ORIGINAL ARTICLE

Thrombocytopenia in Malaria: Can Platelet Counts Differentiate Malaria from Other Infections?

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Abstract

Objective: To determine the accuracy of thrombocytopenia as a diagnostic marker for malaria.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Medicine, 1 Mountain Medical Battalion (Bagh, Azad Kashmir) from July to September 2013.
Methodology: Adult patients presenting with a short history of fever without any localizing symptoms or signs were included. Exclusion criteria included patients with fever of > 7 days duration, those in whom an underlying diagnosis could be easily confirmed on the basis of history and physical examination, those on antibiotics/ antimalarials or antiplatelet agents and patients with Dengue fever. Platelet counts in venous whole blood samples were analysed with Sysmex KX-21 Haematology analyzer. Thick and thin peripheral blood smears were then prepared and examined for malarial parasites. Diagnosis of malaria was established on the basis of smear findings.
Results: There were 245 patients in total. Out of the 109 patients with thrombocytopenia, 61 had *vivax* malaria. Platelets count was normal in 136 patients, including 4 with *vivax* malaria. *Falciparum malaria* was not seen in any patient. All cases with malaria were uncomplicated. Various measures of accuracy thus calculated were sensitivity 93.85%, specificity 73.33%, positive predictive value 55.96%, negative predictive value 97.06%, positive likelihood ratio of 3.52, negative likelihood ratio of 0.08, diagnostic odds ratio 41.94 and diagnostic accuracy of 78.78%.
Conclusion: Thrombocytopenia has an excellent sensitivity and a very good specificity for *vivax* malaria. Normal platelet counts provide very strong evidence against malaria as the etiology of fever without a focus.

Key Words: *Malaria. Thrombocytopenia. Plasmodium vivax.*

INTRODUCTION

Malaria affects 225 million people every year, 40% of whom reside in South-East Asia.[1] The disease is frequently encountered in tropical countries like Pakistan. As reported earlier, the vast majority of cases seen at local setup are caused by *Plasmodium vivax*.[2] The initial presentation of the disease is frequently very non-specific and bears strong resemblance with many viral infections. Though *vivax* malaria is far less deadly as compared to *Falciparum malaria*, disease related complications are being heard of more often in the recent years.[3] This emphasizes the importance of early diagnosis and treatment to reduce the sufferings of patients. The gold standard for diagnosis of malaria is peripheral blood smear examination for parasites. This technique, though easily available, takes time and is operator dependent.[4] Another diagnostic modality, therefore, needs to be identified that can provide an objective evidence of on-going malaria infection in febrile patients quickly and confidently.

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Hematological blood analysers are now being used routinely for presumptive diagnosis of malarial infection. A number of parameters have been studied in the past. Briggs *et al*. explored the use of standard deviation volume of lymphocytes and monocytes for detecting the presence of malarial parasites.[5] Similarly, the roles of pseudoeosinophilia and white blood cell differential scatterplot abnormalities have also been evaluated.[6,7] However, all of these techniques have inherent limitations especially in terms of a relatively low sensitivity. A trained haematologist is also required for proper interpretation but may not be available at smaller healthcare setups like the one where this study has been carried out.

Thrombocytopenia is a hallmark of malaria, with enough evidence to suggest that it is commoner in *vivax* malaria as compared to *Falciparum malaria*.[8] A previous observational study found out that thrombocytopenia was seen in more than 90% of patients with *vivax* malaria.[2] The mechanism is not fully known but it is believed that the *Plasmodium vivax* has a direct lytic effect on platelets, which may be mediated both immunologically and non-immunologically.[9] Since there is no reduction in megakaryocytes in bone marrows of the affected patients, direct bone marrow suppression is not to be blamed as was thought previously.[10] Fortunately, despite the marked reduction in platelet counts, bleeding complications are infrequent because

of enhanced platelet activation and their ability to aggregate.

Analysis of platelet counts using an automated analyzer takes much less time than examining a smear and the technique does not require significant expertise. This study was, therefore, designed to determine the role and utility of thrombocytopenia as a diagnostic marker for malaria in an endemic region.

METHODOLOGY

This cross-sectional study was carried out at the Department of Medicine, 1 Mountain Medical Battalion (Bagh, Azad Kashmir), from July to September 2013, coinciding with the maximum incidence of malaria in the region. Study was approved by the ethics review committee of the hospital and patients were enrolled after they provided informed written consent. A minimum sample size of 243 was calculated assuming that thrombocytopenia has a sensitivity of 95% (SN=0.95) with a confidence interval of 5% (W=0.05) to diagnose malaria and that 30% patients in the study population would be having malaria (p=0.3). Following formula was used for sample calculation: n = (TP+FP)/P; where: TP+FP = z^2 x SN (1-SN) / W^2

Adult patients presenting in outdoor clinic with a short history of fever (< 7 days) without any localizing symptoms or signs were included. Exclusion criteria included patients with fever of > 7 days duration, those in whom an underlying diagnosis could be easily confirmed on the basis of history and physical examination, those already started on antibiotics/ antimalarials, patients with dengue fever, those using antiplatelet drugs and unwilling patients.

A detailed history was taken and physical examination done to look for a possible cause of fever. Venous whole blood samples (2.5 ml) were collected in EDTA bottles and analysed with Sysmex KX-21 Haematology analyzer to determine the complete blood counts and platelet counts. Thrombocytopenia was defined as platelet counts < 150,000/µl. Giemsa stained thick and thin peripheral blood smears were then prepared from the same sample by another experienced laboratory technician (who was blinded to the results of blood counts) and examined for malarial parasites (rings and trophozoites). Diagnosis of malaria was established on the basis of smear findings alone. Presence of platelets clumps was also ruled out while examining the smears. Since this study involved outdoor patients, smears were made from a single sample only and repeat testing was not done in patients with smears negative for malarial parasites.

Stata version 12 and MedCalc software (version 12.7.7) were used for statistical analysis. Different quantitative variables were described as mean ± standard deviation and compared amongst patients with thrombocytopenia and those without thrombocytopenia using independent samples t-test. Thrombocytopenia was classified into three classes depending on the platelet counts: mild (100,000 - 149,000/µl), moderate (50,000 - 99,000/µl) and severe (< 50,000/µl). Number of patients falling into these categories was calculated both for those having malaria and those not having malaria. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of thrombocytopenia for the diagnosis of malaria were calculated using standard formulas applied on a 2 x 2 table.

RESULTS

This study was carried out on 245 patients (156 males and 89 females), whose clinical and laboratory characteristics are depicted in Table I. Thrombocytopenia was present in 109 patients, the severity of which is shown in Figure 1. Chi-square test to compare this distribution amongst patients with and without malaria yielded a significant difference (p=0.004). Patients with thrombocytopenia were younger than those without thrombocytopenia, but the duration of fever and the haematological parameters including haemoglobin, TLC and platelets were equal in both the groups. All of the 65 patients diagnosed to have malaria on the basis of peripheral blood smear examination had *vivax* malaria. *Falciparum malaria* was not seen. Moreover, none of the patients with malaria had any feature of severe disease. Out of 65 patients with

Table I: Characteristics of the study population.

	Total (n=245)	Patients with thrombocytopenia (n=109)	Patients without thrombocytopenia (n=136)	p-value
Age (years)	30.33± 10.86	28.88± 8.30	31.49± 12.45	0.062@
Duration of fever (days)	2.40 ± 1.33	2.61 ± 1.43	2.24 ± 1.23	0.033#
Haemoglobin (g/dl)	13.43 ± 1.75	13.99 ± 1.41	12.97 ± 1.87	< 0.001#
TLC ($X10^9$/l)	8.13 ± 4.02	6.85 ± 3.28	9.16 ± 4.27	<0.001#
Platelets ($X10^9$/l)	182.98 ± 100.80	101.59 ± 31.65	248.21 ± 89.04	<0.001#

TLC = Total leucocyte count. # Significant. @Not significant

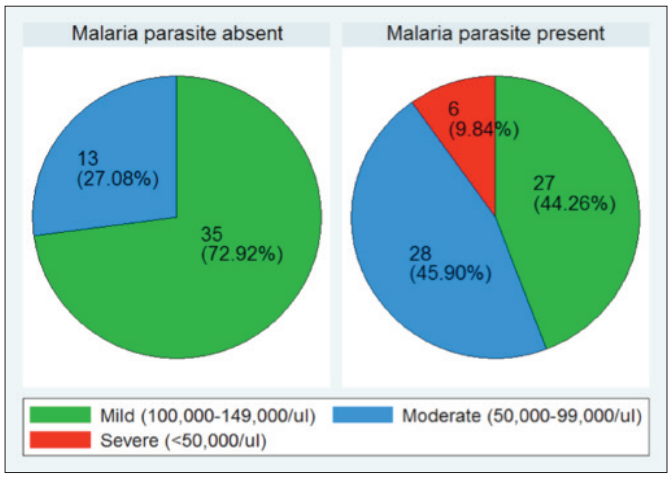

Figure 1: Degree of thrombocytopenia in study population.

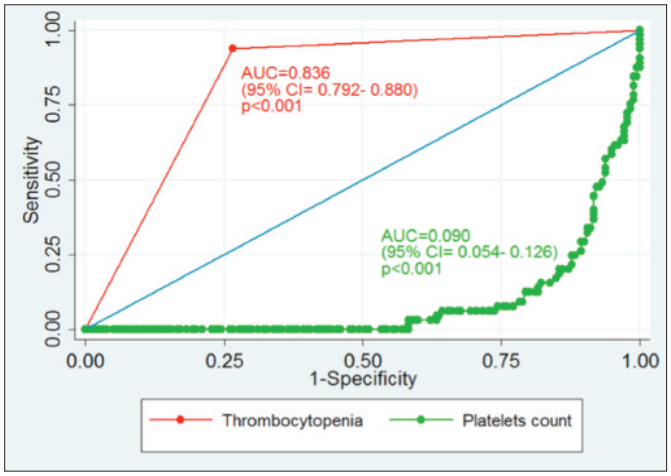

Figure 2: ROC curves for thrombocytopenia and platelet counts. (AUC = Area under curve; CI = Confidence interval)

positive Malaria Parasite (MP), 61 had thrombocytopenia. Out of the 180 patients with negative MP, 132 had normal platelet counts. The diagnostic accuracy thus obtained from these figures was sensitivity of 93.85% (95% CI = 84.97 - 98.26%), specificity of 73.33% (95% CI = 66.24 - 79.64%), positive predictive value of 55.96% (95% CI = 46.13 - 65.46%) and negative predictive value of 97.06% (95% CI = 92.63 - 99.18%). Positive likelihood ratio was 3.52 (95% CI = 2.74 - 4.52) whereas the negative likelihood ratio was 0.08 (95% CI = 0.03 - 0.22). Diagnostic odds ratio was 41.94 (95% CI = 14.47 - 121.56) whereas, the diagnostic accuracy was 78.78% (95% CI = 74.22 - 80.97). ROC curve analysis for thrombocytopenia showed an area under curve of 0.836 as shown in Figure 2.

DISCUSSION

Thrombocytopenia has been evaluated as a marker for malaria in the past as well. For example, a study done on Americans travelling back from endemic areas found a sensitivity of platelet count for diagnosing malaria to be 100%, specificity 70%, positive predictive value 86% and the negative predictive value 100%.[11] These values are very close to those found in this study. Mahmood *et al*. found results comparable to ours in Liberian patients (sensitivity 80.11%, specificity 81.36%, positive predictive value 63.87% and negative predictive value 90.86%).[12] However, they encountered *Falciparum malaria* which is endemic in that region. A study done in Karachi found that thrombocytopenia did not have a good sensitivity or specificity for the diagnosis of malaria.[13] One reason could be the fact that various statistical measures of diagnostic accuracy vary with the degree of thrombocytopenia, as has been observed previously.[14] Another explanation for this gross difference in results of this study is that the authors assessed patients with platelets below 50,000/µl only, whereas in this study, all patients with platelets count less than 150,000/µl have been labeled as having thrombocytopenia. Platelets count below 150,000/µl have been used to define thrombocytopenia in many other studies in the past as well.[14-16] Lathia *et al*. conducted a similar study in India nearly 10 years ago.[17] The striking difference in their results was a much lower sensitivity (60%) and negative predictive value (21%). The authors themselves stated in discussion that their study may be limited by selection biases related to a possible inclusion of a greater proportion of thrombocytopenic patients. ROC curve analysis revealed an area under curve greater than 0.8 meaning that thrombocytopenia is a good discriminatory test for the presence or absence of malaria.

Thrombocytopenia had an excellent negative predictive value for *vivax* malaria in this study. This figure, unlike sensitivity and specificity, is dependent on the prevalence of malaria in the particular population studied.[18] The prevalence was obviously higher considering the time frame during which this study was carried out. Caution must be exercised when applying the negative predictive value to other patient populations, especially ones in non-endemic regions and at other times of the year. Similarly, the negative likelihood ratio was very low thus proving that a normal platelets count is a very strong indicator against the presence of malaria.

In addition to providing a clue from diagnostic point of view, the degree of thrombocytopenia may be related to parasite load and severity of the disease. Erhart *et al*. demonstrated a correlation between the level of parasitemia and degree of thrombocytopenia in Thai patients with *vivax* malaria.[19] A significant negative correlation between parasite density and platelet counts was also shown in Brazilian patients with *vivax* malaria.[20] Results opposite to this were obtained in a study on 546 patients with *vivax* mono-infection that failed to show a statistically significant correlation between parasite load and platelet counts.[21] Similarly, Muley *et al*. have proved that thrombocytopenia is associated with more severe clinical manifestations of *vivax* malaria infection.[22] However, such association could not be evaluated during this study because none of the 65 patients had any features suggestive of severe malaria. The failure to methodically look into other conditions causing thrombocytopenia is another limitation of this study.

The results of this study have strong implications considering the fact that in the resource-limited settings, antimalarials are often given on clinical suspicion alone.[23] This practice can undoubtedly lead to emergence of resistant strains of the parasite. It is suggested that anti- malarial treatment should be started empirically in patients presenting with short febrile illness and thrombocytopenia; a strong emphasis should also be laid on meticulous peripheral blood smear

examination to confirm the diagnosis in such cases. However, in patients with normal platelet counts, treatment may be withheld pending the results of blood smear findings.

CONCLUSION

Thrombocytopenia has an excellent sensitivity and a very good specificity for *vivax* malaria. More importantly, the presence of a normal platelets count effectively rules out malaria considering the extremely high negative predictive value of thrombocytopenia.

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