

2.2 Patients

During the study period, any patient having Acute undifferentiated fever (AUF) of more than 5 days, visiting the medicine or paediatric OPD and/or admitted in the emergency ward were enrolled for the study. A predesigned standard proforma was used to record the socio-demographic details of the enrolled subjects. History of fever, jaundice, convulsions, nausea and vomiting along with the duration of illness was taken. The history of bleeding from any site as well as any drug intake, history of any similar complaints in the past and the family history were also taken.

2.3 Methodology

Laboratory investigation of Malaria:

Two -three ml of veni-puncture blood was collected in 2 EDTA vacutainer vials with aseptic precautions.

One EDTA vial was submitted to the Department of Microbiology of the Hospital.

It was tested for malarial parasite (MP) by microscopic examination of Peripheral blood smear (PBS) – a thin and thick smear after Giemsa staining and by Rapid Malarial Antigen Test (RMAT) (Medsource Ozone Biomedicals Pvt Ltd, India) which detects histidine rich protein 2(HRP-2) specific to P. falciparum and parasite specific Lactate DeHydrogenase (pLDH) specific to Plasmodium species in the human blood sample. Each microscopically positive peripheral smear was further evaluated for the Plasmodium species identification and the parasitic load by WBC tally method. They were further classified into low, moderate and severe parasitemia depending on whether the parasite load was below 100/µl, 101 to 10000/µl and more than 10001/µl respectively.[4]

For hematological investigation, a second EDTA vial was submitted to the Department of Laboratory Medicine. The following parameters were recorded namely Total RBC (x 10^6/µl), Total leukocyte Count (TLC; x 10^3/µl), Platelet count (PC; x 10^3/µl), Haemoglobin level (Hb; g/dl), Mean corpuscular volume (MCV; fL), Mean corpuscular Haemoglobin (MCH; pg/cell) and Mean corpuscular hemoglobin concentration (MCHC; g/dl). Anaemia, Thrombocytopenia, Leukocytosis and Leukopenia. These were defined according to the WHO criteria and were further classified.[5]

2.4 Ethics approval

Ethical clearance was taken from the Ethics Committee of the institute. A written informed consent was obtained from each adult study participant and a Legal Authorized Representative consent form was signed by the guardian of the minor.

2.5 Statistical Analysis

All the statistical analysis was done using a copy of SPSS version 16. For correlation of the values: mean, median and odds ratio were calculated. P value <0.05 was recorded as statistically significant.

3. Results

Of the 200 cases enrolled in the study with AUF, 150 were found to be positive for Malarial parasite by both RMAT and Peripheral blood smear examination(PBS). Samples which were positive on PBS were considered as true positives. The age of the participants ranged from 1 to 58 years with a median age of 17 ± 14 years. Majority of patients, 92 cases (92/150; 61%) belonged to 0-15years age group while 58 cases (58/150; 39%) were above 15 years of age. The Male: Female ratio in the study was 1.3:1.

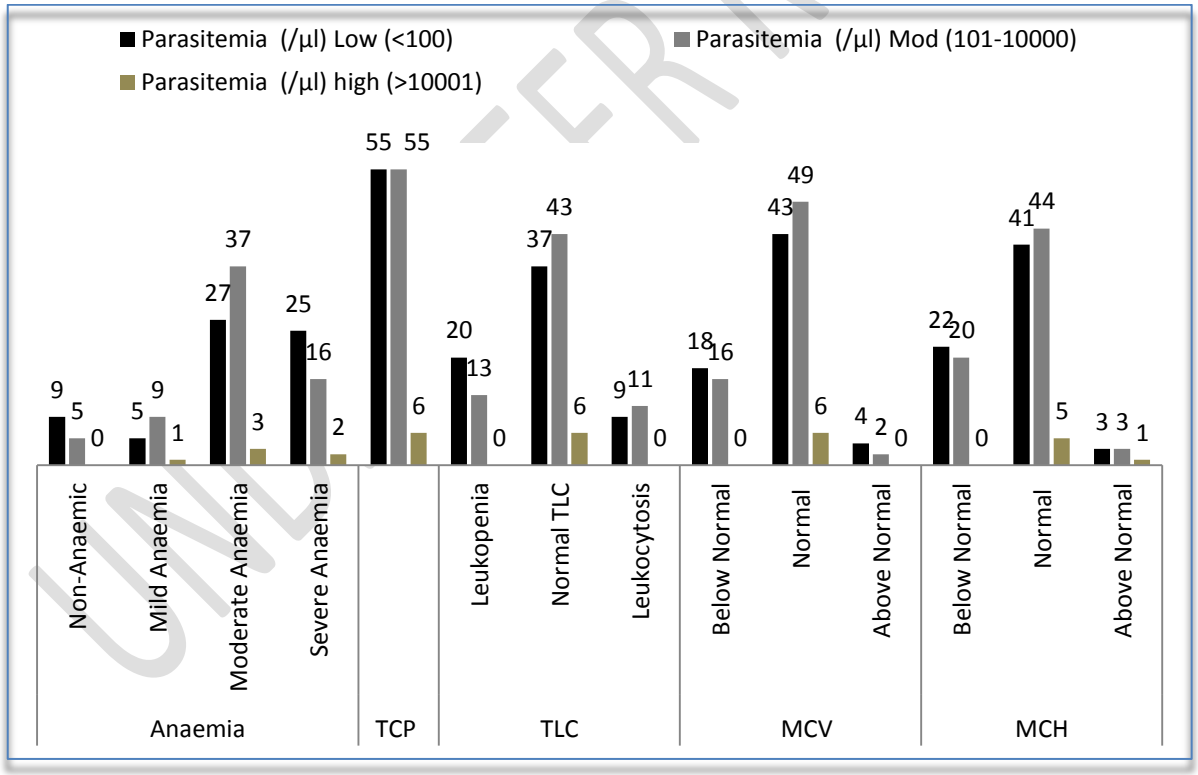
Of the 150 (n=150) patients with Malaria, 138 cases (138/150; 92%) were positive for Plasmodium vivax, 10 cases (10/150;7%) were positive for P. falciparum and 1 case (1/150;1%) was categorised as mixed Plasmodium species infections. As only ten (10) cases of Plasmodium falciparum and one (01) cases of mixed infection were diagnosed, it was difficult to draw any statistically significant value for these cases. So only the Plasmodium vivax cases (n=139) were further studied in detail. Of the

Plasmodium vivax positive cases (n=139), low, moderate and high parasite load was found in 66 (47.5%), 67 (48.5%) and 6 (4%) respectively as shown in Fig1.

Each parasitic load sub group was further analysed based on the age of the patient and divided into 2 groups- children (0-15 yrs) and adults (>15 yrs). In children, it was found that P. vivax infections caused low and moderate parasitemia in 37 (37/73;50.6%) and 36 (36/73; 49.4%) cases respectively. There were no cases of high load parasitemia. Whereas in adults- low, moderate and high load parasitemia was found in 29 (29/66;43.9%), 30 (30/66;45.4%) and 7 (7/66; 10.6%) respectively.

Out of the 139 P.vivax cases, anaemia, thrombocytopenia, leukopenia, leucocytosis, varied MCV and MCH were observed in 125 (125/139;89.9%),116 (116/139;83.4%), 33 (33/139; 23.7%), 20 (20/139;14.4%), 34 (34/139; 24.5%), 6 (6/139; 4.3%) 42 (42/139; 30.2%) and 7 (7/139; 5%) cases respectively as shown in Figure 2.

Figure 2. No of cases (n=139) of Plasmodium vivax in each parasitic load sub group and haematological changes



Note: All the classification as per WHO guidelines. [5] TCP –thrombocytopenia

The haematological profile of all 139 P.vivax cases were further analysed and correlated with the parasite load. It was observed that low and moderate parasitemia were observed with moderate anaemia and thrombocytopenia, the association was found to be statistically significant($p<0.05$).

Detailed analysis of Plasmodium vivax cases with haematological parameters in both the children and adults showed the following association (Table 1). It was found that in children with both low and moderate parasitemia, the mean value of TRBC was 3.2±0.7 $x10^6$/µl, which is below the normal value of 4.5$x10^6$/µl as per national guidelines.[6]

Table 1: Haematological profile of children and adults cases of P.vivax at low, moderate, and high parasitic load

Lab Parameters	Children (n=73)			Adults (n=66)		
	Low PL (37)	Mod PL (36)	High PL	Low PL (29)	Mod PL (30)	High PL (7)
Total RBC ($x10^6$/µl)	3.2±0.7	3.2±.7	-	3.1±0.8	3.5±.7	2.8±.9
Total LC ($x10^3$/µl)	8.1±6.9	7.5±6.9	-	5.8±3.3	6.9±7	6.3±3.4
Platelet count ($x10^6$/µl)	111±99	91.8±99	-	67.2±57.1	81.7±99	69±98.9
Hb(g/dl)	8.3±2.4	8.4±2.4	-	9.4±2.6	9.8±2.4	9.0±2.4
MCV (fl)	81±9.6	82.1±9.7	-	86.8±8.6	88.4±9.7	90.8±9.7
MCH (pg/cell)	27.1±5.1	25.7±5	-	27.7±4.4	29.5±5	29.5±5.0
MCHC (g/dl)	34.1±3.6	31.2±3.2	-	31.2±3.8	33.3±3.2	32.4±3.2

Note: In children none of the malaria case diagnosed with high parasitaemia. The no inside the bracket represent no of cases in each parasitic count range. Low PL- low parasitic load (<100/µl);Mod PL- moderate parasitic load (100-10000/µl); High PL- high parasitic load (>10001/µl)

As shown in Table 1, the mean haemoglobin in cases with low and moderate parasitic count was calculated and found to be 8.3±2.4 and 8.4±2.4 g/dl. This is also very low as compared to the reference standard (which is 14mg/dl in children).[6] The mean value of TRBC and Hb indicates that moderate anaemia was associated significantly with low and moderate parasitemia in children. The mean values of TLC i.e 8.1±6.9 $x10^3$/µl with low parasitic counts were much nearer to the normal values than with the moderate parasitic counts, where mean TLC was 7.5±6.9 $x10^3$/µl. However this correlation was not found to be statistically significant. The mean value of total platelet count in cases having both low and moderate parasitic count i.e 111±99 $x10^6$/µl and 91.8±99$x10^6$/µl respectively, was much lower than the normal count of 150 $x10^6$/µl found in the healthy children. This clearly indicates that with increase in parasitic count, the platelet count decreases($p<0.0001$). The mean MCV, MCH and MCHC were found to be lower than the standard cut off. Statistically significant lowering of these parameters was observed with increase in parasitic count.

When haematological parameters of adults were compared with the different parasitic load, the following findings were noted (as shown in Table1). The mean of total RBC, Haemoglobin level and

platelet count decreased with increase in the parasitic count and this association was found to be statistically significant. The pattern of TLC and MCV did not correlate well with the parasitic count.

4. Discussion

Malaria is a major cause of morbidity and mortality in developing countries like India. Delhi is an urban area and it has been reported by the Indian Council of Medical Research (ICMR) that P.vivax is on the rise in urban India.[9] In this study the incidence of Plasmodium vivax was found to be 92% . Similar findings have been observed in other studies from North India with incidence rates of 85% and 63% respectively.[7,8] We found that Plasmodium vivax infections caused more number of cases of low and moderate parasitemia in both children and adults. Various other studies have also reported P.vivax to cause malaria with low parasite counts.[3,10] On the contrary Muluken Birhanu et al have found P.vivax causing severe malaria with a high parasitic count.[11] Although haematological abnormalities are considered to be a hallmark of malaria especially in Plasmodium falciparum infections, but these abnormalities were also seen in P. vivax infections. In our study we observed that anaemia, thrombocytopenia, leukopenia, leukocytosis, decreased and increased MCV and MCH were found in 89.9%, 83.4%,23.7%, 14.4%, 24.5%,4.3%, 30.2% and 5% cases respectively. We also found a statistically significant association of thrombocytopenia and anaemia with moderate and high load of parasite count in 31% and 83.3% cases respectively. These findings are in accordance with other studies done across the world, which have also shown a strong association between P. vivax malaria with anaemia and thrombocytopenia.[12, 13] The presence of thrombocytopenia can be explained by the immune-mediated platelet destruction, adherence of platelets to parasitised RBC and oxidative stress to membrane components which occurs in malaria, whereas sequestration of blood cells in the spleen may contribute to both anaemia and thrombocytopenia.[3]

In the present study, it was found that in both children and adults, the total RBC, Haemoglobin, Platelet count, MCH and MCHC significantly decrease with increase in the parasitic load, thereby increasing the severity of malaria. These findings are in concordance with the findings of Manas Kotepui et al and Maina R et al., which showed significant correlation of these haematological parameters in the malaria patients. [12,14] On the other hand there was no significant change in the TLC (Total leucocyte count) with increase in the parasitic load. This is contrary to the findings of Manas Kotepui et al and Inam Ullah et al .[12,13] On comparison of the parameters, it was also noted that even though TLC was comparable to the parasitic load, but the DLC (differential white blood cell count) was not comparable with the parasitic load. Another limitation of this study was the relatively small number of patients in this group. As malaria spikes are seen only once in a year, during the monsoon season, hence collection of more number of samples during this period would have greatly increased the validity of the study.

The present study has laid emphasis on the severity of malaria in patients with Plasmodium vivax infection. But study of the changes in the haematological parameters also gives an insight into the prediction of the parasitic load, the severity of malaria as well as the prognosis of the patients in the remote areas in those areas where there may not always be facility and expertise to report on the parasite load except that of conducting a complete blood count (CBC). Many more studies are needed in the near future to validate the findings of RMAT and PBS for MP along with a strong molecular quantification of the malaria parasites.

Conclusion:

The patients infected with Plasmodium vivax exhibit important changes in many haematological parameters especially total red blood cell count, haemoglobin level and platelet count, which determine the severity of the infections. The correlation between the haematological parameters with the parasitic load, which are both diagnostic as well as prognostic markers in patients with P. vivax malaria, often helps the clinicians to determine the severity of the illness.

Conflict of interest statement

We declare that we have no conflict of interest.

References:

1. World Health Organization. Fact sheet: World Malaria report 2017. Available at https://www.who.int/malaria/publications/world-malaria-report-2017/en/.

2. Kaul A, Bali P, Anwar S, Sharma AK, Gupta BK, Singh OP, Adak T, Sohail M. Genetic diversity and allelic variation in MSP3α gene of paired clinical Plasmodium vivax isolates from Delhi, India. Journal of Infection and Public health. 2019 Jul 1;12(4):576-84.

3. Wickramasinghe SN and Abdalla SH. BaillieÁ re's Clinical Haematology;Harcourt Publishers Ltd;2000.

4. World Health Organization. Malaria Parasite Counting. Malaria microscopy standard operating procedure – mm-sop-09.

5. World Health Organization. Vitamin and Mineral Nutrition Information System. Geneva, 2011. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Available at http://www.who.int/vmnis/indicators/haemoglobin.

6. Nathan, David G, Oski, Frank A. Hematology of Infancy and Childhood; 3rd addition, 1987.

7. Sharma R, Gohain S, Chandra J, Kumar V, Chopra A, Chatterjee S et al. Plasmodium vivax malaria admissions and risk of mortality in a tertiary-care children's hospital in North India, Paediatr Int Child Health 2012;32:3:152-7.

8. Kochar DK, Tanwar GS, Khatri PC and Kochar SK. Clinical Features of Children Hospitalized with Malaria - A Study from Bikaner, Northwest India 2010. Am. J. Trop. Med. Hyg;83:5: 981-9.

9. Indian Council of Medical Research. Annual Report 2017-18 by Dr. Chanchal Goyal.

10. World Health Organization. 2014; Tropical Medicine and International Health is published by John Wiley & Sons.

11. Birhanu M, Asres Y, Adissu W, Yemane T, Zemene E and Lealem Gedefaw. Hematological Parameters and Hemozoin-Containing Leukocytes and Their Association with Disease Severity among Malaria Infected Children: A Cross-Sectional Study at Pawe General Hospital, Northwest Ethiopia. Interdisciplinary Perspectives on Infectious Diseases 2017.

12. Kotepui M, Piwkham D, Phuech BP, Phiwklam N, Chupeerach C, Duangmano S. PLOS ONE 2015; 45-56.

13. Ullah I, Ali MU, Saeed A, Rafiq A, Sattar Z, Hussain S. Hematological Profile of Patients Having Malaria-positive Peripheral Blood Smears: A Cross-sectional Study at a Diagnostic Research Center in Khyber Pakhtunkhwa, Pakistan. Cureus 10:9.

14. Maina R, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L et al. Impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya. Malaria J 2010; 9: 03–04.