



Original article

Correlation between thrombocytopenia and malaria infection in Medical Military Hospital, Khartoum State-Sudan

Ibrahim Mohammed Eisa¹, Tayseer Elamin Mohamed Elfaki^{*2}, Gwahir Ali Nafee Mustafa³, Ihood Imad Eldin Mahmoud³, Mawada Mohammed Hamed³, Nosiba AL-Tayeb Adam AL-Toom³, Mohammed Ahmed Ibrahim Holie⁴, Mohamed Mobarak Elbasheir¹

1- Department of Parasitology and Medical Entomology, Faculty of Medical Laboratory Science, ALzaiem ALazahari University, Khartoum North, Sudan.

2- Department of Parasitology and Medical Entomology, College of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum, Sudan.

3- B.Sc. student, Department of Parasitology and Medical Entomology, Faculty of Medical Laboratory Science, Karary University, Omdurman, Sudan.

4- Department of Microbiology and Immunology, Faculty of Medical Laboratory Science, ALzaiem ALazahari University, Khartoum North, Sudan.

ARTICLE INFO

Article history:

Received 27 September 2020

Received in revised form 27 October 2020

Accepted 28 October 2020

Keywords:

Thrombocytopenia
Malaria Infection
Medical Military Hospital
Parasitaemia
Khartoum State

ABSTRACT

Background: This study was conducted on 72 blood samples collected from patients attending in Medical Military Hospital in Omdurman province. **Results:** Out of the blood samples examined, 32 were found positive for malaria infection. This constituted a prevalence rate of 44.4%. The study included 40 males. Among them, 18 were infected with malaria with a prevalence rate of 56.3 %. The study also included 32 females with 14 infected with malaria constituting a prevalence rate of 43.8 %. The difference in prevalence rates was found to be statistically insignificant. The study showed that there was a negative relation between the platelet count and parasite density as the correlation coefficient (rho) was found to be (-0.376) demonstrated that when the parasite density was high the platelet was low. The study showed that there was a negative relation between the age and parasite density as the correlation coefficient (rho) was found to be -0.023 showing that the levels of parasitaemia was decreasing by age increase.

Introduction

Malaria is a disease of global importance that result in 300-600 million cases annually and an estimated 2.2 billion people are at risk of infection [1]. Numerically the most important of the life-threatening protozoan disease is malaria, which is responsible for at least 750,000 deaths a year, mostly in young children in Africa [2,3]. The agent of malaria is an obligate intracellular sporozoan in the genus *Plasmodium*, which contains four species: *P. malariae*, *P. vivax*, *P. falciparum* and *P. ovale* [4].

The human and some primates are the primary vertebrate hosts for these species, which are geographically separate and show variations in the pattern and severity of disease. Over half of the world's population is at risk from catching malaria.

Malaria is currently endemic in 109 countries in four continents and of the 500 million cases of malaria estimated to occur annually, approximately one million results in death. Most of the fatalities are in children under the age of five years old and pregnant women [5]. Malaria accounts for at least \$12 billion in economic losses each year in Africa and a reduction in annual economic growth estimated at 1.3 percent [6].

Changes in hematological parameters are likely to be influenced by any disease condition including endemic diseases, such as malaria, that can affect health of mankind with various clinical presentations [7]. Hematological changes are some of the most common complications in malaria and they

play a major role in malaria pathogenesis. These changes involve the major cell types such as RBCs, leucocytes and thrombocytes [7]. Malaria infected patients tended to have significantly lower platelets, WBCs, lymphocytes, eosinophils, RBCs and Hb level, while monocyte and neutrophil counts were significantly higher in comparison to non-malaria infected patients [8]. One study showed patients with higher WBCs count compared with community controls [9].

The most common complication during malaria infection is thrombocytopenia [10]. Microscopic slide examination of peripheral blood remains the most widely used test and is the gold standard for detecting malaria infection, however, it requires technical expertise and smear examinations is time-consuming [11]. Hematological changes during malaria infection, such as thrombocytopenia and leucocytosis or leucopenia are well recognized. Diagnostic value of these haematological alterations may be easily obtained and may be useful in people living in malaria endemic areas [12].

The main objectives of the current study to determine the association between malaria infection and platelets count in patients attending Medical Military Hospital, Khartoum State- Sudan, to investigate the prevalence of malaria infection among patients according to gender, to investigate the correlation between parasite density of malaria among patients and age groups and to determine the association between parasitemia and platelets count in patients attending Medical Military Hospital, Khartoum State- Sudan.

Materials and Methods

Study design

This was a cross-sectional facility-based study.

Study area

The study was conducted in Medical Military Hospital which is located 15 kilos far away from Khartoum center.

Study population

The study was carried out on 72 referred patients suspected to have malaria like symptoms. The patients were categorized according to age and gender.

Sample size

72 blood samples were collected from the patients under the study which were selected randomly.

Samples collection

Blood was collected using sterile disposable plastic needle EDTA vacutainer, using aseptic standard non-traumatic vein puncture technique and immediately complete platelets count was done and blood films were prepared.

Data collection

A questionnaire was designed to collect data regarding age, gender and signs and symptoms if any.

Data processing and analysis

Data collected was analyzed manually by using master sheet and computerized by using SPSS version for windows program. Frequency, mean, percentages, Chi-square test and correlation coefficient (ρ) were used. Then data was presented in tables and figures.

Ethical consideration

The approval was taken from Karary University- Faculty of Medical Laboratory Science and Medical Military Hospital administration as well as verbal consent was taken from the patients after explaining the study purpose.

Methods

Preparation and examination of blood films

To make thick smear, the collected blood was stirred with a corner of slide until an appropriate thickness obtained. To make thin smear, the edge of spreader was placed just in front of the drop of blood. Then it was drawn back until it touches the drop of blood. The blood was allowed to run along the edge of spreader. The spreader was then pushed to the other end of slide with smooth movement. Then the slide was allowed to dry.

Both blood films were stained using Geimsa stain. Thin films were fixed with absolute methanol for 1-2 minutes. Then slides were covered with 10% Geimsa solution for 10 minutes. All slides were washed using clean water and allowed to dry by air. The slides were then examined using light microscope with oil immersion lens. Thick film was used for detection of malaria parasites. For levels of malarial parasitemia, data were grouped into hyperparasitemia (more than 10 parasites/ 1 oil field) considered as (++++), high parasitemia (1–10 parasite/ 1 oil field) considered as (+++), moderate parasitemia (10–100 parasite/100 oil field) considered as (++) and low parasitemia (less than 10 parasites/ 100 oil field) considered as (+) [12]. The thin films were used for identification of species.

Platelet counts by automated hematology analyzer (Sysmex KX-21N)

Well mixed EDTA blood sample were tested with automated hematology analyzer, easily fitting into any laboratory and ideal as a backup analyzer to the Sysmex full differential analyzer system.

Sysmex proceeded

Before placing blood (5ml) mixed with EDTA in clean tube in the machine, the machine was calibrated by ease of system operation and maintenance, does not require highly, fully automatic sample aspiration, dilution and analysis for 18 parameter test results, Compact instrument “foot print” and daily maintenance was automatically performed at every start up and shut down. After onset of the machine, the reading approved and was recorded.

Results

Frequency of malaria according to gender

A total number of 72 cases were included in this study. The distribution of gender among the 72, 40 were males (55.6%) and 32 were females (44.4%), the deference between frequency of males and females was insignificant at P value= 0.346 (Table 1).

Frequency of malaria according to species

Among the 32 patients of malaria there were 30 (93.8%) *P.falciparum* cases against 2 (6.2%) *P.vivax*

Table 1. Frequency of malaria according to gender.

Gender	Frequency	Percent (%)	Positive result	Percent (%)	<i>P.value</i>
Males	40	55.6%	18	56.3%	0.346
Females	32	44.4%	14	43.7%	

Table 2. Frequency of malaria according to species.

Spp.	Frequency	Percent (%)	<i>P.value</i>
<i>P.falciparum</i>	30	93.8	.000
<i>P.vivax</i>	2	6.2	

Table 3. Parasite density in all study patients.

Parasite density	Frequency	Percent (%)	<i>P.value</i>
+	17	53.1	.724
++	15	46.9	

Table 4. Correlation between platelets count and parasite density.

	Platelets count	<i>P. value</i>
Parasite density	-0.376 (rho)	0.006

cases, the frequency distribution of species was significant ($p.value = .000$), that means the *P.falciparum* was significantly more frequent than *P.vivax* (Table 2).

Parasite density in all study patients

Parasite density was determined in thick Giemsa stained blood film. Among the 32 patients of malaria there were 17 (53.1%) had one (+) parasitaemia and those with two (++) parasitaemia were 15 (46.9%) (Table 3).

Parasite density and platelet count

The results showed that there was a negative relation between the platelets count and parasite density as the correlation coefficient (rho) was found to be -0.376 and the result statistically was found to be significant at $P= 0.006$ (Table 4, Figure 1) demonstrated that when the parasite density was high the platelets was low.

Parasite density and age

The results showed that there was a negative relation between the age and parasite density as the correlation coefficient (rho) was found to be -0.023 and the result statistically was found to be insignificant at $P= 0.689$ (Table 5, Figure 2) showing that the levels of parasitemia was decreasing by age increase.

Figure 1. Correlation between platelets count and parasite density.

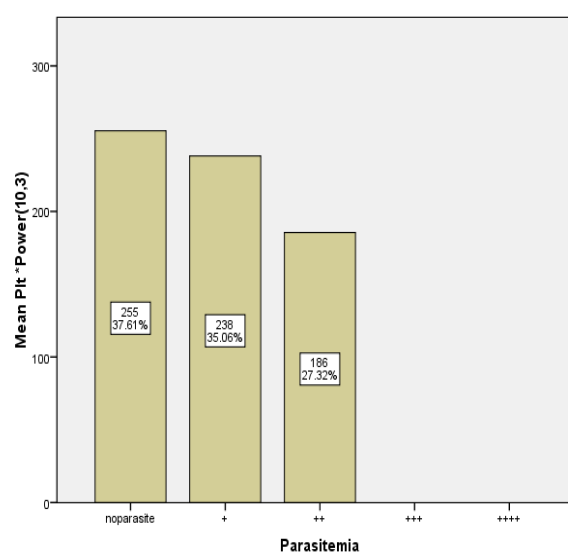
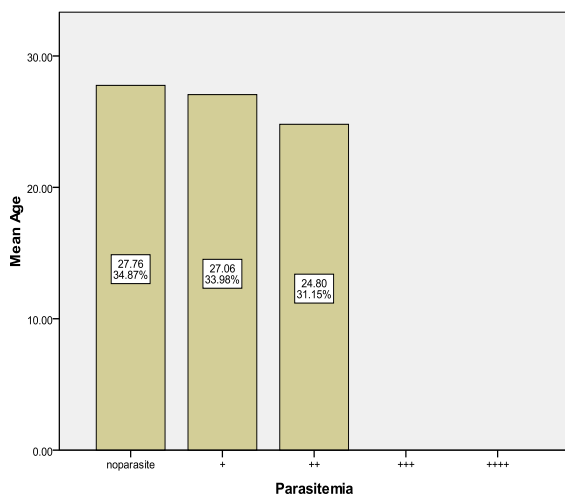


Table 5. Correlation between age and parasite density.

	Age	P.value
Parasite density	-0.023 (rho)	0.689

Figure 2. Correlation between age and parasite density

Discussion

Mechanism of thrombocytopenia in malaria infection stated that platelets engulf malaria parasites, in the process of engulfing platelets are damaged and thus being removed from circulation reduced platelet counts during malaria infection result from platelet activation; splenic pooling and the clumping of platelets have also been suggested as the reasons for thrombocytopenia [13]. The study showed that 93.8% of the studied population were infected with *P.falciparum*, while 6.2% were infected with *P.vivax* and no mixed infection with both *P.falciparum* and *P.vivax*. These results were comparable to the study carried out by **Shah et al.** [14] who showed that 80 % of cases were infected with *P.falciparum* and 18.7% of cases infected with *P.vivax* and no mixed infections were reported out of total 100 cases.

Among the various haematological changes in malaria, thrombocytopenia in the most consistent one. In this study high parasitaemia was associated with thrombocytopenia. This finding was in agreement with the finding obtained by **Ladhani et al.** [9] who showed that thrombocytopenia was strongly associated with the degree of parasitaemia. The present study reflected that there was significant difference between low platelets count and high parasitaemia, these findings were in line with the finding of **Abdalsayed** [15] who reported that there was significant difference between low platelets count and high parasitaemia. The present study

indicated that all age groups ranging from 5-70 years were affected by malaria. These findings were similar to findings obtained by **Harani et al.** [16] who reported the age was ranged between 2-72 years was affected by malaria. The current study illustrated that the levels of parasitaemia were decreased by age increased. This finding was in line with the finding obtained by **Mahmood and Yasir** [10]. As far as gender was concerned, the results showed that the difference in rates of malaria infection was found to be statistically insignificant; this finding was consistent with the finding obtained by **AbdAlsayed** [15].

Conclusion

This study concluded that *P.falciparum* was significantly more frequent than *P.vivax* and there was a negative relation between the platelet count and parasite density. From the finding of the study even if other hematological parameters were altered in malaria infection, reduced number of platelet count was a significant marker for the malaria infection. Identification of thrombocytopenia was important not only as a screening tool for identification of malaria in endemic regions but also has important prognostic significance. In an endemic area the platelet count has to be checked, because the presence of thrombocytopenia may indicate malaria infection.

Recommendations

More efforts control infection in Omdurman province should be applied. Eradication of the vector responsible of malaria transmission in the area under study. A physician that suspects a patient for malaria diagnosis using clinical manifestations only can use platelet parameter as another supporting marker. If laboratory technologists register severe thrombocytopenia in parallel with marked anemia in patients that live around malaria endemic area, malaria diagnosis should be considered. Since finding of low platelet count in patients significantly indicates the presence of malaria, laboratory technologists are highly recommended to consciously scan, detect and identify malaria parasite before reporting it as negative for hem parasite.

Acknowledgement

The authors are grateful to Medical Military Hospital Administrations for their co-operation and kindness. Also are thanks all patients who donate blood samples.

Funding: None declared.

Competing interests: None.

References

- 1-**Singh N, Shukla MM, Shukla MK, Mehra RK, Sharma S, Bharti PK, et al.** Field and laboratory comparative evaluation of rapid malaria diagnostic test versus traditional and molecular techniques in India. *Malaria Journal* 2010; 9:191.
- 2-**Greenwood D, Barer M, Slack, R, Irving W.** *Medical Microbiology*. 18th edition, China. 2012; 642.
- 3-**World Health Organization (WHO).** Malaria fact sheets. 2015. Available at: <http://www.who.int/mediacentre/factsheets/fs094/en>.
- 4-**Talaro KP, Chess B.** *Foundation in Microbiology*, 8th edition. Mc Graw-Hill, New York. 2012; pages 31- 35.
- 5-**Lamb TJ.** *Immunity to Parasitic Infection*. 1st edition, New Delhi- India. 2012; pages 91-104.
- 6-**National Institutes of Health (NIH).** Malaria quick facts. 2010. Available at: <http://www.niaid.nih.gov/topics/Malaria/understandingMalaria/Pages/facts.aspx>.
- 7-**Bakhubaira S.** Hematological parameters in severe complicated *Plasmodium falciparum* malaria among adults in Aden. *Turk J Haematol* 2013; 30:394-399.
- 8-**van Wolfswinkel ME, Vliegthart-Jongbloed K, De Mendonca MM, Wever PC, McCall MB, Koelewijn R, et al.** Predictive value of lymphocytopenia and the neutrophil-lymphocyte count ratio for severe imported malaria. *Malar J* 2013; 12:101.
- 9-**Ladhani S, Lowe B, Cole AO, Kowuondo K, Newton CR.** Changes in white blood cells and platelets in children with *falciparum* malaria: relationship to disease outcome. *Br J Haematol* 2002; 119(3):839-847.
- 10-**Mahmood A, Yasir M.** Thrombocytopenia: a predictor of malaria among febrile patients in Liberia. *Infect Dis J* 2008; 14:41-44.
- 11-**World Malaria Report.** 2010. Available at www.who.int/malaria/publications/atoz/9789241564106/en/.
- 12-**Kotepui M, Phunphuech B, Phiwklam N, Chupeerach C, Duangmano S.** Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border. *Malaria Journal* 2014; 13:218.
- 13-**Igbeneghu C, Odaibo AB.** Impact of acute malaria on some haematological parameter. *Journal of medical principle and practice*. Nigeria 2013; 13 (9):36-38.
- 14-**Shah UB, Shah AM, Dave KK, Sakera BL, Gonsai RN.** Comparative study of microscopic detection methods with hematological changes and coagulation profile in malaria. *I.M.A.G.S.B. News Bulletin* 2007; 2(7): 37- 40.
- 15-**AbdAlsayed MO.** Association between malaria parasites and platelets count. M.Sc. thesis, Alzaiem Alazhari University 2010.
- 16-**Harani MS, Beg MA, Khaleeq, L, Adil SN, Kakepoto GN, Kurshid M.** Role of ICT Malaria Immunochromatographic Test for Rapid diagnosis of Malaria. *Journal of Pakistan Medical Association* 2006; 56:4.