CORRELATION OF TYPE OF SPECIES AND PARASITE DENSITY IN MALARIA WITH PLATELET COUNT, MEAN PLATELET VOLUME AND PLATELET DISTRIBUTION WIDTH

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ABSTRACT

BACKGROUND

Malaria is an important infectious protozoan disease and it has been estimated that worldwide there are 300 - 500 million cases of malaria per year and 1.5 - 2.7 million deaths. The early diagnosis of malaria is the key feature for its prompt treatment and prevention of complications. The study was conducted to statistically analyse other haematological parameters like platelet indices that is Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) along with platelet count. Correlation of the above was done with parasite density and type of species. An attempt was made to study whether platelet indices and counts are indicative of type of infection.

MATERIALS AND METHODS

The study was conducted prospectively over a period of one month in the Haematology Laboratory of Kasturba Medical College Hospital, Mangalore. It included cases of acute malaria which presented with fever within a week caused either by *Plasmodium vivax* (PV), *falciparum* (PF) or dual infection (PV + PF). All cases were diagnosed by demonstration of the respective parasite by Quantitative Buffy Coat assay. Haematological parameters which included platelet count (PC), mean platelet volume (MPV) and platelet distribution width (PDW) were recorded and statistically analysed.

RESULTS

The study included 100 cases of malarial infection with maximum cases in the age group of 10-30 years. Out of 100 cases 86 were PV positive, 6 cases were PF positive and 8 cases of mixed infection. From above graphs, there was no correlation between *falciparum* parasitaemia with either PDW ($P = 0.58$) or MPV ($P = 0.16$), similar to *vivax* infection. The drop in platelet count with increasing parasitaemia of *vivax* schizonts was highly significant ($P < 0.001$), but not *falciparum* infection ($P = 0.00$). The increase in PDW was significant with increasing MPV ($P = 0.003$) and simultaneous decrease in platelet count ($P = 0.00$).

CONCLUSION

Increased *vivax* parasitaemia, unlike *falciparum* parasitaemia, was significantly associated with thrombocytopaenia. The increase in PDW was significantly associated with increase in MPV and simultaneous decrease in platelet count. Correlation of parasitaemia in either of malaria species with changes in MPV and PDW, was not significant.

KEYWORDS

Plasmodium Vivax (PV), *Plasmodium Falciparum* (PF), Platelet Count (PC), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW).

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BACKGROUND

Malaria is a Protozoal disease caused by infection with parasites of the genus *Plasmodium* (PI).⁽¹⁾ They are transmitted to humans by certain species of female Anopheline mosquito, which are infected.⁽¹⁾ Five species of *Plasmodium* (*Plasmodium Vivax*, *Plasmodium Falciparum*, *Plasmodium Ovale*, *Plasmodium Malariae* and *Plasmodium Knowlesi*) cause malaria in humans.⁽¹⁾ An estimated 243 million cases of malaria were present in 2008, of which 85% were in African region followed by South-East Asia region

(10%) and 4% were East Mediterranean.⁽¹⁾ Approximately, 863,000 people died of malaria of which 89% were from African region.⁽¹⁾ Malaria is an important infectious protozoan disease and early diagnosis of malaria is a key feature of its prompt treatment and prevention of complications.⁽²⁾ Main complications of malaria are coma, hypoglycaemia, acidosis, renal failure and pulmonary oedema.⁽²⁾ Microscopic diagnosis is needed for confirmation of malaria, but it requires technical expertise and may be at times unreliable when poorly executed.⁽³⁾ Haematological changes which include low platelet count, haemoglobin concentration and haematocrit have been reported to be associated with malaria.^(4,5) Many recently conducted studies have shown the role of platelet indices to determine the various causes of malaria and thrombocytopaenia.⁽⁶⁾ This study was conducted to statistically analyse other haematological parameters like Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) along with the platelet count. Correlation of the above was done with parasite density and type of species. An attempt was made to study whether platelet indices and counts are indicative of type of infection.

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MATERIALS AND METHODS

The present study was conducted prospectively over a period of one month in the Department of Pathology, Kasturba Medical College, Mangalore, a premier tertiary care centre in coastal Karnataka. Cases were collected from Manipal Hospital Jyothi Circle and Manipal Hospital Attavar. Sample size of 100 cases was taken. The study included cases of acute malaria which presented with fever within a week caused either by Plasmodium Vivax (PV), Plasmodium Falciparum (PF) or dual infection (PV + PF). All the cases were diagnosed by demonstration of the respective parasite by Quantitative Buffy Coat Assay. Haematological parameters which included Platelet Count (PC), Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) was recorded. The automated analyser used was Beckman coulter LH780. Quantitative Buffy Coat (QBC) was done using QBC centrifuge and QBC capillary blood tube at 12000 rotations per minute for 5 minutes using Acridine Orange stain to highlight the parasite. The fluorescent microscope used was Olympus Cx21i. The parasites were viewed under QBC immersion oil with a magnification of 60x at objective and 10x at eye piece. The parasites were graded as 1+ when parasite density was less than 1 per High Power Field (HPF), 2+ when parasite density was between 1 - 10 per HPF, 3+ when between 10 - 100 per HPF and 4+ when more than 1000 per HPF. Statistical analysis was done using ANOVA (Fisher's test). P value of < 0.001 was found to be statistically significant.

RESULTS

Hundred cases which were proved positive by QBC technique were selected. Out of 100 cases 90 were PV schizonts positive, 64 were PV trophozoites positive and 18 were PF trophozoites positive. Age range for selected cases was 1 to 79 years with mean being 34.7. Platelet count, PDW and MPV was noted. Platelet count range was 9000 to 305,000 with mean being 114,700. Similarly, PDW range was 15.6 to 19.4 with mean being 17.4 and MPV range being 6.1 to 11.6 with mean being 8.91. Schizonts of PV were seen in 90 out of 100 cases of which 29 were 1+, 25 were 2+, 18 were 3+ and 18 were 4+. PV trophozoites were seen in 64 cases out of which 11 were 1+, 20 were 2+, 21 were 3+ and 12 were 4+. PF trophozoites were seen in 18 cases out of which 8 were 1+, 4 were 2+ and 6 were 3+.

	Frequency	Percent
< 10	3	3.0
10 - 20	16	16.0
20 - 30	35	35.0
30 - 40	15	15.0
40 - 50	11	11.0
50 - 60	10	10.0
> 60	10	10.0
Total	100	100.0

Table 1. Age Group Distribution

		N	Mean	Std. Deviation	F	p
pdw	1+	29	17.2966	.87606	1.33	.27
	2+	25	17.3120	.82579		
	3+	18	17.6889	.92666		
	4+	18	17.6278	.63599		
mpv	1+	29	8.4345	1.12449	2.72	.05
	2+	25	9.0760	1.32893		
	3+	18	9.2056	1.10638		
	4+	18	9.2278	.94918		
platelet	1+	29	152068.9655	74523.21356	6.60	< 0.001 VHS
	2+	25	109080.0000	65485.95269		
	3+	18	96777.7778	48093.72658		
	4+	18	75000.0000	41621.54420		
	Total					

Table 2. Plasmodium vivax Schizont

There was no correlation between parasitaemia of vivax schizont with either PDW (P = 0.27) or MPV (P = 0.05). There was no correlation between parasitaemia of vivax trophozoites with either PDW (P = 0.83) or MPV (P = 0.71).

There was no correlation between parasitaemia of falciparum with either PDW (P = 0.58) or MPV (P = 0.16). However, drop in platelet count with increasing parasitaemia of vivax schizonts was highly significant (P < 0.001).

		N	Mean	Std. Deviation	F	p
pdw	1+	11	17.4364	1.04812	.29	.83
	2+	20	17.3250	.91068		
	3+	21	17.4905	.77647		
	4+	12	17.6083	.71154		
mpv	1+	11	8.6091	1.19202	.47	.71
	2+	20	9.0200	1.12699		
	3+	21	9.0571	1.15697		
	4+	12	9.1000	1.10618		
platelet	1+	11	134545.4545	72526.35884	2.54	.07
	2+	20	117050.0000	61865.28233		
	3+	21	88476.1905	48675.06451		
	4+	12	82666.6667	39823.09365		

Table 3. Plasmodium vivax Trophozoites

		N	Mean	Std. Deviation	F	p
pdw	1+	8	17.1750	.68400		
	2+	4	17.2750	.63966		
	3+	6	16.8000	.98387	.56	.58
mpv	1+	8	9.0375	.89752		
	2+	4	9.7750	.60208		
	3+	6	8.6833	.86583	2.07	.16
platelet	1+	8	133875.0000	64368.22086		
	2+	4	61500.0000	30005.55504		
	3+	6	115666.6667	74518.90141	1.78	.20

Table 4. Plasmodium falciparum Trophozoites

		MPV	Platelet
pdw	R	.291	-.543
	P	.003	.000
	N	100	100
mpv	R		-.500
	P		.000
	N		100

Table 5. Correlations

The increase in PDW was significant with increasing MPV (P=0.003) and simultaneous decrease in platelet count (P=0.000).

Sex			MPV	Platelet
Male	pdw	R	.343	-.514
		P	.004	.000
		N	69	69
	mpv	R		-.515
		P		.000
		N		69
Female	pdw	R	.315	-.655
		P	.085	.000
		N	31	31
	mpv	R		-.480
		P		.006
		N		31

Table 6. Correlations

DISCUSSION

Malaria is caused by *Pl. vivax* and *Pl. falciparum*, which is endemic in many parts of India.⁽¹⁾ Malaria is a true haematological disease, which affects almost all blood components.⁽¹⁾ Most frequently malaria associated haematological complications are thrombocytopenia and anaemia.⁽¹⁾ Malaria has been reported as the major cause of low platelet counts in the endemic areas.⁽¹⁾ In some places, it is used as an indicator of malaria in patients presenting with fever as a characteristic feature. Platelets count of less than 150,000/cumm increases the chance of malaria 12-15 times.^(7,8,9)

Pl. vivax trophozoite infection was most common in our study with a positivity of 64%. *Pl. falciparum* trophozoites were seen in 18% of the cases. A similar observation was made by Gupta et al, which showed 56.51% positivity for *Pl. Vivax* and 39.13% positivity for *Pl. falciparum* infection. Similar results were seen in a study done by Faseela et al.⁽¹⁰⁾

In our study, increase in parasitaemia of *Pl. vivax* was highly significant with the drop in platelet count with p value being < 0.001. In a study done by Gupta et al,

thrombocytopenia was seen in 78% of cases.⁽¹⁾ Similar observations were made by Colonel et al, which showed thrombocytopenia in 72% of cases.⁽¹¹⁾ A study done by Jamal et al on paediatric patients reported thrombocytopenia in 72% of patients with malaria infection.⁽¹²⁾

Exact mechanism of thrombocytopenia is still unknown in malaria. Fajardo and Tallent demonstrated a direct lytic effect of the parasite on platelets and also *Pl. vivax* within platelets.⁽¹³⁾

Both non-immunological destruction as well as immune mechanism⁽¹⁴⁾ involving specific platelet associated IgG antibodies that bind directly to malarial antigen in the platelets have been recently reported to play a role in the lysis of platelets.⁽¹⁵⁾

Oxidative stress damage of platelets has also been implicated in the aetiopathogenesis based on the finding of low levels of platelet superoxide-dismutase and glutathione peroxidase activity and high platelet lipid peroxidation levels in malaria patients when compared to those of healthy subjects.⁽¹⁶⁾

Decreased thrombopoiesis has been ruled out, because platelet forming megakaryocytes in the marrow are usually normal or increased.⁽¹⁶⁾ A good tolerance of low platelet count is well-known in malaria.

In our study, *Pl. vivax* malaria was found to have more significant thrombocytopenia. More cases of thrombocytopenia in *vivax* malaria infection may attribute to a possible development of a new genotype of *Pl. vivax*.⁽¹⁶⁾ Recent studies have shown that thrombocytopenia is equally or even more common in *Pl. vivax* malaria contrary to the popular belief that it may be observed in *Pl. falciparum* malaria.⁽¹⁷⁾

It is recently seen in India that thrombocytopenia exhibited a heightened frequency and severity among patients with *Pl. vivax* infections.⁽¹⁸⁾ Recent studies conducted from Indian subcontinent have found significant thrombocytopenia in *Pl. vivax* malaria.^(19,20) Studies from Qatar and Venezuela had shown the similar results.^(21,22) There is no matched control group. This is one of the limitations of this study.

CONCLUSION

Higher frequency of mild-to-severe thrombocytopenia is observed in patients suffering from malaria. The above finding can have therapeutic implications in context of avoiding unnecessary platelet infusion in malaria patients. Presence of thrombocytopenia in a patient with acute febrile illness in the tropics increases the possibility of malaria. This may be used in addition to the clinical and microscopic parameters to heighten the suspicion of this disease and prompt initiation of the treatment.

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