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Effect of Plasmodium vivax malaria and their density on some Haematological parameters in infected patients admitted to Wad Medani teaching hospital in Gezira state, Sudan

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Abstract---Background: Despite the great effort of the malaria control program in Sudan, *Plasmodium vivax* malaria has remained a major

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challenge recently, causing significant morbidity with a variety of haematological changes. Objective: This study aims to investigate the effect of *Plasmodium vivax* malaria and their density on some haematological parameters in patients admitted to Wad Medani teaching hospital in Gezira state, Sudan. Methods: Some haematological parameters of 160 participants, 80 infected with vivax malaria (47 male and 33 female) and 80 non-infected with malaria, who were admitted to Wad Medani teaching hospital in Gezira state, Sudan during high transmission season between August and November 2018, were evaluated for some haematological parameters. Results: The parameters (haemoglobin, haematocrit, counts of red blood cells, platelets, white blood cells, lymphocytes, monocytes and eosinophils) were significantly lower in infected patients than malaria negatives. The platelets and haemoglobin were inversely correlated to parasite density in positive cases. Conclusion: The exhibition of some haematological parameters changes was closely related to patients infected with vivax malaria versus non-infected, and these changes could be used as a diagnostic criterion for vivax malaria diagnosis in endemic regions.

Keywords---Haematological parameters, *Plasmodium vivax*, Malaria, Parasite density, Gezira State, Sudan.

Introduction

Vivax malaria is a protozoan mosquito borne disease caused by *Plasmodium vivax* which is one of the five Plasmodium species that cause malaria in humans ¹. Globally the prevalence of malaria was 229 million malaria cases in 2019 in 87 malaria endemic countries and 409 000 deaths in 2019 ². The malaria caused by the species vivax of *the genus Plasmodia* is the most prevalent and leads to 13.8 million clinical cases annually, including serious illness and death ²⁻⁴. Despite the frequency of Duffy-negative individuals in Africa, there were many reports highlighting the emergency of *Plasmodium vivax* malaria in some African countries^{5,6}. Although the pathogenicity of *Plasmodium falciparum* and *Plasmodium vivax* is different, vivax malaria cause severe malaria with serious complications as well as falciparum malaria ^{4, 7, 8}. Recent evidence has indicated that the former view of malaria caused by vivax as a benign infection need to be changed as the vivax malaria can also result in severe complications and death ⁹⁻¹¹. Patients with severe vivax malaria have been reported in Eastern Sudan⁴.

Haematological changes were indicated in vivax malaria patients, such as anemia, thrombocytopenia, and atypical lymphocytosis. The variations in hematological parameters may be affected by any infection and inflammatory conditions like malaria, which is one of the common systemic complications, which causes serious illness and severe complications ^{12, 13}. Raised and decreased white blood cells, neutrophil, eosinophil, and monocyte count were reported in patients with vivax malaria ¹⁴⁻¹⁶.

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Severe complications of malaria have been more commonly seen in P. falciparum infections, and those caused by P. vivax have been considered benign. However, the literature has alarming reports of complicated malaria seen in vivax infections in recent times¹⁷. In Sudan *Plasmodium falciparum* was the main species that was found in Sudan and distributed in all states, the *Plasmodium vivax* representing about 5% of malaria cases ¹⁸. Also, the percentage of *P. vivax* may differ in Sudan states, elevated mainly in Central Sudan and Eastern Sudan ^{11, 18}.

A study done in New Halfa, which located in an irrigated scheme in Eastern Sudan, indicated that *vivax* malaria represented nearly about 3% of malaria cases in 2005, this increasing to 6.1% in 2012^{19} in Eastern and Central Sudan *Plasmodium vivax* malaria cases increased and represent 10 - 15% of malaria cases ¹⁰. A recent study reported that the prevalence of malaria caused by *Plasmodium vivax* represented 26.6% of all malaria cases in Sudan ²⁰. In Sudan clinicians requested complete blood count for most patients with fever, to help in deferential diagnosis for many infectious and non-infectious diseases. This study aimed to investigate the effect of *Plasmodium vivax* malaria and their density on some hematological parameters in Gezira State, Sudan.

Methods Ethical Consideration

Ethical approval was first obtained from the Ethical Clearance Committee of Gezira State Ministry of Health (reference MU/2019). Then a written letter from the general head director of Wad Medani teaching hospital in Gezira state was obtained. Verbal consent was also obtained from each study participant after explanation of the study aim, procedures, potential risks, and benefits of the study. The confidentiality of the result was secured by use of correspondence codes rather than written participant's name. The results were referred to a clinician for appropriate treatment and the infected patients were recorded as having had received antimalarial chemotherapy according to the National Malaria Control Program, Federal Ministry of Health, Republic of Sudan.

Study design

This is a cross-sectional laboratory based- study aimed to investigate the effect of *Plasmodium vivax* malaria and their density on some haematological parameters in Gezira State, Sudan

Study area

The study was done in Wad Medani Teaching Hospital, in Wad Medani City which is the capital of Gezira State, located in Central Sudan, near the Blue Nile River, 187 kilometers south of Khartoum, where the Irrigated Scheme is found. Gezira State located between the Latitude: 14.6672° or 14° 40' 1.8" north and Longitude: 33.2224° or 33° 13' 20.5" east. Malaria is endemic in Gezira State with two high transmission seasons. *Plasmodium falciparum* is the predominant species and the second species are *Plasmodium vivax*.

Criteria of selection

Patients aged equal or more than 18years old with positive vivax malaria were included in our study. The patients under malaria chemotherapy, and/or haemoglobinopathies, patients infected with other species than vivax, mixed infection were excluded from this study.

Sample collection and processing

The diagnosis of patients with vivax malaria was based on the making of blood film and identification of malaria parasite by light microscope. The blood was collected from finger-pricks on clean glass slides. Thick and thin blood films were made for detection of parasite density and differentiation of malaria species respectively. The films were left to air dry and the thin film was fixed with absolute methanol (CRESCENT Diagnostics, Jeddah, KSA). Then, both blood films were flooded with working reagent of Giemsa stain at 10% concentration and left for 10 minutes, then washed with tap water, air dried, and examined through an 100× oil immersion objective.

To determine the *Plasmodium vivax* parasite density: independently two highly qualified medical microscopists counted instances of the asexual stage of *Plasmodium vivax* parasite in the thick blood film against 200 WBCs for each of the vivax malaria patients. Then, the averaged results of the two microscopists were taken for calculation of *Plasmodium vivax* parasite density. The estimation of the parasiteamia was calculated according to the formula stated by WHO-basic malaria microscopy ²¹.

Prior to treatment, three ml of venous blood were collected under aseptic conditions into an EDTA container (CRESCENT Diagnostics, Jedda, KSA) from all subjects with vivax malaria as well as the control group. The analysis of full blood count were done for all the EDTA blood samples using a fully automated hematological Sysmex analyzer (EX300 German) after calibration and running of daily control material of the machine and the results obtained just after passing of control materials. For quality assurance standard operation procedures were followed in all the laboratory tests.

Data analysis

After verification of data completion and coded. The data was entered into a computer and analyzed by using SPSS version 22.0. The mean, standard deviation, and percentages of the study parameters were obtained. The independent-sample t-test was used to compare the mean hematological value of the malaria cases with malaria negative individuals. Pearson correlation analysis was used to test the association between two continuous variables. P<0.05 was considered as statistically significant in all statistical tests.

Results

Demographic data and Plasmodium vivax parasite density among positives cases

A total of 160 participants were enrolled in this study, 80 were infected with *Plasmodium vivax* malaria and the remaining were not infected. The age of infected patients ranged between 18-70 years with mean 32.3 ± 18.7 standard deviation, among the infected patients 58.75% were males and 41.25% were females table 1. Based on the parasite density the positives cases were categorized into three groups, these are labeled <400 parasite/µl (23.8%), 401-4000 parasite/µl represent (73.8%), and > 4000 parasite/µl represent (2.6%) table 2.

The values of Some Hematological parameters of infected and non-infected participants

The mean of some hematological parameters of the positive cases were compared with those of the negative malaria participants using an independent-sample t-test, to identify the some hematological changes of P. vivax infections. The hematological parameters (hemoglobin, hematocrit, red blood cells, platelets, white blood cells, lymphocytes, monocytes and eosinophils) were significantly lower in infected patients than malaria negatives P value <0.05. With the exception, the neutrophil parameter was significantly higher in positives cases when compared with negatives, P value <0.05, Table 3.

Anemia-low Hgb concentration, defined as; Hgb <12 g/dL for females and Hgb <13g/dL for males. Among the infected patients, 54(67.5%) had low Hemoglobin. Of the total 80 positive cases, 20(25%) presented with increased total white blood cells count (leukocytosis), 7(8.75%) showed leucopenia and the remaining 53(66.25%) with normal white blood cells counts. In vivax malaria patients neutrophilia was recorded in 32(40%), neutropenia in 4 (5%), the remaining 44 (55%) had normal neutrophil values. Among positive subjects 38 (47.50%) showed lymphopenia, 13 (16.25%) presented with increased lymphocytes count (lymphocytosis) and 29 (36.25%) presented with normal lymphocytes count. Of the total 80 positive subjects monocytopenia was recorded in 44 (55%), while normal monocyte count presented in 36(45%). No monocytosis was observed in *Plasmodium vivax* malaria patients. Among the positive participants eosinopenia was recorded in 39(48.75%) and the remaining 41(51.25%) recorded normal eosinophil count. Thrombocytopenia is defined as platelets count less than 150,000/µl. the prevalence of thrombocytopenia was 61(76.25%) among positives cases, where platelets count less than $150,000/\mu$ l. and the remaining 19(23.75%)had normal platelet count table 4. Among the positive cases there was no patient with thrombocytosis and thrombocytopenia was the most common hematological abnormality finding observed in vivax malaria in our study. There was an inverse correlation between plasmodium vivax density and platelets count, and hemoglobin level /dl (r=0.43, P vale 0.001 and r=0.39, P value 0.037) respectively. table 5.

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Discussion

Changes in hematological parameters due to malaria infection are included in the three main cells in the blood, specifically erythrocytes, leucocytes and thrombocytes, with various clinical presentations. According to this study the mean of red blood cell count was significantly lower in those infected with *Plasmodium vivax* when compared with participants with no malaria. Similar results were reported in other studies done in Thailand ^{22, 23} and India. Based on these results, the hemoglobin level and packed cells volume were significantly lower than in non-infected subjects, and this could be due to short half-life of deformed and destroyed red cells by *Plasmodium vivax* malaria parasite in their life cycle, and/or reduction of erythropoiesis by some anti-inflammatory reaction and removal of deformed red cells, parasitized red blood cells by the spleen, causing anemia. Our findings revealed that the total white blood cell count of infected patients with vivax malaria was lower, when compared with non-infected subjects. This is a significant difference and our result agree with studies conducted in Thailand ^{14, 22} and India ¹⁷ which stated that these changes could reflect the hypersplenism. Malaria-infected patients with leucopenia have been frequently seen and reported by other researchers to have demonstrated low white blood cells count ^{24, 25}, this disagreeing with another published report that had demonstrated leukocytosis ²⁶. In our present study neutrophil count was slightly increased when compared with the result from non-infected subjects. The increased neutrophil number in this study associated with *Plasmodium vivax* malaria was similar to previous studies in Thailand ^{22, 23, 26} and in Ethiopia ²⁷, suggesting that the increased number of neutrophil may due to the activation of production of neutrophil, or released from the red bone marrow The slight increase of neutrophil count could be due to concomitant bacterial infection and/or due to acute malaria infection. In this study a slight decrease in lymphocyte count was observed in patients with Plasmodium vivax malaria in comparison to the non-infected control group, this is in agreement with other studies in Thailand^{22, 23} and in India ²⁸, which suggests the association between malaria infection and decreased lymphocyte counts could reflect the sequestration of lymphocytes by the spleen.

Monocyte count was significantly decreased in Plasmodium vivax malaria patients, when compared with the non-infected control group and this result is similar to studies done in Thailand ^{22, 23}, but contrasts with study conducted in India ²⁸, which suggests an increase in monocyte count due to contribution of monocyte response to malaria infection. Eosinophil count of malaria subjects was significantly decreased when compared with non-infected subjects. Similar results were documented in Thailand ²⁸, suggesting that the causes of peripheral blood eosinopenia, which occurs in acute malaria, are not fully understood. This suggests that the destruction of eosinophil is more than the decreased production of eosinophils, which is responsible for the decreased eosinophil counts in severe malaria infection. According to this study the mean platelets count is $(117.275 \times$ 10^9 \l and standard deviation is 67.79) for the total population which was significantly low, when we compared it to the platelet count with the healthy population. In comparison with other published data, it was similar to studies done in Thailand ^{22, 23}, India ^{29, 30}, Pakistan ^{31, 32}, and in Sudan ³³. In the present study there was an inverse correlation between hemoglobin level, platelet count

and *Plasmodium vivax* parasite density. This could be due to increased destruction of red blood cells during life cycle and immunological interactions. There are many hypotheses for thrombocytopenia in malaria infection, such as consumption by disseminated intravascular coagulopathy, sequestration of platelets by spleen and peripheral destruction ^{31, 33, 34}. This in agreement with the study conducted in South Ethiopia ³⁵.

Limitations of the study

The study did not follow up the patients to show the hematological parameters progression after treatment.

Conclusion

Some hematological parameter changes were closely related to patients being infected with vivax malaria. A similar study with large sample size is recommended for further evaluation of the effect of *Plasmodium vivax* on haematological parameters and their usefulness in the prediction of vivax malaria infection.

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Conflicts of interest:

The authors declare there are no conflicts of interest.

Authors' contributions statement: Albadawi A Talha designed the research plan of this study and wrote the manuscript. Lana M Elamin and Sanaa E. Hussein coordinated the analysis of data, and also contributed to the manuscript writing. Esraa A. Goda carried out all laboratory analysis. Adam D A Salim, Elhadi A. Ahmed, Bakri Y M. Nour and Abozer Y Elderdery participated in the main role of editing the manuscript and wrote the manuscript. Final version of the manuscript was reviewed and approved the all authors.

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Table 1	
Demographic data of infected p	atients

Variable	Age/year	n(%)
	18-28	15(18.75%)
	29-38	21(26.25%)
Age (mean32.3±18.7)	29-48	18(22.5%)
	49-58	17(21.25%)
	>59	911.25%)
Condor	Male	Female
Gender	47(58.75%)	33(41.25%)

Table 2
Distribution of Parasite density among positive cases

Parasite density per microliter	n (%)
<400	19(238%)
401-400	59(73.8)
>4000	2(2.5%)

Table 3 Mean values (±SD) of Some Hematological parameters of infected and noninfected participants

Parameter	Infected	Non-infected	P. value
	Mean ±SD	Mean ±SD	
Red Blood Cells (106/ µL)	4.35±1.01	4.6±0.5	0.032
White Blood Cells $(10^3/\mu L)$	6.250±1.54	7.7 ± 2.1	0.035
Platelets (10 ³ /µL)	117.27±67.79	321.2±96.0	0.000
Hemoglobin (g/dL)	10.6 ±2.44	12.9±2.3	0.006
Packed Cell Volume (%)	33.61±7.46	38.1±63	0.027
Neutrophil (%)	69.10±13.94	43.4±9.7	0.001
Lymphocyte (%)	28.07±14.21	46.4±10.7	0.003
Monocyte (%)	1.97±1.15	6.8±3.1	0.002
Eosinophil (%)	0.58±0.66	2.9±1.1	0.000

Data are presented as mean ± standard deviation. The P value <0.05 is considered significant

Table 4Prevalence of some hematological parameters changes among positive cases

Parameter	N(%)
Anemia	54 (67.5%)
Leucocytosis	20 (25%)
Leucopenia	7 (8.75%)

Neutrophilia	32 (40%)
Neutropenia	4 (5%)
Lymphopenia	38 (47.50)
Lymphocytosis	13 (16.25%)
Monocytopenia	44 (55%)
Eosinopenia	39 (48.75%)
Thrombocytopenia	61 (76.25%)

Table 5 Results of Some Hematological Parameters based on Plasmodium vivax parasite density

Parasite density/ µL	<400	401-4000	>4000	P value
variable	Mean ± SD	$Mean \pm SD$	$Mean \pm SD$	
Red Blood Cells (10 ⁶ / µL)	4.5±1.2	4.3±0.97	3.9±0.4	0.387
White Blood Cells	6.53±1.6	6.21±2.4	4.25±0.63	0.178
(10³/µL)				
Platelets $(10^3/\mu L)$	154±86.5	101±53.2	103±10.6	0.001
Hemoglobin (g/dL)	11.58 ± 2.1	10.5±2.4	7.4±2.4	0.037
Packed Cell Volume (%)	36.7±7.3	32.8±7.4	28.0±1.4	0.086
Neutrophil (%)	69.97±14.8	68.94±13.7	65. 5±21.9	0.374
Lymphocyte (%)	31.50±4.7	28.1±4.1	27.5±4.7	0.243
Monocyte (%)	2.1±1.19	1.9 ± 1.2	2.0±1.4	0.559
Eosinophil (%)	0.42±0.7	0.61±0.6	1.5±0.7	0.095

Data are presented as mean ± standard deviation. The P value <0.05 is considered significant