Can Thrombocytopenia be Used to Make a Presumptive Diagnosis of Malaria in Patients with Acute Febrile Illness

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ABSTRACT

Objective: To ascertain the diagnostic accuracy of thrombocytopenia in making a presumptive diagnosis of malaria in patients with acute febrile illness, keeping thick and thin films as the gold standard.

Study Design: Cross-sectional study.

Place and Duration of Study: Pak Emirates Military Hospital Rawalpindi Pakistan, from May to Oct 2017.

Methodology: A total of 145 patients meeting the inclusion criteria i.e., patients with fever of 10 or less days, age of ≥12 and ≤65 years of either sex were included in the study. Sample for a complete blood picture was taken in Ethylenediaminetetra-acetic acid (EDTA) vacutainer. Simultaneously, two slides each with a thick and thin blood smears were made to ascertain absence or presence of plasmodium vivax species (smear positive and smear negative). The diagnostic accuracy was measured using two by two table and applying formulae for specificity, sensitivity, negative and positive predictive value. Likelihood ratio and ROC (Receiver operator curve) was also calculated.

Results: Out of 145 subjects, ninety-four patients (64.8%) were males and 51 were (35.2%) females with mean age of 36.76 ± 14.749 years. The specificity, sensitivity, negative and positive predictive value and diagnostic accuracy of thrombocytopenia in making a presumptive diagnosis of malaria in patients having short history of fever, keeping thick and thin films as the gold standard was 68.8%, 81.4%, 64.7%, 84.0% and 77.2% respectively.

Conclusion: Hematological abnormalities are encountered in malaria. Thrombocytopenia has a very good sensitivity and good specificity for malaria.

Keywords: Acute febrile illness, Malaria, Thick and thin films, Thrombocytopenia.

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INTRODUCTION

Malaria is caused by infection with *Plasmodium parasites* and is a growing health issue throughout the world. About 214 million malaria cases and 438000 malaria related mortalities worldwide were reported in 2015 World Malaria Report. Plasmodium species causing malaria in humans are (*p.malariae, p.ovale, p. vivax* and *p.falciparum*). Infection with *P.falciparum* is associated with considerable morbidity and mortality.[1] Southeast Asian region houses about 40% of world's population at risk of malaria.[2] Although Vivax malaria is relatively benign as compared to Falciparum malaria, disease related complications are being reported more often nowadays.[3] In Pakistan, *P.vivax* infection is mainly responsible for causing malaria, however *P.falciparum* and mixed infections also occur.[4] The prevalence of malaria has been reported to be as high as 30% in South Waziristan.[5] Clinical diagnosis of malaria can be a challenge since it can present with a wide spectrum of symptoms and signs showing considerable overlap with other febrile illnesses like viral fevers e.g., Dengue fever, enteric fever, leptospirosis etc. Clinical features alone cannot predict a diagnosis of malaria or differentiate between the infecting species.[6] Patients having a short history of intermittent fever with chills are irrationally prescribed anti-malarial without making a microscopic diagnosis.[7] As per the recommendations of World Health Organization, all suspected cases of malaria must have a confirmatory parasitological diagnosis.[8] "Gold standard" for diagnosing malaria involves microscopic detection of plasmodium species using Giemsa stained thick and thin blood smear slides.[9,10] Hematological abnormalities like anemia, thrombocytopenia, leukopenia etc, are a hallmark of malarial infection and are the most common complications encountered in malaria. Thrombocytopenia has been suggested to be highly sensitive and specific for diagnosing malaria, sensitivity of 93.85% and specificity of 73.33%. Despite the recommendations, reliable

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laboratory facilities/qualified pathologists are not available at many setups in our country. Considering the resource limitations, it is hypothesized in local studies that thrombocytopenia could potentially be used as a surrogate marker for malaria.[10] This study has therefore been planned to ascertain the diagnostic accuracy of thrombocytopenia in making a diagnosis of malaria in patients reporting with short history of fever, keeping thick and thin films as the gold standard.

METHODOLOGY

This observational cross-sectional study was carried from May to October 2017 at Pak Emirates Military Hospital Rawalpindi Pakistan. Sample size calculated by WHO calculator was found to be 145, considering the prevalence of vivax malaria in our population as 10%.[4] Following formula was used for sample calculation: n =(TP+FP)/P; where: TP+FP=z2 x SN (1-SN)/W2, where SN, sensitivity of thrombocytopenia=95%, W, confidence interval=5%, z=1.96.[12] Sampling technique used was non-probability sampling.

Inclusion Criteria: All the patients og age ≥12 years and ≤65 years of either gender, with any grade of fever of 10 or less days were included in the study.

Exclusion Criteria: Patients, fever with an obvious cause established on history, examination or laboratory investigations (e.g. chest X-ray, Urine RE, Stool RE, Blood Cultures, LFTs etc.), patients who were on antibiotic or anti-malarial therapy, any patient requiring management in Intensive Care Unit, any patient who was taking anti-platelet drugs, known case of Idiopathic Thrombocytopenia or low platelet count secondary to other illness e.g. Decompensated chronic liver disease (DCLD), Hypersplenism etc., and patients who did not give consent were excluded.

For collection of data, first the approval was duly taken from the Ethical Committee, Pak Emirates Military Hospital Rawalpindi. Priorin formed consent was sought from the participants. All confounding variables were identified. Their exclusion was done in accordance with the exclusion criteria. Patients reporting with fever were admitted to Inpatient facility from Medical OPDs and ER. Detailed history was taken followed by a thorough general physical and systemic examination. Patients who were afebrile at the time of admission were monitored for development of fever. Blood sampling was done when patient was febrile and prior to initiation of antibiotics and anti-malarial therapy. Smear negative patients had their smears repeated after every 08 hours. About 3ml of venous blood sample was taken from the patients in EDTA vessels and was analyzed using Sysmex KX-21 Hematology analyzer. Quality control was ensured by making daily checks as per the manufacturer's instructions by a pathologist. Hematological indices including Hemoglobin (Hb), red blood cell count (RBC), hematocrit (Hct), Mean Cell Volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count (PC), total leukocyte count (TLC), and differential leukocyte count which included percentages for neutrophils, basophils, eosinophils and monocytes.[13] Blood smear thick and thin slides, stained with Giemsa, and were prepared simultaneously for microscopy by a hematologist. Smear positive and smear negative for vivax speciescases along with presence or absence of thrombocytopenia were then determined. Smear Positive were defined as identification of malarial parasites in any of the three samples sent at the time of admission or 8 hourly there after whereas, Smear Negative was defined as those not having malarial parasites detectable on either of three samples. Thrombocytopenia was considered as a platelet count <150 x 10^9/L.

Data were entered in IBM SPSS Statistics 24 software for analysis. Age and platelet count, duration of illness was expressed as mean ± SD. Frequency of thrombocytopenia in age, gender and area was calculated for smear positive and smear negative patients for vivax malaria. The diagnostic accuracy was measured using two by two table and applying formulae for sensitivity, specificity, positive and negative predictive value. Likelihood ratio and ROC (Receiver operator curve) was also calculated. Stratification was used to curtail effect modifiers in the current study. After the process of stratification, the diagnostic accuracy was measured.

RESULTS

An aggregate of 145 patients with short history of fever were selected to conduct this study. The mean age was 36.76 ± 14.7 years. Ninety-four patients (64.8%) were males and 51 patients (35.2%) were females as shown in Table-I. The mean duration of fever was 5.63 ± 2.3 days.

Table-I: Baseline characteristics of study population.

Parameters	n(%)
Male	94 (64.8 %)
Female	51 (53.2%)
Age	36.7 ± 14.749 years
Fever duration	5.63 ± 2.366 days

The area of current employment was Rawalpindi in 42 patients (29.0%), Islamabad in 32 (22.1%), FATA in 33 (22.8%) and Peshawar in 38 patients (26.2%). Chi-square test to compare this distribution amongst patients with and without malaria yielded a significant difference (p=0.001).

Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of thrombocytopenia in making a presumptive diagnosis of malaria in patients with short history of febrile illness, keeping thick and thin films as the gold standard found in our study was 81.4%, 68.8%, 84.0%, 64.7% and 77.2% respectively as shown in Table-II.

Table-II: Diagnostic accuracy table

Test	Smear Positive	Smear Negative	Total
Thrombocytopenia (+)	33	18	51
Thrombocytopenia (-)	15	79	94
Total	48	97	145

Positive likelihood ratio was 2.53, whereas the negative likelihood ratio was 0.27 as shown in Table-III.

Table-III: Diagnostic parameters of thrombocytopenia keeping smear positive (Thin & Thick Film) as gold standard in making persumptive diagnosis of malaria in patients with acute febrile illness.

Sensitivity	81.4%
Specificity	68.8%
Postive Predictive Value	84.0%
Negative Predictive Value	64.7%
Diagnostic Accuracy	77.2%
Postive Liklihood Ratio	2.53
Negative Liklihood Ratio	0.27

ROC curve analysis for thrombocytopenia showed an area under curve of 0.836 as shown in Figure.

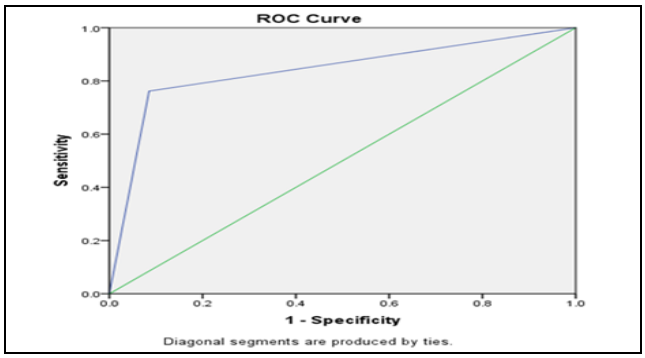

Figure: ROC curves for thrombocytopenia and smear positive results. (AUC = Area under curve = 0.83).

DISCUSSION

Although hematological changes are well-recognized with malarial infection, background haemoglobinopathy, nutritional status, demographic factors and malarial immunity plays a major role in specific changes in that geographical region. These parameters are well studied in *P.falciparum* infection, but now recent studies have indicated that these changes do occur in *P.vivax* infection also.[14,15]

Thrombocytopenia has been evaluated as a marker for malaria in the past as well. A study done in India at Hakeem Abdul Hameed Centenary Hospital, found a sensitivity of platelet count for diagnosing malaria to be 92.1%, specificity 88.2%, positive predictive value 63.8% and the negative predictive value 98.1%.[14] 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%).[15] However, they encountered Falciparum malaria which is endemic in that region. Few studies done in previous years showed that thrombocytopenia did not have a good sensitivity and specificity for diagnosing malaria. One reason could be the fact that variousstatistical measures of diagnostic accuracy vary with thedegree of thrombocytopenia, as has been observedpreviously. Another explanation for this grossdifference in results of these studies is that the authors assessed patients with platelets below 50,000/µl only, whereas in this study, all patients with platelets countless than 150,000/µl have been labeled as having thrombocytopenia. Platelets count below 150,000/µl have been used to define thrombocytopenia in manyother studies in the past as well.[16-18]

The major hematological change seen in the present study was thrombocytopenia which was seen in 97.5% patients. Only 2 (2.5%) patients had normal platelet count at presentation. Severe thrombocytopenia (counts <20 $x10^9$/l) was seen only in one patient, while in majority of patients platelet count was between 50 x 10^9/l to 100 $x10^9$/l. The pathogenesis of thrombocytopenia consists of a myriad of pathogenic mechanisms. Possible mechanism of thrombocytopenia can be either platelet sequestration by spleen under the influence of parasite antigen bound to the surface of platelets or suppression of thrombopoiesis by parasite that access the bone marrow.[19]

However, it has been observed in clinical practice and several studies that malaria associated thrombocytopenia tends to improve and platelet count returns

to normal limits after initiation of appropriate antimalarial therapy in a week's time.

A study from Uttarakhand region of India, which demonstrated manifestations of severe malaria in vivax infected patients had also shown thrombocytopenia as the major haematological change.[20]

Lathia *et al*, conducted a similar study in India nearly 10 years ago.[21] 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 population studied. 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 likelihood ratio of anegative test 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. Khatib *et al,* demonstrated a correlation between malarial parasitic index and hematological and biochemical abnormalities.[22] 72.3% of malaria patients had Thrombocytopenia. There was a proportional relation observed between severity of malaria and the degree of parasitemia. Also, hematological and biochemical abnormalities were more prevalent in patients having a higher Parasitic Index. Similarly, Muley *et al*, have proved that thrombocytopenia is associated with more severe clinical manifestations of vivax malaria infection.[23] However, such association could not be evaluated during this study because none of the patients had any features suggestive of severe malaria. The failure to methodically investigate other conditions causing thrombocytopenia is another limitation of this study.

The results of this study have strong implications considering the fact that in the resourcelimited settings, anti-malarials are often given on clinical suspicion alone. This practice can undoubtedly lead to emergence of resistant strains of the parasite. It is suggested that antimalarial 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.

CONCLUSION

Hematological abnormalities pertaining to plateletsare encountered in malaria. Thrombocytopenia has a very good sensitivity and a good specificity for vivax malaria. Normal platelet counts provide very strong evidence against malaria as the etiology of fever without a focus.

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Conflict of Interest: None.

Authors' Contribution

AA: Design, manuscript writing, MG: data collection, KMU: conception, MG: data interpretation, NA: editing, AA: data analysis

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