

ORIGINAL RESEARCH

A Study on Observation of Platelet Status in Malaria

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ABSTRACT

The present study was conducted in the Department of medicine, Gandhi Hospital, Secunderabad. 100 malarial fever cases were taken up for the study, which consisted of 79(79%) males and 21% females. Among this, 55% cases were of *p.vivax* and 45% *p.falciparum*. The maximum number of cases fell in the age group of 15-35 years (62%). 100% cases presented with fever, chills and rigors. Other presenting symptoms were headache (25%) and vomiting (33%). Splenomegaly was seen in 32% cases, while hepatomegaly in 22%, 15% showed pallor and 8% cases had icterus. Complete hematological tests were done on cases taken for study and analyzed. Anemia ranged from 6.0 to 15.6gm/dl, leukocyte count varied from 1.8 to 17.7cu/mm. Platelet count was done on every patient on day 1, 2, and 3. Thrombocytopenia was seen in 31% (31) cases of *p.vivax*, 24% of *p.falciparum* patients. Association of thrombocytopenia and effect of treatment was completely analyzed in both *p.falciparum* and *p.vivax* the effect of antimalarial drugs on platelet count was observed. On Day 1 platelet count varied from 15,000 to 1,94,000. On Day 2 platelet count varied from 14,000 to 1,84,000. On Day 3 platelet count varied from 26,000 to 1,98,000.

Keywords: *P.falciparum*, Splenomegaly, Thrombocytopenia, hepatomegaly, Antimalarial drugs.

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INTRODUCTION

Humanity has three great enemies Fever, famine and war; of these by far the most terrible is fever. The most common cause of fever is malaria. Malaria is the most prevalent parasitic infection in the world. It continues to pose a challenge in view of its resurgence in the recent years. Malaria is an endemic in tropics,^[1] and has posed major health problem.

It was observed in nineties that malarial fever had its course with diminished platelet counts. Thrombocytopenia was mostly seen in plasmodium falciparum infections but lately it is also observed in plasmodium vivax. Therefore, this work has been taken up to thoroughly investigate different types of Malaria and hematological changes occurring in them especially the changes in thrombocytic status. The results have been fully analyzed and changes occurring in different malaria have been analyzed. Thrombocytopenia has been observed in different clinical settings in severe viral infections like dengue, bone-marrow depression, such cases will be excluded from the present study. Correlation of thrombocytopenia with parasitic index will have been analyzed.

The clinical presentation of both the malaria species presented with fever with chills rigors, headache and vomiting, however some of the patients had unusual complaints like diarrhea, hypoglycemia.

The treatment with Anti-malarial drugs made a significant change in the thrombocyte count. Initially in some cases, thrombocytopenia also continued on second day also, but on third day, Anti-malarial treatment produced beneficial effects and platelet counts increased in both the groups.

Aim & Objectives:

1. To study the clinical profile of malaria.
2. To study the incidence of thrombocytopenia in different types of malaria.
3. Association of plasmodium vivax with thrombocytopenia.
4. To compare the effects of treatment in different types of malaria in relation to thrombocytopenia.

MATERIALS & METHODS**Setting:**

This study was carried out on patients admitted in Gandhi Hospital, Secunderabad.

Study Duration:

1½ years (October 2020 to January 2022).

Number of cases: 100

Inclusion Criteria of Cases:

- 1) Age > 15 years
- 2) Clinical presentation of malaria
- 3) Positive smear slide (vivax or falciparum).

Exclusion Criteria: Patients with following conditions were excluded.

- 1) Dengue
- 2) Leptospirosis
- 3) Other causes of thrombocytopenia like
 - (a) Leukemia's, aplastic anemia
 - (b) Drugs like chloramphenicol and frusemide.
- 4) Patients who have been transfused with platelets (in smear positive).

Examination of Patients on Arrival to Hospital:

- 1) Chief complaints
- 2) Personal history
- 3) On General Examination: Pulse

Blood pressure:

Pallor, icterus, clubbing, cyanosis; Present or not

4) Systemic Examination:

A). Per Abdomen:

- a. Inspection
- b. Palpation; Checked for hepatosplenomegaly.
- c. Percussion
- d. Auscultation.

B) Respiratory System:

- 1) Inspection
- 2) Palpation

- 3) Percussion
- 4) Auscultation: Air entry equal on the both the sides or not. Any added sounds

C) Cardiovascular System:

- 1) Inspection
- 2) Palpation
- 3) Percussion
- 4) Auscultation.

Central Nervous System

- Level of consciousness
- Cranial nerves
- Motor system
- Sensory system
- Reflexes

Investigation's done during the Course of Hospitalisation

a) Complete blood picture :(CBC)

- 1) Hemoglobin
- 2) Total Leukocyte Count
- 3) Total Erythrocyte Count.
- 4) Neutrophils, Eosinophils, Monocytes, Basophils.

b) Malarial Parasite with thin and thick film.

c) Parasitic Index:

d) Platelet count on

Day 1

Day 2

Day 3

* 2 cc of Blood taken in an EDTA bulb for examination.

e) S.Creatinine, Blood urea nitrogen

- f) Liver function Tests: 1) Total Bilrubin: 
- 2) SGOT
 - 3) SGPT

*3cc of blood sent in plain bulb

g) X-RAY Chest PA view

Treatment Given To the Patients in Hospital Stay:

- 1) Strict Temperature and Blood pressure monitoring.
- 2) Injection Artesunate 120 mg intravenously stat followed by 60 mg after Hours Followed by 60 mg for four days as Antimalarial
- 3) Injection Ceftriaxone 1 gm intravenously twice daily to avoid complications
- 4) Cap.Doxy 100 mg twice daily for 5 days as Antimalarial
- 5) Anti-Emetics, Anti Histaminic to prevent nausea, vomiting.
- 6) I.V fluids to avoid Dehydration.
- 7) Tab. Crocin if fever had come.

RESULTS

Table 1: Genderwise Distribution of Malaria Cases in Present Study

Gender	No. of Cases	Percentage
Male	79	79 %
Female	21	21 %

This table shows gender wise distribution of the 100 cases of malarial fever in the current study. They compromised of 79 (79%) males and 21(21%) females.

Table 2: Incidences of Malaria Cases in Present Study

Gender	No. of Cases	Percentage
P- Vivax	55	55%
P- Falciparum	45	45%

The above table depicts the number of cases of plasmodium vivax and plasmodium falciparum. In the study 55 cases (55%) plasmodium vivax and 45 cases (45%) of plasmodium falciparum. This correlates statistically with the increasing trend of plasmodium vivax malaria.

Table 3: Age Group Wise Distributions of Malaria Cases in Present Study

Age Group (Yrs.)	No. of Cases	
	Male	Female
15 - 25	32	8
26 - 35	30	7
36 - 45	4	3
> 46	13	3

This is the age group wise distribution of patients in present study, it shows that the maximum number of cases were in the age group of 15-25 years while the minimum were in the age group of 36-45 years. Out of the 40 cases in the age group of 15-25, there were 32 males and 8 females. On the other hand males were 4 and females were three in the age group of 36-45.

Table 4: Frequencies of Symptoms in Malaria Cases in Present Study

Symptoms	Frequency	Percentage
Fever	100	100 %
Chills & Rigors	100	100 %
Headache	25	25 %
Vomiting	33	33 %
Others	5	2%

In the current study the most common symptom's with which patient presented Were Fever (100%), chills and rigors (100%). Twenty five Percent (25%) of the patients had come with complaints of headache along with fever; thirty-three (33%) people had vomiting along with fever. Five percent (5%) of patients presented with unusual complaints like loose motions, generalized weakness, high colored urine.

Table 5: Frequency of Signs in Malaria Cases in Present Study

Signs	Frequency	Percentage
Pallor	15	15 %
Icterus	12	12 %
Splenomegaly	32	32 %
Hepatomegaly	22	22 %
Others	19	19 %

The most common signs present on examination were splenomegaly (32%) followed by hepatomegaly (22%), pallor in 15(15%), icterus (12%) and other findings like high colored urine in 19 %.

Table 6: Parasitic Index Wise Distribution of Cases

Parasitic Index	P-Vivax	P-Falciparum
0 - 1.0	46	37
1.1 - 2.0	6	4
2.1 - 3.0	2	2
3.1 - 4.0	1	2

The maximum number of patients according to parasitic index were in the range of 0 to 1.0 per 1000 red blood cells, 46 cases in p.vivax and 37 in p.falciparum were seen. Minimum number of cases were in the group of 3.1-4.

Table 7: Observation of Plateletcount in P-Falciparum and P.Vivax (DAY 1)

Platelet Count/Cmm	P-Falciparum	P-Vivax
< 50000	14	16
50000 - 150000	10	15
> 150000	21	24

14 cases of p.falciparum and 16 cases of p.vivax showed a fall in platelet count less than 50,000 on day 1, 10 cases of p.falciparum and 15 cases of p.vivax showed a fall in platelet count between 50,000 to 1.5 lakh, whereas 21 cases of p.falciparum and 24 cases of p.vivax did not have thrombocytopenia on day 1.

Table 8: Observation of Platelet Counts In P-Falciparum and P-Vivax (Day- 2)

Platelet Count/Cmm	P-Falciparum	P-Vivax
< 50000	16	19
50000 - 150000	8	12
> 150000	21	24

The table shows changes in the platelet count on day 2. There was further reduction in platelet count below 50,000 in 16 cases of p.falciparum and 19 cases of p.vivax.

In the range of 50,000 to 1.5 lakh, they were 8 cases of p.falciparum and 12 cases of vivax, which showed further reduction in platelet count. The 21 cases of p.falciparum and 24 of vivax malaria did not have any change in their count on the 2-nd day. They remained in the category of normal level.

Table 9: Observation of Platelet Counts In P-Falciparum and P.Vivax (Day- 3)

Platelet Count/Cmm	P-Falciparum	P-Vivax
< 50000	10	12
50000 - 150000	14	19
> 150000	21	24

On the day 3, the platelet count improved with treatment, 12 cases of p.vivax and 10 cases of falciparum, platelet count remained below 50,000. In comparison to day 2, the figures showed improvement in the platelet count. 19 cases of p.vivax and 14 cases of p.falciparum were in the group of 50,000 to 1.5 lakh. The remaining cases had no change in platelet count

Table 10: Effect of Antimalarial Treatment on Platelet Counts in both P.Falciparum and Vivax Malaria.

Platelet Count/Cmm	Day 1	Day 2	Day 3
< 50000	30	35	22
50000 – 150000	25	20	33
> 150000	45	45	45

The table shows the effect of antimalarial treatment on the platelets. Majority showed a decline in platelet count on day 2 in both the groups, but on day 3 there was an appreciable increase in platelet count.

The cases with no thrombocytopenia remained same on 3rd day also.

Table 11: Comparing Thrombocytopenia and Effect of Treatment in P.Falciparum And P.Vivax

Effect	No. of Cases		TStatistic	P-Value < 0.05	Remarks
	P.Falciparum	P.Vivax			
Thrombocytopenia	24	31	2.03	0.021	Significant
Treatment	24	31	2.14	0.001	Significant

The incidence of thrombocytopenia in p.falciparum and p.vivax malaria when compared stastically had a significant value. (0.021) On statistical analysis, the effect of antimalarial treatment, on thrombocytopenia revealed significant values. (0.001)

Table 12: Statistical Analysis of Hematological Variables in Malaria

Sr. No.	Variable	Range	Mean	± Sd	Se
1	Parasitic Index	0.2 – 4.0	0.89	0.728	0.073
2	Platelets: Day 1				
A	P.Falciparum	13000 – 180000	101577.8	62363.5	6236.3
B	P.Vivax	20000 – 194000	98600.0	58340.8	5834.1
	Day 2				
A	P.Falciparum	12000 – 184000	98600.0	58340.8	5834.1
B	P.Vivax	18000 – 188000	986000	58340.8	5834.2
	Day 3				
A	P.Falciparum	26000 – 196000	115444.4	58648.6	5864.9
B	P.Vivax	27000 – 198000	127509.1	57444.0	5744.4
Sr. No.	Variable	Range	Mean	± Sd	SE
3	Complete Blood Count				
3.1	HB	6.0 – 15.6	11.21	2.057	0.206
3.2	TEC	1.3 – 7.9	4.08	1.043	0.104
3.3	TLC	1.8 – 17.7	5.19	2.087	0.209
3.4	L	31.0 – 90.0	64.71	13.611	1.361
3.5	E	0.0 – 13.0	1.56	2,204	0.202
3.6	M	0.0 – 79.0	30.98	16.051	1.605
3.7	B	0.0. – 5.0	0.90	1.395	0.140
4	Liver Function Test				
4.1	T.Bilirubin	0.1 – 17.3	2.10	2.691	0.269
4.2	Sgot	19.0 – 398.0	59.63	59.662	5.966
4.3	Sgpt	23.0 – 273.0	56.86	38.457	3.846
5	Serum Creatinine	0.4 – 2.1	0.73	0.281	0.028

DISCUSSION

Malaria is the most common fever prevalent these days in navi mumbai. Nerul is an endemic area for malaria. Of late the most common complication of malaria is thrombocytopenia, Incidence of thrombocytopenia has been observed mainly in p.falciparum malaria in the past. The occurrence of thrombocytopenia in p.vivax was noticed by martelo et alin 1969 and lately many other workers found thrombocytopenia in p.vivax also. Therefore this study was

taken up to find the platelet status in malarial fever and to find out the incidence of thrombocytopenia in *P. vivax* and *P. falciparum*. 100 cases of malarial fever admitted in Gandhi Medical College/General Hospital, were taken for the study. Cases with dengue and leptospirosis were excluded from the present study. Out of the 100 cases taken for the study, 79(79%) cases were male patients and 21(21%) female patients. The preponderance of male in the present study may be because of higher number of migrant males coming to Mumbai than females. The laborers live in unhygienic conditions at construction sites, where mosquitoes breed more and laborers are thus prone to mosquito bites. In the present study, 55 cases were of *P. vivax* and 45 cases were *P. falciparum*. The comparison of incidence of *P. falciparum* and *P. vivax* were statistically significant. *[Table 2]. The result was in accordance with the results published earlier. Priscilla Robinson et al (2001),^[1] in their retrospective case study of 246 patients had seen that there were 182 (68.9%) of *P. vivax* and 71(26.9) cases of *Plasmodium falciparum*. This study also supports the increase in incidence of *P. vivax*. Beg MA et al. (2007),^[2] studied comparative features and outcomes of malaria at a tertiary care hospital in Karachi in which records of 521 hospitalized patients during four and a half year period were analyzed. They found that infections were caused by *P. vivax* (51.8%), *P. falciparum* (46.5%).^[3] The increase in *P. vivax* malaria cases could be because of ineffective treatment of hepatic reservoir in cases of *P. vivax*. Many a time a presumptive diagnosis of malarial fever is done; such cases should be treated as *P. vivax* cases. They should be given primaquine; if not given may recur. The present study consisted of patients belonging to the age group from 15 to 85 years of age. There was more number of patients in age group of 15. Malaria is the most common fever prevalent these days in Navi Mumbai. Nerul is an endemic area for malaria.^[4] Of late the most common complication of malaria is thrombocytopenia, Incidence of thrombocytopenia has been observed mainly in *P. falciparum* malaria in the past.

The occurrence of thrombocytopenia in *P. vivax* was noticed by Martelo et al.^[5] in 1969 and lately many other workers found thrombocytopenia in *P. vivax* also. Therefore this study was taken up to find the platelet status in malarial fever and to find out the incidence of thrombocytopenia in *P. vivax* and *P. falciparum*. 100 cases of malarial fever admitted in Dr.D.Y.Patil medical college, Nerul were taken for the study. Cases with dengue and leptospirosis were excluded from the present study. Out of the 100 cases taken for the study, 79(79%) cases were male patients and 21(21%) female patients.^[6] The preponderance of male in the present study may be because of higher number of migrant males coming to Mumbai than females. The laborers live in unhygienic conditions at construction sites, where mosquitoes breed more and laborers are thus prone to mosquito bites. To 45 years, out of which males were 64% and females 18%, whereas in the age group of more than 45, they were 26% males and 9% females. The reason could be male preponderance in the migrant labor. Preponderance of males has also been found in previous studies done by Layla A.M et al. (2002),^[3] in his study of 727 patients the age group had a wide range (from 2 months to 74 years), mean age was 25.43+14.43 years, there was a male predominance with a male to female ratio of 15:1. The results of the present study are again in agreement with Khaled et al 2007.^[7] Who in their study on 103 patients had 77 male (74.8%) and 26 (25.2%) females; their ages ranged from 18 to 45 years. The usual clinical features of malaria include fever with chills and rigors. This is also associated with hot, cold and sweating phase. Headache and vomiting is also associated with malaria. Some of the cases may also present as algid malaria, black water fever and with other complications like cerebral malaria, ARDS. In the present study, 100 % patients presented with fever, chills and rigors, headache was seen in 25%, vomiting was seen in 33% cases.^[8] Some of the patients came with unusual symptoms like loose motions, generalized weakness, and abdominal pain in around 5(2%). *[Table 4] the similar clinical pictures were also seen in previous work done by Marcela Echeverri et al.

(2003).^[9] in his study had fever (99%) chills (91%), headache (99%), This was positive in 91% of patients out of 103. this study supports the current study. Malarial fever usually present with fever (intermittent), anemia and hepato-splenomegaly. Of late malarial hepatitis is recognized quite often. In the present study, intermittent fever was seen in 100% of the patients, pallor seen in 15 cases (15%), 32 patients had splenomegaly (32%), and hepatomegaly was seen in 22 patients (22%) and icterus in 12 patients (12%). *[Table 5] Similar findings were observed by Marcela echeverri et al. (2003),^[4] in his study on 104 patients had splenomegaly in 10 patients (10%) and hepatomegaly in 18 patients (18%), pallor was seen in 48 % of cases while icterus was seen in 15% of patients. Parasitic index indicates the average degree of parasitemia. In the current study parasitic index was done to see the number of parasites present per 1000 red blood cells. In the current study significant statistical association between parasitic index and thrombocytopenia was not observed However, Layla A.M et al. 2002,^[41] in their study proved higher levels of parasitemia in plasmodium falciparum malaria.

The present study could not get similar findings.^[10]

Malarial fever usually have anemia, which may be due to haemolysis of R.B.C s or bone marrow suppression. The patients of parasitic infection show leucopenia. They also have associated infections; there may be leucocytosis. Eosinophilia may also be observed. In the present study hematological changes in malaria were analyzed, Hemoglobin values varied in the range was 6.2-15.6 gm/dl. There were 15 patients who had anemia (15%). Marcela Echeverri et al. Data analyzed on 69 malarial patients to assess hematological manifestations. Anemia was significant finding ($p=0.0060$), approximately half of the patients had $Hb \leq 12$ g/dl and had 15 cases (22%) $Hb < 9$ gm/dl. In the present study patients had leucopenia (6%) and leucocytosis (4%). Parasitic infections are known to cause leucopenia Reduction in platelet count has been known to occur in plasmodium falciparum malaria in the past. But of late, the vivax malaria cases also have been associated with marked thrombocytopenia. Hence the present study has been taken up to study the incidence of thrombocytopenia in both falciparum and vivax malaria. The results were documented on day 1, 2, 3 and were analyzed *[Table 7-9].

In the present study, platelet count was categorized into three groups.

- 1) Less than 50,000 k/ul
- 2) 50,000 to 1,50,000k/ul
- 3) $> 1, 50,000$ (normal count).

On day1, 16 cases of p.vivax and 14 cases of p.falciparum, showed a fall in platelet count less than 50,000. 15 cases of vivax and 10 cases of falciparum showed fall in platelet count between 50,000 to 1.5 lakh whereas 24 cases of vivax and 21 cases of falciparum did not have thrombocytopenia.

On day 2, 19 cases of p.vivax and 16 cases of p.falciparum had further reduction in platelet count below 50,000.12 cases of p.vivax and 8 cases of p.falciparum showed further reduction in platelet count less than 1.5 lakh on day 2.

24 cases of p.vivax and 21 cases of p.falciparum did not have any change in their platelet count; they remained in category of normal level.

On day 3, 12 cases of p.vivax and 10 cases of p. falciparum had platelet count less than 50,000. In comparison to day 2, the figures showed improvement in platelet count.19 cases of p.vivax and 14 cases of p. falciparum had platelet count in range of 50,000 to 1.5 lakh.

The group with normal range of platelets had no change on day 3.

The incidence of p.vivax malaria was observed in 1969 by MARTELO et al 1969,^[5] in their study of 173 cases of malaria, 93% were p.vivax, but only 15% had thrombocytopenia. So initially thrombocytopenia in vivax malaria was less observed.

The incidence of thrombocytopenia in *p.vivax* cases was also observed by the work done by Khaled taha et al (2007)^[6] in their study on hematological changes in malaria in relation to plasmodium species has showed that the mean platelet count with falciparum malaria was normal (168.8) while the mean count in patients with vivax showed mild thrombocytopenia (107.06). Analysis demonstrated a significant low platelet count in cases with vivax malaria ($p=0.001$).

Platelet counts were investigated in 26 patients of *p.falciparum* and 39 patients of *p.vivax* before after treatment. Before treatment 22 of the 26 patients of falciparum and 30 of the 39 patients of *p.vivax* had decreased platelet counts below 1, 50,000. Platelet count improved after 3 days of treatment.^[6]

The results of the present work revealed thrombocytopenia in both *p.vivax* and *p.falciparum* malaria. The thrombocytopenia was observed more in *p.vivax* malaria than in *p.falciparum*. Similar results were found by

Layla A.M et al (2002)^[3] in their study on hematological values found that platelet counts were lower most in *p.vivax* cases (74.7%) compared to *p.falciparum* (59.9%) in their case study of 727 patients. Showing a p value =0.0018 which is a significant value. In their study it was observed that thrombocytopenia was more common in *p.vivax* than in *p.falciparum*.

The recent observation of thrombocytopenia in *p.vivax* malaria has been analyzed by previous workers who postulated some of the following mechanisms:

1) Increase level of cytokines.^[7]

2) Immunological destruction due to Anti platelet IgG.^[8]

Oxidative stress^[8]

3) Shortened life span in peripheral blood and sequestration in non-splenic areas.^[10]

4) Genetic changes might be occurring in parasites while going

The observation of thrombocytopenia in *p.vivax* malaria in the present study is statistically significant*[Table 11]

CONCLUSION

- Plasmodium vivax infections in the community have a rising trend.
- Thrombocytopenia is seen in both *p.falciparum* and *p.vivax* malaria.
- The of late observation, that *p.vivax* malaria is associated with increased incidence of thrombocytopenia, is confirmed in this study.
- Parasitic index did not have any bearing on thrombocytopenia.
- The thrombocytopenia in both *p.vivax* and *p.falciparum* improved after the anti-malarial treatment.

REFERENCES

1. Priscilla Robinson, Adam W et al, Imported malaria treated in Melbourne, Australia, J Travel med 2001; 8:76-81.
2. Beg MA sani, N, Mehraj.V et al.2007 Comparative features and outcomes of malaria at a tertiary care hospital in Karachi, Pakistan. J infects Dis 2007. 3 (110:24-30.
3. Layla A.M, Bashawri et al, Malaria: hematological aspects, Annals of Saudi medicine, 22, (5-6); 2002. 33-45
4. Marcela Echeverri; Alberto Tobon; Gonzalo Alvarez et al 2003 Rev.Inst med trop. S. Paulo Jan./feb.2003
5. Martello O.J, Smoller M, Saladin. Malaria in American soldiers, Arch int. Med 1969:383-7
6. Khaled Taha et al; Hematological changes in malaria; relation to plasmodium species, Kuwait medical journal 2007, 39(3):262-267.

7. Park JW, Park SH, YeomJS, and serum cytokine profiles in patients with p.vivax malaria: a comparison between those who presented with and without thrombocytopenia; *Ann trop med parasitol* 2003; 97:339-344.
8. Ohtaka M, Ohyashiki K, Iwabuchi H, Lin K.Y, case of vivax malaria with thrombocytopenia suggesting immunological mechanism. *Rinsho ketsueki* 1993; 34; 410-412.
9. Erel o, vural H, aksoy N, Aslan g, oxidative stress of platelets and thrombocytopenia in patients of vivax malaria.
10. Karanikas G, Zedwitz-Liebenstein k, Eidherr H, Schuetz M, Saverman R, Du dezak. R, Winkler S, Pabinge I, Kletter.k. Platelet kinetics and scintigraphic imaging in thrombocytopenic malaria patients, *thrombhaemost* 2004; 99:553-557.