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Research Article

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Association of Thrombocytopenia, Urine Malaria Antigens, and Blood Groups with Malaria Parasite Density among Sudanese Malaria Patients at Sharg Al-Nile District in Khartoum State

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Abstract

Background: The malaria parasite (sporozoite) infect a human after biting by infected female anopheles mosquito, the erythrocyte which develop of normoblast finally developed to mature erythrocytes, the malaria parasites (*P. falciparum* and *P. vivax*) most common endemically in Khartoum.
Aim: A study was aimed to investigate the association of malaria parasite density with three biomarkers (thrombocytopenia, urine malaria antigens, and ABO & RhD groups).
Methods: A gold standard thick blood film to demonstrate a malaria stained using Gaimsa stain, a density of malaria expressed by number of crosses, then by immune-chromatography test (ICT) to detect malaria antigens according to species from urine deposit, the platelets was estimated by automated haematology analyser (Sysmex KX21 & Mindray) and peripheral blood picture to assess and confirm the platelets count, the ABO and RhD were detected using simple slid method and direct tube method to confirm results, the data were analyzed using SPSS version 22 and the statistical significant was expressed in P value < 0.05.
Results: In 63 malaria patients with irrespective to age, the density of malaria parasite show 39 (+), 16 (++) and 8 (+++) respectively, there were significant associations of malaria parasite density (+++) with thrombocytopenia and urine malaria antigens *P. value 0.000, 0.004* respectively and insignificancy in (ABO & RhD) and malaria parasite density the *P. value 0.959*.
Conclusion: A present finding explicit both thrombocytopenia, and urine malaria antigens may associate with high malaria parasite density, but insignificancy association of ABO, RhD phenotypes with density of malaria parasites.

Keywords: Malaria, parasite density, thrombocytopenia, urine malaria antigens, ABO & RhD, Khartoum

Introduction

Malaria is one of the haemo-parasitic infection that invade the red blood cells lead to destruction and lysis of red blood cells (RBCs), this caused by protozoan parasite *Plasmodium* species, malaria is a major cause of deaths in the tropical region of the world such as Sudan the *P. falciparum*, and *P. vivax* most common malaria parasite in the Sudan [1]. The blood is a fluid connective tissue that composed of cellular portion (erythrocytes, Leukocytes and thrombocytes) and liquid portion (blood plasma). A normality of haematological blood cells parameters include white blood cells and differential, red blood cells and platelets were important to achieve each of functions, previous studies were found malaria infected patients tended to have significantly lower platelets count, were normal in comparison to non-malaria infected patients [2, 3]. Thrombocytopenia may be associated with bleeding tendency which is one of the impotent severe manifestations of *P. falciprum* malaria [4]. Although severe thrombocytopenia is commonly reported to be associated with *P. falciparum* infection and has been reported to occur in patients with co-infection of both *P. falciparum* and *P. vivax*, its occurrence has been rarely reported in cases of *vivax* malaria [5]. Urine as non-invasive specimen for malaria detection was suggested by previous studies that both malarial antigens and antibody are possibly released into the urine during course of malaria infection, much new information has emerged since a relationship between ABO and malaria was first suggested more than 50 years ago. However, the correlation of density of malarial infection to the patient blood group has been of recent interest, the observation by Hadley et al that human erythrocytes lacking the duffy blood group antigen are refectory to invasion by *P. vivax* parasites indicate the usefulness of studying the association of blood group with malaria [6]. Similar studies have been undertaken in India, Sri Lanka and other countries around the world, with mixed result [7].

This study was aimed to investigate of thrombocytopenia, urine malaria antigens and blood groups and the association with parasite density among Sudanese malaria patients at Sharg Al-Nile district in the Khartoum state during January to August 2017.

Materials and Methods

A descriptive cross-sectional laboratory based study, was conducted in Sharg Al-Nile district in the Khartoum state during January to August 2017, the present study was enrolled of 63 patients attending to Aedbabiker and Marabea Al-sharef medical centers and newly diagnosed, clinically with laboratory confirmed for malaria, specimens were collected randomly, 2.5 ml venous blood in EDTA k_3 vacutanier by fully aseptic collection procedure and matched 5 ml urine in universal urine container was collected, specimens were processed for investigated parameters, ethical consideration was obtained from the Nile university medical board and the ministry of health - Sudan and informed consent was obtained from each participants, data were collected and regulated by instructed questionnaire, statistical analysis of data was performed using SPSS version 22 as computer software program, statistical significant was expressed in *P. value* < 0.05 using Chi square and one way ANOVA tests.

Blood specimens were processed and laboratory detection of malaria parasite was done using gold standard thick blood film, stained by Giemsa's stain, the parasite was detected by professional microscopy specialist and confirmed by ICT for malaria antigens and species were differentiated by thin blood film stained by Leishman's stain, platelets was measured by two automated haematology analyzers (Sysmex KX 21N and Mindray) were calibrated and well controlled by inserting of homogenized blood into sample probe and then aspirated by ordering of device, then after few seconds result was printed and record of platelets count for each patient separately, urine sediment was prepared by light speed centrifugation (3000 RPM); sediment was collected and 100 ul was added in the specimen window of malaria antigens ICT cassette, and three drops of kit buffer were added on buffer window then left at bunch (10-30 min) and result was recorded, ABO and RhD blood grouping was performed by simple slid method, and results were confirmed by direct tube method and D^U method for RhD negative was performed and results were recorded.

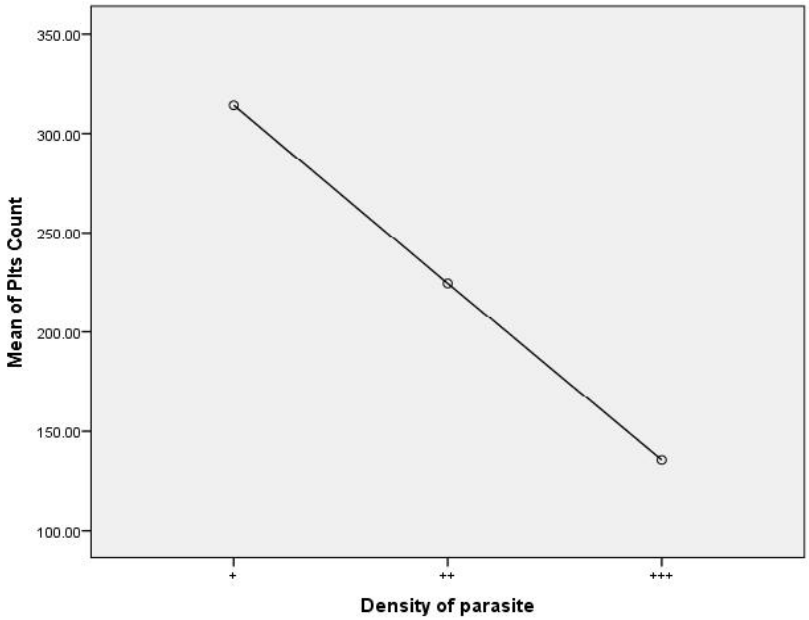
Results

The 63 patients was includes 34 (54%) males and 29 (46%) female with irrespective to the age of the patients, were detected 54 (85%) patient infected by *P. falciparm* and 9 (15%) patient infected by *P. vivax*, the density of malaria parasite expressed by number of crosses in the stained thick blood film show below table, and was detected 39 of patients with one cross (+) and 16 of patients with two crosses (++) and 8 of patients with three crosses (+++), the frequency of thrombocytopenia includes 11 patients with platelets < 150 x 10^3/ul and 5 patients with elevated > 450x10^3/ul and 57 patients with platelets within normal range 150-450 x 10^3/ul, there was significant association between the density of malaria parasite (+++) and urine malaria antigens *P. value* 0.004, there was insignificancy association between blood antigens phenotype (ABO & RhD) and density of malaria parasite (+++) the *P. value* 0.959 and there was significant association between density of malaria parasite (+++) and thrombocytopenia, the platelets count * 10^3/ul shows 63.00, 121.00, 123.00, 135.00, 145.00, 156.00, 166.00 and 177.00 respectively *P. value* 0.000, there was significance association in the comparing the mean of platelets * 10^3/ul with density of malaria parasite by one way ANOVA test show *P. value* 0.000, this was represented in below figure.

Table No. 1 Describe parasite density with study parameters of the study population:

Parameters		Density of malaria parasite			Total
		One cross (+)	Two crosses (++)	Three crosses (+++)	
[a] Platelets count / ul*10^3	Number of cases	39	16	8	63
	Mean	314.1282	224.5000	135.7500	268.7143
	Minimum	60.00	111.00	63.00	60.00
	Maximum	764.00	364.00	177.00	764.00
[b] Urine malaria Ags ICT	Detected	3	6	4	13
	Not-Detected	36	10	4	50
[c] ABO & RhD phenotyping	A, RhD positive	7	4	2	13
	B, RhD positive	9	4	1	14
	O, RhD positive	21	7	5	33
	AB, RhD positive	1	0	0	1
	O, RhD Negative	1	1	0	2

[a] = there was *p. value* 0.000.
[b] = there was *p. value* 0.004.
[c] = there was *p. value* 0.959.



A figure show the one way ANOVA test was compared the mean of platelets *10^3/ul with density of malaria parasite.

Discussion

The Sudan one of African endemic regions of malaria infection, and the Khartoum state was structured from heterogeneous Sudanese population, the malaria was expanded through Khartoum according to different studies [1, 8]. Severity of malaria was composed of different clinical presentation with laboratory findings, in this study the peripheral blood malaria parasitemia with high grade (+++) was used as predictor with association of low platelets (thrombocytopenia), present of urine malaria antigens and ABO & RhD blood groups in the study population.

Thrombocytopenia often accompanies malaria and it's usually mild to moderate. It may however be symptomatic and severe, sixty-three percent of patients with malaria showing thrombocytopenia in our study is close to others reporting low platelets as 17.5% and 82.5% is normal in comparing our results with Mahmood and Yasir [9]. In study done in Liberia shown a similar trend, they studied a total of 145 patients who had *P. falciparum* malaria, out of these109 (75.18%) had thrombocytopenia. Another study had considered thrombocytopenia as a predictor for malaria and reported 60% sensitivity and 88% specificity of malaria diagnosis in acute febrile patients [10]. In the important study from India shown a similar trend, Mahmood and Yasir concluded an extended research for malaria parasite in patients having thrombocytopenia on smear [9]. Mild-to-severe thrombocytopenia observed in hospitalized patients was considered enough to alert the possibility of malarial infection, as *P. falciparum* was found to be common species in these patients. Our study also concludes that *falciparum* malaria is more common at lower platelet counts as compared to *vivax* malaria and overall the chances of finding *falciparum* malaria are almost twice than that of finding *vivax* malaria in thrombocytopenic patients. It is a general consensus that thrombocytopenia is very common in malaria [12, 13] and previously it was believed that it is more common in *falciparum* malaria. Recent studies have shown that thrombocytopenia is equally or even more common in *P. vivax malaria* contrary to the popular belief that it may be observed in *P. falciparum* malaria [14, 15].

More recent data in India has shown how thrombocytopenia exhibited a heightened frequency and severity among patients with *P. vivax* infection [16]. Recent studies conducted from the Indian subcontinent have found significant thrombocytopenia in *P. vivax* malaria [17, 18]. Similar results have been reported from Qatar and Venezuela [19, 20]. Studies from Brazil have shown a similar trend [21]. A recent study from Iran shown not a similar trend of our study, the study confirms that they are getting more cases of thrombocytopenia due to *P. vivax* than *falciparum* and attributes this to the possible development of a new genotype of *P. vivax* [22].

Some other study done by Alejandro et al that describes detection of the malaria antigens in urine of patient with acute *falciparum* and *vivax* malaria infection that reflect association between malaria severity and the urine malaria antigen presented the *P. value* <0.05 and was agree with our finding [23, 24]. Different studies across world that show there is no association of malaria density and erythrocyte antigens phenotyping (ABO & RhD) *P. value* >0.05, our result was agree with study done by Fabiola et al that resulted there is insignificant association between malaria and ABO and RhD blood groups *P. value > 0.05,* another recent study done by Singh et al show similar trend, further study done by Fischer et al that conclude there is significant association between ABO & RhD blood groups and density of malaria *P. value* 0.008 that disagree with our results [25-27].

Conclusion and Recommendations

We concludes in our study the thrombocytopenia can be used as predictor biomarker correlated with of malaria parasite density, non-invasive specimen as urine sample can be used for malaria antigens detection and suggested as powerful as biomarker for malaria disease with association of its density after validating of detection kit, and the conclusion of insignificant association of malaria parasite density and ABO & RhD as biomarker for sever malaria this required more sophisticated method to describe of thought miner and major association.

We were recommend furthermore studies to overall the biomarkers used for severity predictor and indicator in the Sudanese malaria patients, using different methods to describe the exact cause of thrombocytopenia during course of malaria disease, other techniques must be used to characterized malaria antigens presented in the urine and using as predictor for association of parasites density and malaria disease severity, phenotypeing and genotyping for erythrocytes surface antigens (ABO & RhD) to determine and describe the role of erythrocyte antigens in the course of malaria disease.

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