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A STUDY OF THROMBOCYTOPENIA IN MALARIA– CORRELATION WITH TYPE OF MALARIA. A PROSPECTIVE OBSERVATIONAL COHORT STUDY

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ABSTRACT

Malaria is one of the most prevalent human infections worldwide and among the oldest of diseases with far reaching repercussions in human history and remains a major cause of mortality and morbidity. Thrombocytopenia has been reported to be associated with malaria, with an incidence ranging from 24% to 94%, with some studies reporting a higher incidence in vivax malaria as compared to falciparum malaria. Various hypotheses are postulated about the Mechanism of thrombocytopenia in malaria but the exact mechanism is not clear yet. Possible mechanisms include Coagulation Disturbances, Immune mechanisms, Oxidative stress, Bone marrow alteration, Direct damage by parasites, Splenic sequestration. The present study was aimed to evaluate the incidence and severity of thrombocytopenia and to correlate the grade of thrombocytopenia with various species of malaria. We also studied the incidence of bleeding manifestations in malaria. This study was conducted in the Department of Medicine, Hindu Rao Hospital, Malkaganj, Delhi from August 2019 to May 2021. A total of 120 patients who were diagnosed to have Malaria fulfilling all the inclusion and exclusion criteria admitted or treated on Outpatient basis were included in the study. Platelet counts, bleeding manifestation, recovery of platelet counts was studied in these patients. In our study, 82.5% of the patients of malaria had thrombocytopenia. Thrombocytopenia was seen in 86.2% of cases of P. vivax, 68% cases of P.valciparum, 100% cases of Mixed infection There is statistically a significant difference present in mean values of platelet count among types of malaria. There is a statistically significant difference present in grades (severity) among the species (p value 0.004).

Keywords: thrombocytopenia; malaria; plasmodium vivax; plasmodium falciparum; mixed.

INTRODUCTION

The disease malaria is caused by the parasite called Plasmodium. Female anopheles mosquitoes transmit the disease. Five species of Plasmodium (Plasmodium falciparum, Plasmodium vivax Plasmodium malariae, and Plasmodium knowlesi, Plasmodium ovale) cause malaria in humans. Approximately 50% of the world population is in danger of this protozoan infection. Many of the cases and mortality happen in Africa (sub-Saharan part). However, the central east, Asia, and some components of Europe are also affected. It continues to be a huge social, economic and health problem, particularly in tropical countries and a threat to non-endemic countries, and a danger to travellers. Malaria continues to create a serious public health issue in India. There is an increase in the incidence of drug resistance of the parasite and insecticide resistance of the vector.[1]

According to WHO report 2020, a major number of 229 million cases have been reported in eighty-seven endemic countries, of which Africa contributes 94 percent. Malaria results in 0.4 million

deaths globally. India accounted for about 86% of all malaria deaths in the WHO in the region of South-East Asia.[2]

The WHO has released the Global Technical Strategy for Malaria 2016–2030, which advocates acceleration of global malaria elimination efforts and has set targets to reduce malaria mortality rate and malaria case incidence globally by 90% by 2030 (baseline 2015); eliminate malaria from at least 35 countries in which malaria was transmitted in 2015; and prevent re-establishment of malaria in all countries that are malaria-free.[3]

Incidence of thrombocytopenia ranging from 24% to 94% has been seen in malaria in various studies, with some studies showing a higher frequency of thrombocytopenia in P.vivax cases compared to P.falciparum cases.[4] In endemic regions, the low platelet count in cases presented with acute fever is an important indicator of malaria.[5] In the last few years, the scenario shows an unexpected development in P.vivax, aside from anaemia and thrombocytopenia other complications like cerebral malaria, AKI, Acute respiratory distress syndrome, Hypotension are recently seen in P. vivax malaria.[6] Initially it had been thought all of those were attributed to mixed infections but, the study by Kocher et al showed that P. vivax was also can liable for these complications which were not observed before in P.vivax malaria.[7]

As Dengue fever is also common in India, the thrombocytopenia in Malaria cases brought interest as avoiding platelet infusions which are not necessary in these cases is of giant benefit.[8] As it decreases the transfusion related complications and aid in better use of blood products in poor resource countries like India where the necessity for blood and its components is more than the availability.[9]

MATERIALS AND METHODS

Place of Study: This study was conducted in the Department of Medicine, Hindu Rao Hospital, Malkaganj, Delhi.

Study Period: 1 year 10 months; August 2019 to May 2021.

Study Design: A Prospective Observational Cohort Study.

Study Subjects: All the patients with a malarial spectrum of fever and identified positive for Malaria parasite on either peripheral smear examination with conventional microscopy and/or Rapid diagnostic card test (PfHrp2/ Pv LDH antigen test), fulfilling all the inclusion and exclusion criteria.

Inclusion Criteria

1. All patients with 13-65 years of age both male and female
2. Cases positive for malaria by either conventional microscopy of blood smear and/or Rapid diagnostic card test (PfHrp2/PvLDH antigen test).

Criteria for Diagnosis: Diagnosis of malaria will be done by a Malaria parasite identification on peripheral smear examination with conventional microscopy and/or Rapid diagnostic card test (PfHrp2/PvLDH antigen test) in patients with malarial spectrum of fever.

The Severity of thrombocytopenia,[51]

Platelet count < 1, 50,000 / mm3 is taken as cut off for Thrombocytopenia, and graded as

Grade 1: 75-150 × 10 3 / mm3
Grade 2: 50-75 × 10 3 / mm3
Grade 3: 25-50 × 10 3 / mm3
Grade 4: < 25 × 10 3 / mm3

Exclusion Criteria

1. Cases with History of Congenital & Hereditary Thrombocytopenia.
2. Cases of Immune induced thrombocytopenia, Drug induced thrombocytopenia.
3. Cases that received partial treatment for malaria before enrolment.
4. Cases with other disorders that can cause thrombocytopenia like aplastic anaemia, leukaemia, Megaloblastic anaemia, HIV infection, Hepatitis, concurrent dengue infection.
5. Cases who are on medication that can cause bleeding manifestations like antiplatelet drugs, anticoagulants.
6. Pregnant females.

Methodology

A total of 120 patients who were diagnosed to have Malaria fulfilling all the inclusion and exclusion criteria admitted or treated on an Outpatient basis at Hindu Rao hospital, Delhi were included in the study. All study subjects were identified positive for Malaria parasite on either peripheral smear examination with conventional microscopy and/or Rapid diagnostic card test (PfHrp2/PvLDH antigen test). Detailed history taking, clinical examinations were done to see the inclusion and exclusion criteria. Platelet count was done on a fully automated, quantitative analyzer (SYSMEX Kx-21), and blood counts with other details were obtained. Sysmex Kx- 21 is an automatic multi-parameter blood cell counter for in-vitro diagnostic use in clinical laboratories. Platelet count is the number of thrombocytes derived from directly measured platelet pulses, multiplied by a calibration constant and expressed in thousands of thrombocytes per microliter of whole blood. Manual platelet count was done in patients with thrombocytopenia to confirm the thrombocytopenia. Other investigations included Complete blood Counts, Liver function test, Renal function test, Dengue serology, HIV serology, if necessary, Blood Culture, Urine Culture, Chest X-Ray, Ultrasound Abdomen.

RESULTS

In the present study, a total of 120 patients who were diagnosed to have Malaria fulfilling all the

inclusion and exclusion criteria admitted or treated on an outpatient basis were included in results are presented below.

Table 1: Age distribution of study population

Age Groups in years	Frequency	%
13 - 20	35	29.2%
21 - 30	47	39.2%
31 - 40	19	15.8%
41 - 50	12	10.0%
>50	7	5.8%
Total	120	100%
Mean ± SD	28.52 ± 12.28	
Min - Max	13 - 65	
Median (IQR)	26 (19.00 - 34.25)	

In our study the majority of the population belongs to 21-30 years. 39.2%(n=47) of population belong to 21 - 30 years followed by 29.2% (n=35) of population belong to 13 - 20 years, 15.8%(n=19) of population belong to 31-40 years, 10.0%(n=12) of population belong to 41-50 years and 5.8%(n=7) of population belongs to above 50 years.

Table 2: Gender distribution

Sex	Frequency	%
Female (F)	33	27.5%
Male (M)	87	72.5%
Total	120	100%

In our study population 72.5% (n=87) belonged to male & 27.5% (n=33) were female.

Table 3: Distribution of Species in the study

Species	Frequency	%
P.falciparum (PF)	29	24.2%
P.vivax (PV)	87	72.5%
Mixed infection (PV+PF)	4	3.3%
Total	120	100%

72.5% (n=87) of study the population had infection with P.vivax (PV) followed by P.falciparum (PF) which was present in 24.2%(n=29). In 3.3% (n=4) of study population has Mixed infection (PV+PF).

Table 4: Incidence of thrombocytopenia

Platelet count	Frequency	%
Normal platelet count	21	17.5%
Thrombocytopenia	99	82.5%
Total	120	100%

In our study population, 82.5% (n=99) had thrombocytopenia and 17.5% (n=21) had normal platelet count.

Table 5: Incidence of thrombocytopenia in Age groups

Age groups inyears	Thrombocytopenia				p value
	No		Yes		
	Frequency	%	Frequency	%	
13 - 20 yrs	5	23.8%	30	30.3%	0.417
21 - 30 yrs	10	47.6%	37	37.4%	
31 - 40 yrs	5	23.8%	14	14.1%	
41 - 50 yrs	1	4.8%	11	11.1%	
>50 yrs	0	0.0%	7	7.1%	
Total	21	100%	99	100%	

P value is 0.417 which means Age has no statistically significant effect on the incidence of thrombocytopenia in malaria.

Table 6: Incidence of thrombocytopenia in Male and Female

Gender	Thrombocytopenia				p value
	No		Yes		
	Frequency	%	Frequency	%	
Female(F)	7	33.3%	26	26.3%	0.510
Male(M)	14	66.7%	73	73.7%	
Total	21	100%	99	100%	

P value is 0.51 which means gender has no statistically significant effect on the incidence of thrombocytopenia in malaria.

Table 7: Frequency of grades of thrombocytopenia

Thrombocytopenia	Frequency	%
Normal platelet count	21	17.5%
Grade 1	53	44.2%
Grade 2	22	18.3%
Grade 3	13	10.8%
Grade 4	11	9.2%
Total	120	100%

In our study population 44.2% (n=53) shoed to grade 1 thrombocytopenia, 18.3% (n=22) Showed grade 2, 10.8%(n=13) showed grade 3 and 9.2%(n=11) showed grade 4. Normal plate count observed in 17.2%(n=21) of study population.

Table 8: Platelet counts in the study

	Mean ± SD	Min - Max	Median (IQR)
Platelet count	104000 ± 72400	8000 - 435000	55800 (89500 - 138000)

In our study mean platelet count values is 104000 ± 72400, min-max value is 8000 – 435000 and median (IQR) value is 55800(89500-13800) at the time of admission.

Table 9: Comparison of Mean Platelet counts in Study Species

		Species			p value
		P.falciparum(PF)	P.vivax(PV)	Mixed infection(PF+PV)	
Platelet count	Mean ± SD	86941.38 ± 59667.87	112467.82 ± 75422.04	37250.0 ± 27645.02	0.016
	Min - Max	9300 - 178000	11800 - 435000	8000 - 71000	
	Median(IQR)	77000 (30500 - 157500)	98200 (68000 - 137000)	35000 (11750 - 65000)	

In our study, the mean platelet counts of study species are 86941.38 ± 59667.87 for P.falciparum (PF), 112467.82 ± 75422.04 for P.vivax (PV) and 37250.0 ± 27645.02 for Mixed infection (PF+PV) with a significant p value 0.016.

Table 10: Species wise incidence of thrombocytopenia

Species	Total cases	Thrombocytopenia		p value
		No	Yes	
		Frequency (%)	Frequency (%)	
P.falciparum (PF)	29	9 (31.0%)	20 (69.0%)	0.069
P.vivax (PV)	87	12 (13.8%)	75 (86.2%)	
Mixed infection (PV+PF)	4	0 (0.0%)	4 (100%)	
Total	120	21 (17.5%)	99 (82.5%)	

In our study species the incidence of thrombocytopenia is majorly observed in P.vivax with n=75(86.2%) in 87 patients followed by P.falciparum with n=20(69%) in 29 patients and Mixed infection(PV+PF) with n=4(100%). This difference is statistically not significant as p value is 0.069.

Table 11: Grades of thrombocytopenia in study species

Species	Total cases	Platelet count					p value
		Normal	Thrombocytopenia				
			Grade 1	Grade 2	Grade 3	Grade 4	
		Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	
P.falciparum (PF)	29	9 (31.0%)	7 (24.1%)	4 (13.8%)	5 (17.2%)	4 (13.8%)	0.004
P.vivax (PV)	87	12 (13.8%)	46 (52.9%)	17 (19.5%)	7 (8.0%)	5 (5.7%)	
Mixed infection PV+PF	4	0 (0.0%)	0 (0.0%)	1 (25.0%)	1 (25.0%)	2 (50.0%)	
Total	120	21 (17.5%)	53 (44.2%)	22 (18.3%)	13 (10.8%)	11 (9.2%)	

In study species, Grade 4 thrombocytopenia was seen in 50% of cases in Mixed infection,13.8% of cases in falciparum, 5.7% of cases in Vivax.

Table 12: Incidence of bleeding manifestations

Bleeding Manifestations	Frequency	%
No	118	98.3%
Yes	2	1.7%
Total	120	100%

In our study, the incidence of bleeding manifestations is observed in n=2 (1.7%) cases out of 120 malaria cases.

Table13: Species wise incidence of bleeding manifestations

Species	Total cases	Bleeding Manifestations		p value
		No	Yes	
		Frequency (%)	Frequency (%)	
P.falciparum (PF)	29	28 (96.6%)	1 (3.4%)	<0.001
P.vivax (PV)	87	87 (100%)	0 (0.0%)	
Mixed infection (PV+PF)	4	3 (75.0%)	1 (25.0%)	
Total	120	118 (98.3%)	2 (1.7%)	

In our study, incidence of bleeding manifestations is in n=2(1.7%) cases and they are observed with n=1 (3.4%) in P.falciparum (PF) and with n=1 (25%) in Mixed Infection(PF+PV).

Table 14: Grade wise incidence of Bleeding manifestations

Bleeding Manifestations	Platelet count					Total	p value
	Normal	Thrombocytopenia					
		Grade 1	Grade 2	Grade 3	Grade 4		
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)		
No	21 (17.8%)	53 (44.9%)	22 (18.6%)	13 (11.0%)	9 (7.6%)	118 (100%)	<0.001
Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (100%)	2 (100%)	

In our study, the incidence of bleeding manifestations with n=2 (1.7%) cases are observed in grade 4 of thrombocytopenia.

DISCUSSIONS

A total of 120 patients who were diagnosed to have Malaria fulfilling all the inclusion and exclusion criteria basis were included in the study.

In our study, the majority of the cases (39.2%) belong to the 21-30 years age group followed by the 13-20 years age group (29.2%). Mean age was 28.52 ± 12.28 (SD). Age has no statistically significant effect on the incidence of thrombocytopenia in malaria. In a study conducted by Bansal Y et al,[10] the mean age of the patients was 28.7 years. The majority 29.3% were from the age group of 21–30 years, followed by 11–20 years 26.9%. 21-30 years was predominantly involved in malaria in studies by Patel P et al,[11] Kumar N et al(12) as well .

In our study males (72.5%) were affected more than females (27.5%). These results are similar to other studies by Bansal Y et al (10) and Patel P et al,[11] Gender has no statistically significant effect on the incidence of thrombocytopenia in malaria. The incidence of malaria is more in men than in women may be due to the working pattern i.e., men are exposed to mosquito bites outdoors whereas females are less exposed & may be due to good clothing of Indian women.

In our study P. vivax cases were seen in 72.5%(n=87), P. falciparum cases were seen in 24.2% (29), mixed infection was seen in 3.3%. Similar to Bansal Y et al,[10] by Patel P et al,[11] and Kumar N et al,[12] P. vivax is more common than P. falciparum followed by Mixed infection.

In our study of 120 cases of malaria, 82.5% (n=99) patients had thrombocytopenia Incidence of thrombocytopenia ranging from 24% to 94% has been seen in malaria in various studies (4). Our study result is similar to Bansal Y et al,[10] Kumar N et al.[12]

The mean platelet count in our study is 1,04,000 ± 72,400 (Mean ± SD), median (IQR) count is 55800 (89500 - 138000). The mean platelet count was 92,000, 71,634 in Study by Bansal Y et al (10), Patel P et al,[11] respectively.

In the study population of 120, the Thrombocytopenia of Grade 1 (platelet count between 75,000-1,50,000) is seen in 44.2%(n=53), Grade 2 (50,000-75,000) is seen in 18.3%(n=22),

Grade 3 (25,000-50,000) is seen in 10.8%(n=13), Grade 4 (<25,000) is seen in 9.2%(n=11). Results show that severe thrombocytopenia is not uncommon in Malaria patients. In Study of Memon AR et al,[13] 70 % of patients with malaria had platelet count between 50000-150000, 22% patients had platelet count between 20000-50000, 8% patients had platelet count <20000. In study of Bansal Y et al,[10] Platelet count <20000 is seen in 3.8% patients of malaria.

Comparison among the Species (P.vivax, P.falciparum, Mixed Infection: Thrombocytopenia was seen in 86.2% of cases of P.Vivax, 68% cases of P.Falciparum, 100% cases of Mixed infection. But this variation is statistically not significant p value (0.069). Our study results were comparable with Patel P et al.[11] There is a statistically significant difference present in mean values of platelet count among species (p value 0.016). In P.Vivax cases Mean platelet count is 1,12,467 ± 75,422, with the minimum platelet count of 11,800. In P.falciparum cases Mean platelet count is 86,941 ± 59,667, with minimum platelet count of 9,300. In cases of mixed infection Mean platelet count is 37250.0 ± 27645.02 with the minimum count of 8,000. the Study of Gill MK et al (13) showed mean platelet count in Plasmodium vivax (Pv) malaria was 1,27,652/µl (SD 78,269) as against Plasmodium falciparum malaria where the mean platelet count was 78,500/µl (SD 51,485).

There is a statistically significant difference present in grades (severity) among the species (p value 0.004). Severe thrombocytopenia is more common in Mixed infection cases followed by P.falciparum than P.vivax. But severe thrombocytopenia was seen in cases of P.vivax also. Platelet count <25,000 (Grade4) is seen in 5.7% cases of P.vivax, 13.8% cases of P.falciparum and 50% cases of mixed infection. Platelet count <50,000 (Grade3 and Grade4) is seen in 13.7% cases of P.vivax, 31% cases of P.falciparum and 75% cases of mixed infection. In study Gill MK et al,[13] platelet count <50,000 is seen in 17.4% cases of P.vivax, 33.3% cases of P.falciparum. . In the study of Jadhav UM et al,[15] platelet count <20,000 is seen in 1.5% cases of P.Vivax, 8.5% cases of P.falciparum. In study of Patel P et al,[11] platelet count <20,000 is seen in 7.7% cases of P.Vivax, 3.67% cases of P.falciparum.

Bleeding Manifestations

In our study, bleeding manifestations were seen in only 2 cases (1.7%) that needed transfusion of platelets. Both the cases belonged to grade1 of WHO bleeding grade (46) and belong to Grade4 thrombocytopenia and platelets count less than 10,000. One case of P.falciparum with platelet count of 9,300 had petechiae. One case of mixed infection with a platelet count of 8,000 had minimal gingival bleeding. In the study of Bansal et al,[10] bleeding manifestations are seen 1 case out of 131 in the form of petechiae. In the study by Gill MK et al,[14] 8.3% of cases presented with bleeding manifestations in the form of petechiae, gingival bleeding and epistaxis. In the study by Limaye CS et al,[16] bleeding manifestations are seen in 8% of cases in the form of mucosal bleed and there was no life-threatening bleeding. There were no bleeding manifestations noted in studies of Gupta NK et al,[17]

CONCLUSION

In our study which is conducted in Delhi, the incidence of P.Vivax cases is higher compared to P.falciparum. Mixed infection cases are less common.

- Thrombocytopenia is a common laboratory finding in malaria patients. Clinicians should keep malaria in the differential diagnosis in patients of acute febrile illness with Thrombocytopenia and should work up & treat accordingly.
- The incidence of thrombocytopenia is more common in mixed infection followed by P.vivax than P.falciparum but the presence of thrombocytopenia is not a distinguishing feature between the types of malaria.
- Severe thrombocytopenia is more common in cases of mixed infection and P.falciparum. but severe thrombocytopenia can also be seen in P.vivax cases.
- Patients with malaria tolerate Thrombocytopenia well, despite severe thrombocytopenia bleeding is seen in a smaller number of cases. Unnecessary transfusions to be avoided to decrease the transfusion related complications.
- Thrombocytopenia in an acute febrile illness patient should be regarded as an important feature of malaria. Prompt initiation of antimalarial therapy can help avoid unnecessary transfusions, thereby preventing serious transfusion-related complications in these patients.

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