Original Article

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Pathology Section

Haematological Alterations in Initially Diagnosed and Relapse/Recurrent Cases of Malaria: A Comparative Study

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ABSTRACT

Introduction: Malaria is endemic in India with an estimated 70-100 million cases each year. An alteration of haematological profile is one of the hallmarks in patients with malaria. A variety of haematological alterations like progressively increasing anaemia, leucocytosis or leucopenia, thrombocytopenia have been reported.

Aim: To evaluate and compare the effect of initially diagnosed cases and cases of relapse/ recurrence of malaria on haematological parameters and their correlation with Parasitic Index (PI).

Materials and Methods: This prospective study included 183 patients of initially diagnosed malaria cases and 64 follow-up cases of malaria. The diagnosis of malaria was confirmed on peripheral blood film and/or malaria antigen test. Complete blood counts were performed using automated Sysmex XN-1000. MP antigen test was done by Alere bioline SD malaria antigen kit. The Pearson's Chi-square test was used for comparison of the patient's data for association between distribution of severe anaemia in initially diagnosed cases of malaria and in cases with relapse/recurrence in relation to PI. The association of Grade III & IV thrombocytopenia in initially diagnosed cases of malaria and in cases with relapse/recurrence in relation to PI was evaluated using the paired Student's *t*-test.

Results: Out of 183 initially diagnosed malaria cases, 163 cases had *P. vivax* and 20 cases had *P. falciparum*. All the cases of *P. vivax* and 75% of cases of *P. falciparum* had anaemia. All the cases had thrombocytopenia. Among 64 follow-up cases, 57 had *P. vivax* and 7 cases had *P. falciparum* infection. Approximately 50% cases of *P. vivax* and 40% cases of *P. falciparum* had normal Hb levels. Normal platelet counts were observed in 14% of *P. vivax* and 28.5% of *P. falciparum* cases. Majority of the cases (≅ 85%) of both the groups had PI ≤10.

Conclusion: Anaemia and thrombocytopenia are frequent findings in malaria with severity of thrombocytopenia related to PI. Thrombocytopenia is a constant finding in initially diagnosed cases of malaria but in cases of relapse/recurrence platelet counts may be normal.

Keywords: Anaemia, Parasitic index, Severity, Thrombocytopenia

INTRODUCTION

Amongst the numerous infectious diseases known to man, malaria is considered to be a menace in most of the developing countries since long. This disease is endemic in India with an estimated 70-100 million cases each year. Malaria is a protozoan disease caused by the *Plasmodium* species. There are five species of *Plasmodium* viz., *P. falciparum, P. ovale, P. vivax, P. malariae, P. knowlesi* that are known to cause malaria in humans [1-3].

Despite extensive worldwide efforts towards reduction of malarial transmission, it remains the most serious and widespread protozoan disease. In tropical countries malaria is considered as the main differential diagnosis of acute febrile illness. An alteration of haematological profile is one of the hallmarks in patients with malaria. A prompt and accurate diagnosis is the key to effective management and reduction in morbidity as well as mortality [1].

Microscopic examination is the established method and gold standard for the laboratory confirmation of malaria that requires technical expertise and repeated smear examinations [1].

Being the parasite of blood it induces a wide array of haematological changes. A variety of haematological alterations like progressively increasing anaemia, leucocytosis or leucopenia, thrombocytopenia and rarely Disseminated Intravascular Coagulation (DIC) have been reported with various species of *Plasmodium* [1,3-7].

So much work has already been done on haematological alterations in cases of malaria with emphasis on newly diagnosed cases only [8-17]. There is paucity of data in literature on haematological derangements seen in cases of relapse/recurrence. Despite extensive search no study in the literature comparing the haematological alterations in initially diagnosed cases of malaria and cases of relapse/recurrence was found. It is strongly felt that the knowledge of haematological changes in cases of malaria relapse/recurrence will further aid in diagnosis and appropriate treatment planning. With this background the present study was undertaken to evaluate and compare haematological alterations in initially diagnosed cases of malaria and cases of relapse/recurrence.

MATERIALS AND METHODS

This was a prospective cohort study carried out in the department of Pathology at Shri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun. An informed written consent was taken from patients and the study was approved by ethical committee of the institute (SGRR/REC/54/13). The study was conducted for consecutive three years from Jan 2014 to Dec 2016 and all indoor as well as outdoor cases diagnosed with *Plasmodium* infection by peripheral blood smear examination and/ or rapid antigen test were included in the study. Relevant history, clinical findings and routine haematological investigations were recorded on a pre-designed proforma.

A total of 183 cases were taken during the study period. In all the cases Haemoglobin, Total leucocyte count and Platelet count were done by automated method using Sysmex XN-1000. Platelet count was also done manually to check for EDTA induced pseudothrombocytopenia and to look for giant platelets which

may falsely result in low platelet count [18,19]. All the cases of *Plasmodium* infection were sub-categorised based on haemoglobin levels, TLC and platelet count as follows [18,20,21]:

Normal Hb: 12 gm/dL and above, Mild Anaemia: 10–12gm/dL, Moderate Anaemia: 7 – 10gm/dL, Severe Anaemia: < 7gm/dL

Normal leucocyte count: 4000 – 11000/L, Leucopenia < 4000/L, Leucocytosis > 11000/L.

Normal platelet count: 1,00,000/cumm and Above, Grade I thrombocytopenia: 75000 – 1,00,000/cumm, Grade II thrombocytopenia: 50,000–75000/cumm, Grade III thrombocytopenia: 10,000 - 50,000/cumm, Grade IV thrombocytopenia: 10000/cumm and below.

MP antigen *test* was done by Alere bioline SD malaria antigen kit. Peripheral blood smears were prepared, stained with leishman stain and examined. PI was calculated as number of parasitized Red Blood Cells (RBCs) per 1000 (RBCs). Asexual forms of parasites i.e., ring form; trophozoite and schizont were included to calculate PI. The parasitic densities were graded as 0-5%, 6-10%, 11-15%, 16-20% and 21% & above [9].

Further, a subset of patients whose symptoms subsided after taking complete treatment of malarial infection presented again within 14 days with complaints of fever, were considered as relapse/recurrence and studied. The same haematological parameters and PI were observed and results were compared with the previous group.

STATISTICAL ANALYSIS

The Pearson's Chi-square test was used for comparison of the patient data for association between distribution of severe anaemia in initially diagnosed cases of malaria and in cases with relapse/ recurrence in relation to PI.

The association of Grade III & IV thrombocytopenia in initially diagnosed cases of malaria and in cases with relapse/recurrence in relation to PI was evaluated using the paired Student's *t*-test.

Adjustments were made using backward stepwise logistic regression and included all variables. All tests were 2-tailed. Significance was declared at $p < 0.05$.

All statistical tests were conducted using Statistical Package for the Social Sciences (SPSS) for Windows version 21.0 (SPSS Inc., Chicago, IL). Descriptive analysis was used, and the data was represented in the form of tables. The tables were created in Microsoft excel and Microsoft word.

RESULTS

The study included 183 cases of initially diagnosed *Plasmodium* infection, of which 163 cases had *P. vivax* infection and 20 cases had *P. falciparum* infection. This group included 105 males and 78 females with a male to female ratio of 1.34:1. The mean age of these patients were 29.7 with the highest proportion in 21-30 years age group. All the cases of *P. vivax* and 75% of cases of *P. falciparum* had anaemia. Total leucocyte count was normal in more than 50% cases (*P. vivax*-68.1% and *P. falciparum*-50%). All the cases had thrombocytopenia although majority (> 60% of *P. vivax* and 50% of *P. falciparum*) had grade I thrombocytopenia. The PI was ≤10 in majority of cases (85%) [Table/Fig-1].

A total of 64 cases presented with relapse/ recurrence. Out of these, 57 had *P. vivax* infection and seven cases had *P. falciparum* infection. This group had male to female ratio of 1.28:1 with 36 males and 28 females. The mean age was 29.9 with maximum cases in 21-30 years age group. Approximately 50% cases of *P. vivax* and 40% cases of *P. falciparum* had normal Hb levels. Both the infections had almost similar number of cases with normal leucocyte counts (around 55%) and PI ≤10 (≅85%). While majority of cases in this group had thrombocytopenia, in some cases platelet counts were normal (*P. vivax* 14% and *P. falciparum* 28.5%) [Table/Fig-2].

Both the groups were compared to correlate the severity of anaemia and thrombocytopenia with PI. The results are shown in [Table/Fig-3,4]. It was observed that there is no significant influence of parasitic densities on severity of anaemia. Grade III and Grade IV thrombocytopenia showed statistically significant correlation with parasitic densities in both initially diagnosed and relapse/recurrence cases.

	P. Vivax	P. Falciparum
Haemoglobin	Initially Diagnosed n=163 (%)#	Initially Diagnosed n=20 (%)#
Normal	0 (0)	05 (25)
Mild anaemia	113 (69.3)	03 (15)
Moderate anaemia	28 (17.1)	05 (25)
Severe anaemia	22 (13.4)	07 (35)
Total leucocyte count		
Normal	111 (68.1)	10 (50)
Leucopenia	42 (25.7)	04 (20)
Leucocytosis	10 (6.1)	06 (30)
Platelet count		
Normal	0 (0)	0 (0)
Grade I Thrombocytopenia	99 (60.7)	11 (55)
Grade II Thrombocytopenia	24 (14.7)	2 (10)
Grade III Thrombocytopenia	36 (22.1)	6 (30)
Grade IV Thrombocytopenia	4 (2.4)	1 (5)
Parasitic index		
0-5	107 (65.6)	7 (35)
6-10	35 (21.4)	10 (50)
11-15	13 (7.9)	1 (5)
16-20	3 (1.8)	2 (10)
21 and above	5 (3.1)	0 (0)

[Table/Fig-1]: Haemoglobin level, Total leucocyte count, Platelet count and Parasitic index of cases initially diagnosed with malarial infection
#figure in parenthesis indicate percentage

	P. Vivax	P. Falciparum
Haemoglobin	Relapse/ Recurrence n=57 (%)	Relapse/ Recurrence n=7 (%)
Normal	30 (52.6)	03 (42.8)
Mild anaemia	5 (8.7)	01 (14.2)
Moderate anaemia	15 (26.3)	02 (28.5)
Severe anaemia	7 (12.2)	01 (14.2)
Total leucocyte count		
Normal	31 (54.38)	04 (57.14)
Leucopenia	15 (26.31)	02 (28.57)
Leucocytosis	11 (19.2)	01 (14.28)
Platelet count		
Normal	8 (14)	02 (28.5)
Grade I Thrombocytopenia	20 (35.1)	02 (28.5)
Grade II Thrombocytopenia	11 (19.3)	01 (14.2)
Grade III Thrombocytopenia	17 (29.8)	02 (28.5)
Grade IV Thrombocytopenia	1 (1.7)	(0)
Parasitic index		
0-5	40 (70.17)	3 (42.8)
6-10	8 (14.03)	3 (42.8)
11-15	7 (12.28)	1 (14.2)
16-20	2 (3.5)	0
21 and above	0	0

[Table/Fig-2]: Haemoglobin level, Total leucocyte count, Platelet count and Parasitic index of relapse/ recurrence cases of malarial infection
#figure in parenthesis indicate percentage

Parasitic Index (PER 1000 RBC)	Anaemia	Initial Cases n=183 (%)	Relapse/ Recurrence n=64 (%)	Pearson Chi-Square	p-value (2-sided)
0-5%	Severe	17 (14.9) n=114	05 (11.6) n=43	11.250	0.259
6-10%	Severe	07 (15.5) n=45	02 (18.2) n=11		
11-15%	Severe	01 (7.1) n=14	0 (0) n=8		
16-20%	Severe	01 (20) n=5	01 (50) n=2		
21% & above	Severe	03 (60) n=5	0 (0) n=0		

[Table/Fig-3]: Distribution of severe anaemia in initially diagnosed cases of malaria and in cases with relapse/recurrence in relation to parasitic index.

Parasitic Index (per 1000 RBC)	Thrombocytopenia	Initial Cases n=183 (%)	Relapse/ Recurrence n=64 (%)	Mean±SD	p-value
0-5%	Grade III + IV	12 (10.5) n=114	2 (4.6) n=43	5.4±2.7	0.011
6-10%	Grade III + IV	15 (33.3) n=45	10 (90.9) n=11		
11-15%	Grade III + IV	10 (71.4) n=14	06 (75) n=8		
16-20%	Grade III + IV	05 (100) n=5	02 (100) n=2		
21%% and above	Grade III + IV	05 (100) n=5	0 (0) n=0		

[Table/Fig-4]: Distribution of Grade III & IV thrombocytopenia in initially diagnosed cases of malaria and in cases with relapse/recurrence in relation to parasitic index
*p-value calculated using paired students *t*-test

DISCUSSION

Malaria causes numerous haematological alterations of which thrombocytopenia and anaemia are the most common. This is so characteristic of malaria that in some places, it is used as an indicator of malaria in patients presenting with fever. There are many studies which indicate that in addition to category of malaria, precise haematological changes may vary with nutritional status, demographic factors, immune status and haemoglobinopathy [9-11].

In the present study, incidence of *P. vivax* was more than *P. falciparum* in both the groups which is in concordance with some previous studies as well [6,8]. However, some of the studies reported higher incidence of *P. falciparum* which could be due to different demographic and geographic profile [11-13].

In the initially diagnosed cases, majority had anaemia (96.7%), although it was mild in *P. vivax* (69.3%) and moderate to severe in *P. falciparum* (60%). Similar results have been observed in previous studies which reported presence of anaemia in 81.2%-94.4% cases [14-16, 22]. In contrast, a study conducted by Richard MW et al., in London, observed anaemia in only 15% [23]. There is a wide variation in anaemia due to malaria infection depending upon the geographical location of the study. In developing countries, majority of the population have iron and folate deficiency due to inadequate dietary intake along with other bacterial and parasitic infections which could also contribute to anaemia.

Changes in WBC are less definite in malaria and there was a wide variation seen amongst various studies. Majority of the initially diagnosed cases in the present study had normal leucocyte count which correlated well with other studies [11,15,17]. Amongst those with abnormal count, it was observed that leucopenia was more common in *P. vivax* (25.7%) whereas in *P. falciparum* infection, there were more cases of leucocytosis (30%). The results were in concordance with study done by Abro AH et al., which reported that leucocytosis was more common in *P. falciparum* infection [11]. Akhtar S et al., in their study reported that 11.11% of *P. vivax* cases had leucopenia as compared to 7.69% cases of *P. falciparum* infection though cases with leucocytosis were almost similar in both the infections [15].

A study by Maina RN et al., concluded that thrombocytopenia as the strongest predictor of malaria [24]. In another study, low platelet counts were more consistently associated with *P. falciparum* infection rather than *P. vivax* [25]. But in our study, thrombocytopenia was present in all the cases of malaria in the initially diagnosed group irrespective of the species of *Plasmodium*. However, the incidence of thrombocytopenia was higher (100%) as compared to previous studies [15,22,25]. In the present study, severe thrombocytopenia was a rare finding in *P. vivax* infection (2.4%) similar to the study conducted by Robinson P et al., [26].

Khatib Y et al., reported 95.41% cases of *P vivax* and 81.57% of *P falciparum* having PI <5 [27]. In the present study, 65.6% cases of *P. vivax* and 35% cases of *P. falciparum* had PI <5 though, majority (≅85%) had P.I < 10.

In the present study it was observed that anaemia and thrombocytopenia were not a consistent finding in the relapse group. Normal Hb levels were reported in 52.6% of *P. vivax* and 42.8% of *P. falciparum* cases and normal platelet counts were observed in 14% of *P. vivax* and 28.5% of *P. falciparum* cases. The possible explanation is that the patients in relapse group have already taken treatment within 15 days for malaria; this may have contributed to normal Hb and platelet counts.

Thus, patients having prior history of malaria even after taking treatment presented again with fever should be investigated keeping malaria also as a possible diagnosis in mind. Malaria antigen test should be performed in cases of suspicion of malaria and peripheral smear should be thoroughly inspected for malarial parasite.

Further, on analysing the influence of parasitic densities on severity of anaemia and thrombocytopenia in both the groups, it was observed that there was not much relation between PI and severity of anaemia as there are numerous factors affecting anaemia in developing country like ours. However, thrombocytopenia is strongly related to PI.

LIMITATION

The present study was a single centre study with a relatively small sample size, which might limit statistical relevance. Further studies with a longer duration of follow-up and larger sample size may give more clinically relevant results.

CONCLUSION

Anaemia is a common finding in malaria, although in developing country like ours, anaemia can be multifactorial and is not much related to the parasitic densities. Thrombocytopenia is a consistent finding in initially diagnosed cases of malaria but cases of relapse/ recurrence may present with normal platelet counts. Severity of thrombocytopenia is definitely related to PI. For low PI, severity of thrombocytopenia increases in initially diagnosed cases but is variable with cases of relapse/recurrence of malaria; however with high PI thrombocytopenia is more marked in initially diagnosed cases of malaria. Therefore, a close watch should be maintained on the platelet count in all cases of malaria to prevent and manage haemorrhagic episodes.

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