Original Article

Thrombocytopenia and Malaria: A Coincidental Co-Existence or A Significant Association? An Analysis

Zeeba Jairajpuri, Safia Rana, Salamah Jaseem, Sujata Jetley

Department of Pathology, Hamdard Institute of Medical Sciences and Research, New Delhi, India

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ABSTRACT

Background: Malaria is a major health problem and a cause of significant morbidity and mortality worldwide especially in the tropics. Anemia, thrombocytopenia, atypical lymphocytosis and infrequently disseminated intravascular coagulation. Leucopenia, leucocytosis, eosinophilia and monocytosis have also been reported.

Methods: The aim of the present study was to detect if an haematological index like thrombocytopenia is a significant indicator of malaria and whether its presence increases the probability of malaria in patients suspicious of suffering from this disease. Clinically suspected cases of malaria with febrile illness presenting at Hakeem Abdul Hameed Centenary, Hospital were included in the study and evaluated.

Results: Among 1248 patients with clinical suspicion of malaria, 230(18%) patients were confirmed by the demonstration of malarial parasite on peripheral blood film mostly in the months of August and September, monsoon season. A male predominance, with a male to female ratio of 2.3:1 was seen. 212 patients out of 230 had accompanying thrombocytopenia. The sensitivity and specificity of this was 92.1% and 88.2% respectively with a disease prevalence of 19%. The positive and negative predictive values were 63.8% and 98.1%with a positive likelihood ratio (LR+) of 7.82 and negative likelihood ratio(LR-) of 0.09.

Conclusions: The malaria-endemic regions of the world are mostly developing countries with limited resources and trained health personnel. The hematological aspects may be used in addition to the clinical assessment, to heighten the suspicion of this disease. Thrombocytopenia is associated with both P. falciparum and P. vivaxinfections. Significant association between malaria and thrombocytopenia has been demonstrated in our study and we suggest malaria should be a consideration in all patients with fever and low platelet counts.

***Corresponding author:**
Dr Sujata Jetley, Professor & HOD, Department of Pathology, Hamdard Institute of Medical Sciences and Research, Jamia Hamdard, New Delhi 110062. India

Introduction

Malaria is a major health problem in the tropics and continues to be a great health problem in some of the most populated countries of the world, posing a burden on the economy of developing countries. Despite advances in knowledge, it is a cause of significant morbidity and mortality worldwide. More so it is one of the most prevailing human infections in the world. Association of malaria with various degrees of hematological complications is a common finding ashematological system is one of the main targets of malaria, hematological abnormalities that have been reported to be consistently associated are anemia, thrombocytopenia, atypical lymphocytosis and infrequently disseminated intravascular coagulation.[1] Leucopenia, leucocytosis, eosinophilia andmonocytosishave also been reported.[2] Thrombocytopenia is reported in up to 70% and severe anemia in 25% of malaria patients whereas leukocyte count may be normal or low; leukocytosis is seen in less than 5% of cases and is a poor prognostic factor.[3]

Thrombocytopenia is a common feature of malaria and occurs in both P. falciparum and P. vivaxinfections regardless of the severity of infection. A low platelet count as assessed on peripheral smear. The absence of the normal quantity of platelets on a peripheral smear] in a case of fever is often a clue to the presence of malaria.[4]The exact mechanisms of thrombocytopenia in malaria is uncertain. Immune-mediated lysis, sequestration in the spleen and a dyspoietic process in the marrow with diminished platelet production have all been postulated.[3]The aim of the present study is to detect if an haematological index like thrombocytopenia is a significant indicator of malaria and whether its presence increases the probability of malaria in patients suspicious of suffering from this disease.

Material and Methods

The present study was an observational study, conducted from January 2012 to September 2013. All clinically suspected cases of malaria with febrile illness presenting at Hakeem Abdul Hameed Centenary, Hospital were included in the study and evaluated. Patients of all ages were included.Patients were excluded on the basis of history and findings suggestive of Dengue, chronic liver disease, bleeding disorder, thrombocytopenia, drug intake or conditions which might have contributed in blood changes. Acomplete blood count (CBC) was done in a three part differential counter (KX-21) and routine hematological parameters includingtotal white blood cell count (TWBC), differential WBC count percentages for neutrophils, basophils, eosinophils, and monocytes, Hemoglobin level (Hb), red blood cell count (RBC), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW) and platelet count (PCT)were obtained.

The diagnosis of malaria was confirmed by thin and thick blood films stained with Leishman's stain for malaria parasite. The slides were initially examined by a resident who was blind folded from the CBC results. All malaria-positive smears were reviewed by a hematologist for confirmation, identification of species and review of smear for platelet count. No disagreement was noted between the two evaluators. Smear examination for malarial parasite was taken as gold standard for the diagnosis of malaria.

Thrombocytopenia is defined as a condition where the platelet counts are below the normal range of the population. In the present study a platelet count of less than 150,000 was taken as thrombocytopenia. Grading of thrombocytopenia was also done, platelet counts of 75000 to 150000 were defined as Grade I, 50000 to 75000 as Grade II, 25000 to 50000 as Grade III and below 25000 as Grade IV thrombocytopenia.[5]The counts were visually confirmed by the hematologist after the counts were done by an auto analyzer machine.The slide positivity rate (SPR) was defined as the percent positive cases among the blood smears examined. SPR was calculated for positive cases.

Results

Out of a total of 1248 patients with clinical suspicion of malaria, 230(18%) patients were diagnosed with malaria, confirmed by the demonstration of malarial parasite on peripheral blood film. Majority of the patients were enrolled in the months of August and September in the monsoon season. A male predominance, with a male to female ratio of 2.3:1 was seen, males were 159(69%) and females 71(31%) in number**. (Figure 1)**

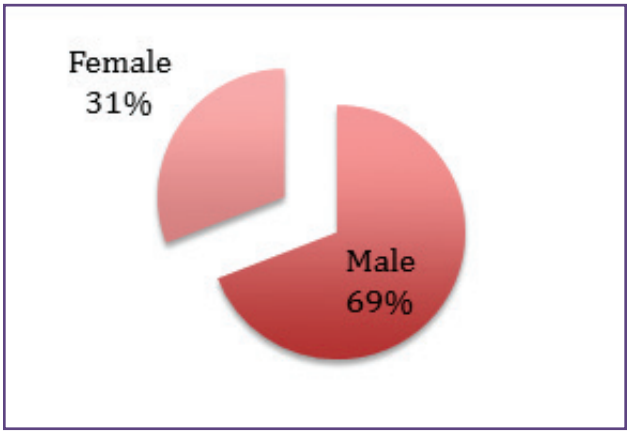

Fig. 1: Pie chart representing distribution of cases according to sex.

The mean age of the patients was 28.9 years with ranging from 11months to 78years. The maximum number of patients 88(38.2%) were between 21- 30 years of age followed by 46(20%) patients in the age range of 31-40years. However, only 6(2.6%) patients each were seen in the age groups 61-70 years and 71-80 years. **(Table 1)**

Table 1: Age-Wise Distribution Of Cases Of Malaria.

AGE GROUP (YRS)	NO.OF PATIENTS(%)
0-10	21(9.1%)
11-20	30(13%)
21-30	88(38.2%)
31-40	46(20%)
41-50	26(11.3%)
51-60	7(3%)
61-70	6(2.6%)
71-80	6(2.6%)

Out of the 230 patients, **(Table 2)** 88.7%were found to be infected with Plasmodium vivax, while 3.5% had Plasmodium falciparum. 7.8% patients were found to have a mixed infection with both Plasmodium falciparum andvivax. All the patients presented with fever of less than 1 week, 218 patients presented with the classical symptoms of fever, chills, rigors while the remaining had just fever.

Table 2: Distribution Of Cases According To Species (N=230)

PLASMODIUM SPECIES	FREQUENCY	PERCENTAGE (%)
Plasmodium vivax	204	88.7%
Plasmodium falciparum	08	3.5%
Mixed infection	18	7.8%

Multiple stages of the hemoparasite were seen including schizonts, trophozoites and ring forms, however gametocytes were more often seen in falciparum while trophozoites and ring stages were common in infection with P. vivax.

The age wise data on malaria case detected in the present study along with the slide positivity rate has been depicted in **Table 3**. Out of 230 malaria cases 88 were in the 21-30years age group with SPR of 22% while in the age group 31-40 years it was 35.6%.

Thrombocytopenia (platelet count <150,000 cumm) was a striking feature in majority of the malaria positive cases with 212(92%) cases having platelet <150,000 and only 18(7.8%) patients having platelet counts more

Table 3: Age group wise Malaria incidence

Age Group (yrs)	□/BSE (SPR %)	Pf/Pv/M
0-10	21/259 (8.1)	2/17/2
11-20	30/241 (12.5)	1/26/3
21-30	88/409 (22)	3/77/8
31-40	46/129(35.6)	0/43/3
41-50	26/121 (21.5)	1/24/1
51-60	7/43(16.3)	0/7/0
61-70	6/31(19.3)	1/4/1
71-80	6/15 (40)	0/6/0

BSE=Blood smear examination, □= no.of malaria positive cases, Pf=Plasmodium falciparum, Pv= Plasmodium vivax, SPR=Slide positivity rate, M=Mixed cas

than 150,000/cumm. It was seen in both P.vivax and P falciparum infections. The severity of thrombocytopenia was graded I to IV in the different species in 212 patients with platelets less than 150,000. **(Table 4)** The maximum number of cases 87(37.8%) were grouped in grade III with platelet counts between 25000-50000 while 19.5% (45) of the cases had platelet count <25,000/cumm labeled as grade IV.

Table 4: Severity of Thrombocytopenia In Malaria Cases and their p-Value

PLATELET COUNT	GRADE OF THROMBO-CYTOPENIA	NUMBER.OF PATIENTS(%) N=212			FISHER'S EXACT TEST p-VALUE
		PV	PF	M	
75,000-150000	Grade I	31	1	3	P = 0.115
50,000-75000	Grade II	39	2	4	P<0.001
25000-50000	GradeIII	79	2	6	P<0.001
<25000	Grade IV	37	3	5	P<0.001

Species wise grading of thrombocytopenia showed a maximum of 38.7% cases of P vivax33.3% cases of mixed infection with Grade III while 37.5% cases of P falciparum were of Grade IV. No patient reported with bleeding episodes.

Significance of Grades of thrombocytopenia was tested by Fisher's Exact test and P value calculated for each Grade. The P value was extremely significant for grades II, III and IV while it was not significant for grade I, hence at lower platelet counts a significant correlationwas observed with diagnosis of malaria.

Out of 1248 patients with fever suspicious of malaria, 230 cases were diagnosed as malarialinfection on smear. 212patients out of 230 had accompanying thrombocytopenia. The sensitivity and specificity of

this was 92.1% and 88.2% respectively with a disease prevalence of 19%. The positive and negative predictive values were 63.8% and 98.1%with a positive likelihood ratio (LR+) of 7.82 and negative likelihood ratio(LR-) of 0.09.

We also statistically evaluated the grades of thrombocytopenia depicted in **Table 5**. The specificity of all the grades was high while the sensitivity was low except for Grade III which had a sensitivity of 38%. A low sensitivity indicated a poor ability of the test to detect true positive cases. An increasing trend in predictive values, LR+ along with diagnostic accuracy was noted with increasing grades of thrombocytopenia.

Post test probability of a given test is a very useful guide to the clinician in estimation the probability of the disease in his clinical settings and in decision making with regards to treatment. The positive post test probability (the probability of having the target condition if the test is positive.) was 58.6% ,highest in Grade IV(platelet count <25000/cumm). **Table 6**

Discussion

Malaria continues to be a great health problem in some of the most populated areas of the world, caused by Plasmodium vivaxand falciparum it is endemic in many regions of India also.For malaria patients, a prompt and accurate diagnosis is key to effective disease management. Haematological abnormalities have been observed in patients with malaria and are considered a hallmark withanaemia and thrombocytopenia being the most common.[6]

Most of the patients in the present study were between 21 to 30 years of age with the mean age of 28.9 years in contrast to other studies which reported mean age of 38.7years [7] Information regarding age and gender related prevalence of malaria is scarce but most of the studies reported generally a high burden in males compared to females, [8,9]our study also showed similar results. A male preponderance of 69% and females 31% was noted in the present study whichwas in concordance with a study by Dungal et al.(Males 68%, Females 32%). The males are generally thought to be at a higher risk due to more outdoor activity and their less protection from mosquito bites.

Malaria transmission in India is a daunting epidemiological challenge and its distribution is hetrogenous largely governed by many climatic and physiological risk factors. [10] P. vivax is the major malarial parasite in the subcontinent contributing to majority of the cases.[11] Review of literature shows many studies with P vivax as the dominant species, however percent positivity varied in the different studies(56.5%[12] 69%[13] 51.6%[14]) P vivax was also the commonest species in our study comprising88.7% of the cases although a small number of P falciparum(3.5%) and

Table 5: Sensitivity, Specificity, Predictive values and Likelihood ratio (LR) and Diagnostic Accuracy for diagnosis of malaria in relation to Grade of Thrombocytopenia.

Grade	Senstivity (%) 95%CI	Specificity (%) 95%CI	PPV (%) 95%CI	NPV (%) 95%CI	LR+ 95%CI	LR- 95%CI	Diagnostic accuracy (%)
I	15.91 10.83-20.52	88.2 71.46-76.92	22.58 8.35-16.01	82.92 76.79-82.02	1.35 0.43-0.82	0.95 1.07-1.22	74.7
II	19.6 14.64-25.29	94.1 92.48-95.47	36 27.61-45.07	83.5 81.2-85.6	3.32 2.32-4.75	0.8 0.80-0.91	78.7
III	38 31.54-44.44	93.3 90.31-93.72	52.1 44.24-59.87	86.7 84.61-88.74	4.81 3.68-6.29	0.67 0.61-0.75	83
IV	19.6 14.64-25.29	96.8 95.48-97.76	57.7 45.98-68.80	84.2 81.97-86.23	6.04 3.94-9.24	0.83 0.78-0.89	82.5

Table 6: Post test probabilities of Grades of Thrombocytopenia.

Post test probabilities of positive of positive and negative test results when pretest probability of malaria is 19%				
Grade of thrombocytopenia	Test result positive		Test result negative	
	LR positive	Positive test probability (%)	LR negative	Post test probability (%)
Grade I	1.35	24	0.95	18
Grade II	3.32	43.7	0.80	15.8
GradeIII	4.81	53	0.67	13.6
Grade IV	6.04	58.6	0.83	16.3

mixed infection (7.8%) were also seen. However, a higher prevalence of P falciparum was noted in studies especially those from the North Eastern states of India.[15]

The SPR in the present study was 18.5% which was much lower than 42.8% seen in a study by Rabha B et. al. An age wise distribution of cases alongwith slide positivity rate was also analyzed which showed a SPR of 35.6% in the age group 0f 31-40 years, followed by 22% and 21.5% in the age group 21-30 years and 41-50 years respectively.The SPR in children less than 10 years was 8.1% while a SPR 0f 44.4% was seen in children less than 5 years in the study by Rabha et.al. of tea estates of North Eastern states, although it was not a exact comparison of the study group but it reflects the trends of malaria prevalence in young ages of the two regions of India. Children are considered to be at more risk of malaria infection due less degree of protective immunity, high mobility and outdoor activity along with insufficient clothing. Significant variation of malaria prevalence in different age groups has been reported.[16]

Thrombocytopenia is a common feature of acute malaria and occurs in both P. falciparum and P. vivaxinfections regardless of the severity of infection.[4] The absence of the normal quantity of platelets on a peripheral smear in a case of fever is often a clue to the presence of malaria.[4]It is so characteristic of malaria that in some places, it is used as an indicator of malaria in patients presenting with pyrexia of unknown origin.[1]92.1% of the malaria positive cases in the present series showed thrombocytopenia (Platelet count <150,000/cumm) which was much higher than other reported studies. 53%of patients with malaria showed thrombocytopenia in a study[17]which was close to others reporting low platelets in 57%[18]and 48%[19]of the patients.

Various observational studies have confirmed the association of thrombocytopenia and malaria but the exact cause of thrombocytopenia is still elusive. The suggested mechanisms leading to thrombocytopenia are disturbances in coagulation profiles, splenomegaly, bone marrow alterations, antibody-mediated platelet destruction, oxidative stress, and the role of platelets as cofactors in triggering severe malaria [20-22] A good tolerance to low platelet count has been observed in malaria and was seen in our study too.[9] A lowest platelet count of 18000/ cumm was seen in two of our patients without evidence of bleeding. In most of the studies including the present study,thrombocytopenia has not been associated with any considerable morbidity or bleeding complications.[9]Platelet activation and enhanced aggregability appears to explain tolerance to low platelet counts.[23]Enhaced hemostatic responses due to hyperactive platelets is thought to be the reason behind lesser bleeding episodes in acute malarial infections despite significant thrombocytopenia. [24]

The sensitivity of the platelet count was considered as a predictor of malaria was 80.11% while specificity was 81.36%[17] this was concordant with our finding of 92.1 and 88.2% respectively. However, in an other study by Patel et al[25] the sensitivity of thrombocytopenia together with the acute febrile syndrome was reported as 100% for malaria diagnosis, with a specificity of 70%. Another study has reported 60% sensitivity and 88% specificity of thrombocytopenia for malaria diagnosis in acute febrile patients.[7]A positive predictive value of 63.87% and a negative predictive value of 90.86% was observed in a study [17]while in another it was 75% and 21% [7],86% and 100% [25] however in our study it was 63.8% and 98.1 % respectively. Although, the PPV were similar in various studies, a wide variation was observed in the NPV. A high LR+ of 7.82 in the present series correlated well with 5.04 observed by Lathia et.al. It is important to mention here that the sensitivity and specificity of a test cannot be used to estimate probability of disease in individual patients**.** **[26]**They can, however, be combined into a single measure called the likelihood ratio which is, clinically, more useful than sensitivity or specificity**.** **[26]**Likelihood ratios are a more practical method of interpreting diagnostic test results and are of immediate clinical relevance. A diagnostic test is considered useful if it provide a high LR+ and a small LR- as seen in the present study. Unlike sensitivity and specificity, which are population characteristics, likelihood ratios can be used at the individual patient level.**[27]**

Grading of thrombocytopenia based on the levels of platelet count was done in the present study similar to other studies. [17,25,28]In the present study the largest group was Group III comprising 37.8% of the cases while the maximum no of patients, 21%were in Grade I group in a study by Khan et.al..In our study we have calculated the sensitivity, specificity, positive and negative predictive value and diagnostic accuracy at different levels of platelet counts and have concluded that the specificity and NPV was high at low platelet counts (Table 5) This was discordant with observations made by Khan et al.[17]However diagnostic accuracy remained almost the same at various counts (around 65%).inthe study by Khan et.al although it was higher for lower platelet counts in our observations.

Pre-test probability (prevalence of disease) in conjunction with likelihood ratios of disease can be used to estimate an individual's post-test probability of disease, that is his or her chance of having disease once the result of a test is known.[26] It may be positive or negative. A positive test may

increase the pre-test probability and a negative test may reduce the pre-test probability.[26]We analyzed the post test probabilities of the different grades of thrombocytopenia in conjunction with their LRs (Table 6), it was noted that positive post test probabilities increased with higher grades i.e at lower platelet counts. We could not find any study to the best of our knowledge that had analyzed the grades of thrombocytopenia. The results of clinical tests are usually used not to categorically make or exclude a diagnosis but to modify the pre-test probability in order to generate the post-test probability. [26]

Conclusions

The malaria-endemic regions of the world are mostly developing countries with limited resources and trained health personnel. Diagnostic parameters in conjunction with clinical suspicion are a realistic option in the clinical diagnosis of malaria. The hematological aspects of malarial infection constitute a very interesting area in various reports and may be used in addition to the clinical assessment, to heighten the suspicion of this disease.Thrombocytopenia constitutes a classical change, its presence in patients who present with acute febrile illness in the tropics, increases the probability of malaria. Thrombocytopenia is associated with both P. falciparum and P. vivaxinfections. Significant association between malaria and thrombocytopenia has been demonstrated in our study and we suggest malaria should be a consideration in all patients with fever and low platelet counts.

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