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Original Article
Haematological Parameters
Pak Armed Forces Med J 2020; 70 (5): 1576-80

EVALUATION OF HAEMATOLOGICAL PARAMETERS IN MALARIA INFECTION AND ITS ASSOCIATION WITH DIFFERENT SPECIES OF MALARIAL PARASITE

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ABSTRACT

Objective: To determine the association of various haematological parameters with different species of malarial parasites.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Haematology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, from Sep 2018 to Sep 2019.

Methodology: Thick and thin blood smears stained with Leishman stain were examined for the presence of malarial parasites under light microscopy. After the organism was detected by examination of thick blood films, identification of malarial parasite species was carried out using thin blood smears. Basic haematological parameters were evaluated using automated analyzer (Sysmex KX-21).

Results: Plasmodium falciparum was identified as the causative organism in 100 (38%) of the malaria cases whereas 100 (62%) of the cases had *Plasmodium vivax*. A trend towards decreased Hemoglobin, Red Blood Cells, platelet, lymphocyte and eosinophil count was observed in *Plasmodium falciparum* group as compared to *Plasmodium vivax* group (p=0.001), whereas, the former showed an increase count of white blood cell, neutrophil and monocyte (p=0.001).

Conclusion: Hematological parameters like Hemoglobin, RBC count, WBC count and Platelet count vary significantly with the type of plasmodium species causing malarial infection.

Keywords: Fever, Hemoglobin, Lymphocyte, Malaria, Plasmodium falciparum, Plasmodium vivax.

INTRODUCTION

Malaria is a common parasitic disease which is transmitted through the bite of a female anopheles mosquito. The parasite termed plasmodium has four species, namely vivax, falciparum, malariae and ovale which differ in geographical distribution. Common plasmodial species in our set up are vivax and falciparum. Sizable efforts have been focused on the diagnosis, management and prevention of malaria at various levels throughout the globe. However, despite intensive global efforts aimed at major reduction in its transmission, malaria still remains a major health hazard in Pakistan. It poses a remarkable burden in terms of mortality and morbidity among all infectious diseases[1]. Its widespread occurrence in our region questions its adequate control and prevention. Plasmodium vivax accounts for 64% cases of malaria whereas 34% cases are attributed to Plasmodium falciparum respectively in our set up[2].

Malaria manifests itself by clinical illness and pathological changes in various body systems. The parasite invades and proliferates in the circulating red blood cells. It causes a variety of hematological changes[3]. Blood is the most easily accessible tissue for diagnosis and screening for various diseases including malaria. Since it affects blood cell physiology, derangements in various haematological parameters ensue and examination of blood is an easy target for the health care providers to look for. Various diagnostic methods for malaria include conventional microscopic examination, concentration techniques {Rapid Diagnostic Test (RDT), Quatitative Buffy Coat (QBC), Immunochromatographic Test (ICT) and molecular methods like Polymerase Chain Reaction (PCR)[4,5]. However these methods vary in their sensitivity, specificity and accuracy.

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Received: 05 Feb 2020; revised received: 05 Mar 2020; accepted: 12 Mar 2020

One of the salient features of malarial infection is variation in blood cell counts. All three cell lines including red blood cells (RBC), white blood cells (WBC) and platelets exhibit variations, leading to anemia, thrombocytopenia and leukocytosis or leucopoenia[6]. Demographic factors, level of malarial endemicity, underlying hemoglobin abnormalities, immune and nutritional status of the patient, are among the various governing factors.

The disease carries high mortality in pregnancy. In highly endemic areas, it can lead to maternal and fetal mortality[7]. In areas of low endemicity it can lead to increased parasite load in the placenta leading to impairment in its physiology resulting in low birth weight of baby.

Pakistan faces numerous challenges in the control and prompt management of malaria. Various issues include lack of adequate diagnostic facilities leading to misdiagnosis, use of empirical treatment and unavailability of artemisinin combination therapy (ACT)[8]. This study was designed with an objective to check the effect of different plasmodium species on various haematological parameters, in order to confirm laboratory diagnosis early and help in institution of specific treatment.

METHODOLOGY

This cross sectional study was carried out in the department of Haematology, Armed Forces Institute of Pathology Rawalpindi, Pakistan.The study was completed over a period of 1 year, from 13th Sep 2018 to 12th Sep 2019. A sample size of 100 was calculated using WHO sample size calculator considering level of significance 5%, power of test 80% and confidence interval of 95%. Sampling technique used was non-probability consecutive sampling.

After taking permission from ethics review board of AFIP (FC-HEM17-31/ READ-IRB/401), allnewly diagnosed cases of malaria were included in the study. Patients with other haematological disorders, immune compromised conditions and those taking any medications especially antimalarial drugs were excluded from the study.Informed consent was taken from patients whose samples were collected. Three (03) ml of blood was drawn after a clean venepuncture under aseptic techniques in an ethylene diamine-acetic acid (EDTA) tube. Complete blood counts were generated through automated haematology analyzer (Sysmex KX-21). Basic haematological parameters including WBC count, Haemoglobin, RBC and platelet count were recorded. Thick smears for malarial parasites were prepared by placing a small drop of blood in the centre of the slide and spreading it out with the corner of another slide to cover an area about four times its original size. The smears were allowed to dry for at least 15 minutes at 37°C before staining. Thick blood smears were made and stained with Leishman's stain as described by Bain and Lewis. The slides were examined by qualified pathologists and malarial parasites were identified. Once the presence of parasites was confirmed, a thin blood smear was examined for recognition of plasmodial species. The smears were examined under 40x and 100x objective oil immersion for species' recognition. In addition differential leucocyte counts were performed on examination of peripheral smear.

Data was analyzed using SPSS version 25.0. Mean and SD were calculated for numerical variables such as age, haemoglobin(Hb), blood cell counts including RBC, platelet, WBC, monocyte, lymphocyte, eosinophil and neutrophils. Percentage and frequency were calculated for categorical variables like gender and type of malarial parasite. Data was divided into groups based on the type of plasmodium parasite identified asa causative agent. Association between these groups and haematological parameters were calculated using independent samples t-test, considering p-value of ≤0.05 to be significant.

RESULTS

A total of 100 patients were included in this study. The age of the patients ranged from 3-67 years with a median age of 22.95 ±5.6 years. Males accounted for 55 (55%) of the study population and 45 (45%) were females. Plasmodium

falciparum was identified as the causative species in 38 (38%) of the cases. However, remaining 62 (62%) cases were caused by *Plasmodium vivax*. No cases of Plasmodium malariae and Plasmodium ovale were found. There was an equal gender distribution of 1:1 in plasmodium falciparum group. However, in plasmodium vivax group male predominance was observed. Ratio of male to female patients observed was 1:0.7 (p=0.3). Summary of hematological parameters in both plasmodium species has been shown in table-I. There was a statistically significant difference between values of various hematological parameters in plasmodium falciparum and plasmodium vivax species (p=0.01).

Table: Comparison of Haematological parameters in plasmodium *falciparum* and *vivax*.

	Species	n	Mean ± SD	*p*-value
HB (g/dl)	Falciparum	38	10.1921 ± .33157	0.001
	Vivax	62	11.3306 ± .29730	
RBC (x10^6 /µL)	Falciparum	38	4.1324 ± .10849	0.001
	Vivax	62	4.4497 ± .11225	
TLC (x10^3 /µL)	Falciparum	38	7.0113 ± .40057	0.001
	Vivax	62	6.3032 ± .10779	
Monocyte (x10^3 /µL)	Falciparum	38	6.8961 ± .18570	0.001
	Vivax	62	5.9018 ± .23817	
Lymphocyte (x10^3 /µL)	Falciparum	38	23.3524 ± .62942	0.001
	Vivax	62	26.1403 ± .45512	
Eosinophil (x10^3 /µL)	Falciparum	38	2.5955 ± .11554	0.001
	Vivax	62	2.9773 ± .12374	
Neutrophil (x10^3 /µL)	Falciparum	38	70.0900 ± 1.52579	0.001
	Vivax	62	65.4161 ± .33111	
Platelets (x10^3 /µL)	Falciparum	38	88.0239 ± 1.17476	0.001
	Vivax	62	98.1763 ± .99502	

DISCUSSION

According to World Health Organization (WHO) estimates, 40% of the world's population is at risk of developing malaria. Studies have reported a global incidence of 300-500 million cases per year with an associated two million deaths per annum. Likewise, in Pakistan, the disease threatens lives of millions of people per year and local literature reveals higher mortality rates among infants, children, and pregnant ladies. A total of 374,513 confirmed malaria cases have been reported from all the public sector health facilities across Pakistan and private health clinics in high malaria burden districts to the federal directorate. Around 6.5 million malaria suspects were screened at these health facilities during 2019. Highest numbers of reported cases were *P. Vivax* (PV) (314,574) 84% followed by *P. Falciparum* (PF) (55,639) 14.9% and Mixed cases (4,300) 1.1%. The National Malaria Control Program of Pakistan has reported a six-fold increase in the incidence of *P. falciparum* malaria that now comprises 42% of all malaria cases reported in the country. Therefore, in addition to being a major public health issue, the disease significantly adds to the country's economic burden.

Various studies in Pakistan have found a widespread variation in the causative agent of malaria in different regions that may be due to local factors that influence harbouring of mosquito, status of health education and awareness, malaria prevention programs, and a genetic tendency to develop malaria immunity. In our study the frequency of *P. vivax* was higher (62%) as compared to *P. falciparum* (38%). Similar outcomes were reported in study conducted by Muhammad and colleagues in Khyber agency showing a higher prevalence of *P. vivax* in patients presenting with malaria like symptoms[10]. Hasina and colleagues also report an increased

incidence of *P.vivax* 97.3% than that of *P falciparum* 2.6% while no case of *P. malariae* and *P. ovale* was detected[11].

Malaria presents with variable clinical features. Classically, patient presents with high grade fever with chills followed by sweating and return of temperature to normal levels. Nausea, vomiting, fatigue and muscle pains accompany the above mentioned symptoms. These clinical symptoms are overlapping with various clinical ailments such as bacterial and viral infections. Malaria is a medical emergency and importance of its prompt diagnosis and management is essential. Delay in diagnosis and mis-diagnosis are the major causes of mortality in such patients[12].

Malaria is associated with a variety of haematological changes. Haematological abnormalities are considered one of the characteristic features of malarial infection. In our study patients of *P.falciparum* showed mean haemoglobin of 10.1 ± 0.3 g/dl which was relatively lower than that observed in patients of *P vivax* group (11.3 ± 0.29 g/dl). Anemia rates as low as 4% and as high as 25% have been reported[13]. Causes of anemia in malarial patients include intravascular hemolysis, inadequate erythropoeitic activity, immune complex adsorption onto erythrocyte membranes and effects of various therapeutic agents on parasitized red blood cells. Haroon & colleagueshave reported anaemia being more prevalent in the *P.falciparum* group[14] with a 100% Positive Predictive Value (PPV).

Our study revealed a decreased erythrocyte count associated with *P.falciparum* infections compared to those of *P.vivax*. This was in agreement with the study conducted by Kotepui & colleagues[15]. Age of RBC's plays a significant role in the infectivity of malarial parasite. Plasmodium vivax and plasmodium ovale prefer invasion of reticulocytes whereas plasmodium malaria targets old circulating RBC's. Infected erythrocyte displays increased clearance by spleen attributed to its altered surface characteristics and reduction in the ability of shape alteration. RBC's which are uninfected and those which are diseased cluster together in a process called cytoadherence. Entrapment in the spleen and rossetting leads to obstruction of the capillary and venules (post-capillary) of several organs[16].

Thrombocytopenia is another hallmark of patients affected with malaria. Our findings support the concept that decreased platelet counts among patients infected with *P.falciparum* in comparison to those of *P vivax* were statistically significant. This was in contrast to the findings reported by Mohtasim and colleagues, which shows that thrombocytopenia was a more common finding in patients having *P. vivax* infection (71.4%) as compared to mixed parasite involvement cases (66.7%) and *P. falciparum* (26%)[17]. Severe thrombocytopenia gives a diagnostic clue to clinicians for a possible underlying malarial infection, with frequency of thrombocytopenia more closely associated complication of *P. vivax* infection[18].

Total leucocyte count of our patients had a mean of 6.57 ± 1.3 x10^3/µL. A greater degree of leukopenia was present in *P. falciparum* group than in the *P.vivax* group. This was in agreement to the findings by Rasheed & colleagues in which leucopenia was present in 22.1%, 20.9%, and 18.4% of subjects in *P. vivax, P. falciparum*, and mixed infections, respectively[19]. In our study differential leukocyte counts reveal higher neutrophil and lower eosinophil responses with *P. falciparum* as compared to *P. vivax* infections. Margination of neutrophils to the sites of inflammation, localization in the spleen, lymphotoxic effects on the serum and recurrent bacterial infections are among the various mechanisms which may be operative behind this[20]. Mean eosinophil counts observed in our study were 2.5x10^3 /µL and 2.9x10^3/µL in *P. falciparum* and *P.vivax* groups respectively. Previous data showed that *P. falciparum* infections have a suppressive effect on pre-existent eosinophilia whereas it is minimally effected by *P. vivax* infections[21].

CONCLUSION

Various laboratory methods are employed for the diagnosis of malaria in the clinical

laboratory. These methods include examination of peripheral blood film for malarial parasites. Presence of malarial parasite on examination of peripheral blood film required experienced observer. If for some reasons malarial parasite is not found on examination of blood films above mentioned haematological changes can be used as surrogate markers for diagnosis of malaria infection. These can help clinicians to start anti-malarial treatment in slide negative patients with clinical features supporting diagnosis of malaria.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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