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(RESEARCH ARTICLE)

Diagnostic and prognostic value of thrombocytopenia severity in Sudanese children with Falciparum malaria

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Abstract

Malaria remains one of the most significant global public health challenges, with more than 200 million clinical cases worldwide each year. Falciparum malaria accounting for up to 87.6 % of malaria cases in Sudan. Platelets abnormalities as thrombocytopenia especially severe thrombocytopenia have been associated with mortality in patients with *P. falciparum* infection. The aim of this study was to study the relationships between thrombocytopenia and their severity with falciparum malaria severity, parasitemia and parasite count. This study was case-control hospital based study conducted among 200 Sudanese children admitted to Wad Medani Pediatric Hospital, Wad Medani, Sudan during period September to December 2018. The study population was divided into two groups. Group 1 (UM) was 100 children with uncomplicated *falciparum* malaria. Group 2 (SM) was 100 children with severe *falciparum* malaria were diagnosed by WHO criteria. A 2.5 ml venous blood sample was collected from each children. The malaria parasitemia was determined from thick blood films using plus system. The parasite count (% of parasitized red cells counting) was counted from thin blood film. Platelets count was determined using the Sysmex XP 300 N automated hematology analyzer (Sysmex, Kobe, Japan) and confirmed and assessed using stained thin blood film. The data were analyzed using SPSS software (V 20.0) and Stat disk software (V 13.0). The mean age and male: female ratio of Group 1 (UM) were 8.83 ± 4.20 years and 1:1.22 respectively and for Group 2 (SM) were 8.63 ± 3.40 years and 1.56:1 respectively. Group 2 (61 %) associated with thrombocytopenia (mean PLTs 160.91 ± 186.24 × 10^9/) more than Group 1 (25 %) (mean PLTs 221.10 ± 98.69 × 10^9/L) (P value = 0.000). The mild, moderate and severe thrombocytopenia account for 19 %, 5 % and 1 % respectively among Group 1; and 22 %, 28 % and 11 % respectively among Group 2 (P value 0.003). Malaria thrombocytopenia was significantly associated (P value = 0.000) and negatively correlated (P value = 0.000; r = - 0.341) with malaria parasitemia. The mean parasite count in malaria thrombocytopenia (0.77 ± 0.51 %) was higher than malaria without thrombocytopenia (0.53 ± 0.36 %) (P value 0.000). Thrombocytopenia severity was significantly positive correlated with malaria parasitemia (r = 258; P value = 0.017) and parasite count (r = 0.229; P value = 0.034). The study concluded that the severe thrombocytopenia associated commonly with severe falciparum malaria and falciparum malaria hyperparasitemia.

Keywords: Platelets count; Severe thrombocytopenia; Severe Falciparum malaria; Falciparum malaria hyperparasitemia; Sudanese children.

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1. Introduction

Falciparum malaria is the most virulent and pathogenic form of malaria (1), it is stills an important threat to public health in sub-Saharan Africa, and outside of Africa, particularly in young children, pregnant women and non-immune adults in communities with poor resources (2, 3). Falciparum malaria accounting for up to 80% of malaria cases globally (WHO, 2018) and 87.6% in Sudan (4, 5).

Falciparum malaria associated with several hematological changes that involve the major blood cell lines such as red blood cells, white blood cells and platelets (6, 7), which play a significant role in severity of falciparum malaria (8, 9). Malaria hematological changes arising from hemolysis, host immune (inflammatory) response, bone marrow suppression, and splenic pooling (10).

Thrombocytopenia (platelet count less than 150 x 10^9/L) is common platelets abnormalities and hematological changes (7, 11) as well as a common feature of malaria due to all plasmodium species particularly in falciparum malaria (occurs in up to 70% of falciparum malaria patients) (11, 12). Platelet survival is reduced to 2-4 days in severe falciparum malaria (7, 13). The pathophysiology of malaria associated thrombocytopenia and reduced platelet survival rate are multifactorial. It has been associated with a variety of hematological insults arising from hemolysis, host inflammatory response, hematopoietic suppression, enhanced splenic uptake or sequestration and DIC (platelets may be removed from the circulation at sites of fibrin deposition) (3, 13). Some but not all studies have shown that there is strong association between thrombocytopenia and severity of malaria (3, 7, 14). Severity and mortality of patients with falciparum malaria are increased with severe thrombocytopenia (3).

Previous studies have shown a consistent inverse correlation between parasitemia and the platelet count (3, 15-17). Patients with platelet counts < 150,000/μL are 12-15 times more likely to have malaria infection than persons with platelet counts > 150,000/μL (7).

The present study aimed to study the relationships between thrombocytopenia and their severity with falciparum malaria severity, parasitemia and parasite count among Sudanese children.

2. Methods

This is study was case control hospital based study, conducted in Wad Medani Pediatric Hospital in collaboration with Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan as part of a wider research project studying the association between TNF-α levels and TNF-α 238 alleles polymorphisms and falciparum malaria anemia. The study population was divided into two groups. Group 1 (UM) was 100 subjects previously diagnosed as uncomplicated *falciparum* malaria by blood film or ICT. Group 2 (SM) was 100 subjects previously diagnosed as severe *falciparum* malaria by blood film and WHO criteria (18).

All study procedures were approved by the Ethics Committees of Ministry of Health, Gezira State and Faculty of Medical Laboratory Sciences, University of Gezira, Sudan. Informed consent was written from each participant parents.

A 2.5 ml venous blood samples were collected by clean venipuncture in K_3EDTA containers. Thin and thick films were prepared immediately. Malaria parasitemia was determined from thick blood films using plus system (19). Parasite count (% of parasitized red cells counting) was counted from thin blood film (18). Platelets count was determined using the Sysmex XP 300 N automated hematology analyzer (Sysmex, Kobe, Japan). The platelets count was confirmed and assessed using stained thin blood film. Thrombocytopenia was defined as a platelets count of less than 150 × 10^9/L (20). Mild thrombocytopenia was defined as a platelets count between 100 and 150 × 10^9/μl, moderate thrombocytopenia was defined as between 50 and 99 × 10^9/μl and severe thrombocytopenia was defined as below 50 × 10^9/μl (20). The data were analyzed using SPSS software (V 20.0) and Stat disk software (V 13.0)..

3. Results

Study conducted on two groups; Group 1 (UM) (100 children with uncomplicated falciparum malaria) and Group 2 (SM) (100 children with severe falciparum malaria). The mean age of Groups 1 and 2 were 8.83 ± 4.20 years and 8.63 ± 3.40 years respectively (P value = 0.712). While the male: female ratio in Groups 1 and 2 were 1: 1.122 and 1.56: 1 respectively (P value = 0.023). Most cases in two Groups in age group between 6 – 10 years (41 % in group 1; 47 % in group 2). Most cases in Group 1 from rural area (70 %), while approximately equal in Group 2 (P value = 0.002) (Table 1).

Table 1 Demographic characteristics of study participants.

Factors	Group 1 (UM) N = 100	Group 2 (SM) N= 100	P value *
Age (years)			
Mean ± SD	8.83 ± 4.20	8.63 ± 3.40	0.712
Age group (years)			
Less than 5 years	24 (24 %)	19 (19 %)	
6 – 10 years	41 (41 %)	47 (47 %)	
11 – 15 years	29 (29 %)	33 (33 %)	0.185
More than 15 years	6 (6 %)	1 (1 %)	
Gender			
Male: Female ratio	1:1.22	1.56:1	0.023
Residence			
Rural	70	49	0.002
Urban	30	51	

* P value > 0.05

The hyperparasitemia (+++ and ++++) account for 93 % in Group 2 and 32 % among Group 1 (P value = 0.000) (Table 1). Most cases in group 1 had parasite count < 0.5 % (80 %) (mean of parasite count % was 0.39 ± 0.30 %), while most cases in group 2 (mean of parasite count % was 0.88 ± 0.42 %) had parasite count > 0.5 % (86 %) (P value = 0.000) (Table 2).

The mean PLTs in Group 2 (160.91 ± 186.24 × 10^9/L) was lower than Group 1 (221.10 ± 98.69 × 10^9/L), giving statistically highly significant differences between them (P value = 0.005) (Table 2). PLTs had significant negative correlation within malaria parasitemia (r = - 0.286; P value = 0.000;) and malaria parasite count (r = - 0.450; P value = 0.000) (Table 8).

Table 2 Clinical characteristics of study participants.

Factors	Group 1 (UM) N = 100	Group 2 (SM) N= 100	P value *
Parasitemia			
+	43 (43 %)	4 (4 %)	
++	25 (25 %)	3 (3 %)	0.000
+++	14 (14 %)	21 (21 %)	
++++	18 (18 %)	72 (72 %)	
Parasite count (%) (Mean ± SD)	0.39 ± 0.30	0.88 ± 0.42	0.000
Parasite count (%) frequency			
< 0.5 %	80	16	
0.5 – 1 %	13	54	0.000
> 1 %	7	30	
PLTs × 10^9/L (Mean ± SD)	191.01 ± 151.69	290.91 ± 97.77	0.000

* P value > 0.05

Group 2 (61 %) associated with thrombocytopenia more than Group 1 (25 %) (P value = 0.000) (Figure 1).

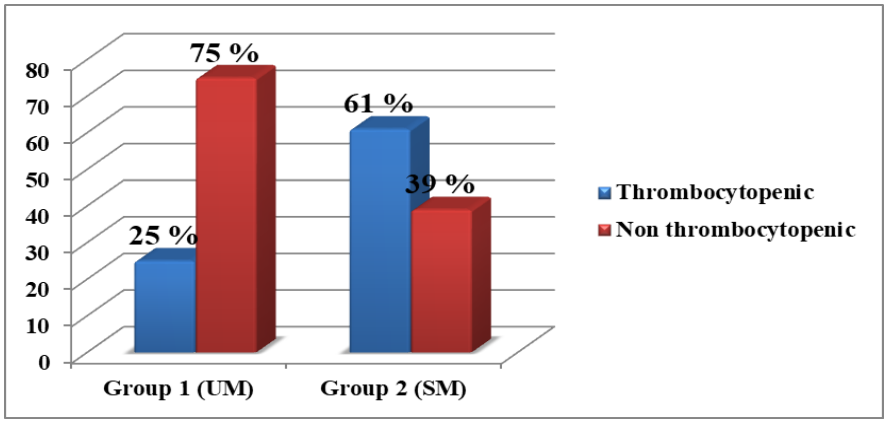

Figure 1 Association between malaria severity and malaria thrombocytopenia (P value = 0.000).

Malaria thrombocytopenia account for 7/47 in (+), 7/28 in (++), 21/35 in (+++), and 51/90 in (++++) giving highly significant association (P value = 0.000) (Table 3) and significant negative correlation (P value = 0.000; r = - 0.341) (Figure 2) between malaria thrombocytopenia and parasitemia.

Table 3 Association between malaria parasitemia and malaria thrombocytopenia.

Parameters	**+** **N=47**	**++** **N=28**	**+++** **N=35**	**++++** **N=90**	**P value ***
Thrombocytopenic	7	7	21	51	0.000
Non-thrombocytopenic	40	21	14	39	

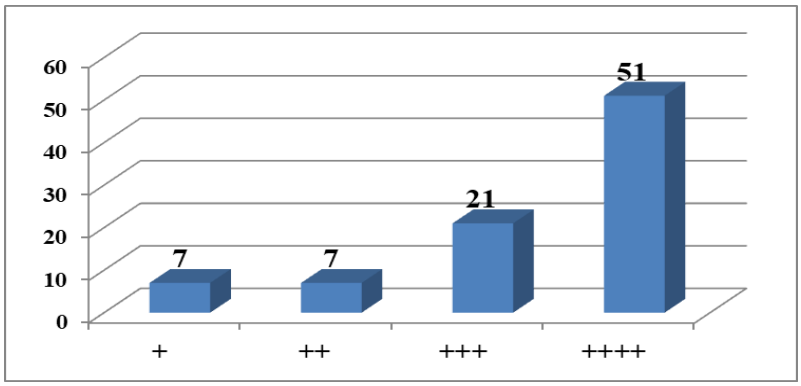

Figure 2 Correlation between malaria parasitemia and malaria thrombocytopenia (P value = 0.000, r = - 0.341).

The mean parasite count in children with malaria thrombocytopenia (0.77 ± 0.51 %) was higher than children with malaria without thrombocytopenia (0.53 ± 0.36 %), giving statistically highly significant differences between them (P value 0.000) (Table 4).

Table 4 Compare of parasite count % between children with thrombocytopenia and without thrombocytopenia.

Parameters	**Thrombocytopenic** **N = 86**	**Non-thrombocytopenic** **N = 114**	**P value ***
Parasite count (%)	0.77 ± 0.51	0.53 ± 0.36	0.000

* P value > 0.05

The mild, moderate and severe thrombocytopenia account for 19 %, 5 % and 1 % respectively among Group 1; 22 %, 28 % and 11 % among Group 2 (P value 0.003) (Table 5).

Table 5 Association between malaria severity and of thrombocytopenia severity.

Thrombocytopenia severity	Group 1 (UM) N = 100	Group 2 (SM) N= 100	P value *
Mild thrombocytopenia	19	22	0.003
Moderate thrombocytopenia	5	28	
Severe thrombocytopenia	1	11	

* P value > 0.05

Despite the most mild, moderate and severe thrombocytopenia cases associated with malaria hyperparasitemia (+++ and ++++), there were no statistically significant association between malaria parasitemia and thrombocytopenia severity (P value = 0.146) (Table 6), and there was significant positive correlation between them (r = 258; P value = 0.017) (Table 8). Severe thrombocytopenia associated with malaria hyperparasitemia (4 with +++, 8 with ++++) (Table 6).

Table 6 Association between malaria parasitemia and thrombocytopenia severity.

Thrombocytopenia severity	+ N=47	++ N=28	+++ N=35	++++ N=90	P value *
Mild thrombocytopenia	6	4	12	19	0.146
Moderate thrombocytopenia	1	3	5	24	
Severe thrombocytopenia	0	0	4	8	

* P value > 0.05

Despite no differences in mean of parasite count % between thrombocytopenia severity (P value = 0.514) (Table 7), there was significant positive correlation between them (r = 0.229; P value = 0.034) (Table 8).

Table 7 Compare of parasite count % between thrombocytopenia severity.

Parameters	Mild Thrombocytopenia N = 41	Moderate Thrombocytopenia N = 33	Severe Thrombocytopenia N = 12	P value *
Parasite count (%)	0.71 ± 0.59	0.81 ± 0.47	0.88 ± 0.22	0.514

* P value > 0.05

Table 8 Correlation between PLTs and thrombocytopenia severity with malaria parasitemia and parasite count.

Parameters		Parasitemia	Parasite count %
Platelets count × 103/µl	Correlation coefficient	- 0.286	- 0.205
	P value*	0.000	0.004
Thrombocytopenia severity	Correlation coefficient	0.258	0.229
	P value*	0.017	0.034

* P value > 0.05

Most children with severe thrombocytopenia associated with severe falciparum malaria (11 out of 12, 91.7 %), malaria hyperparasitemia (4 with +++, 8 with ++++), and parasite count > 0.5 % (11 out of 12, 91.7 %).

4. Discussion

Falciparum malaria is still a major health problem in Sudan accounts for up to 80% of malaria cases globally (21) and about 87.6% of malaria cases in Sudan (4, 5). Poor sanitation and absence of majors protective is significantly leading

to increased prevalence of the disease. Children suffer more malaria episodes and are more prone to severe malaria compared to adults and accounted for 61% (266 000) of all malaria deaths. In fact, about 285,000 children died before their fifth birthdays in 2016 in Africa According to the World Health Organization (21). therefore malaria remains the largest cause of children death in Africa (22). The association of thrombocytopenia (Low Platelets count) with all plasmodium malaria types generally and falciparum malaria particularly is an equivocal. The current study aimed to throw a light on thrombocytopenia severity association with malaria severity, parasitemia and parasite count among Sudanese children.

Study conducted on two groups; Group 1 (UM): 100 children with uncomplicated (mean age 8.83 ± 4.20 years) falciparum malaria and Group 2 (SM): 100 children with severe falciparum malaria (mean age 8.63 ± 3.40 years). Similar studies were reported from different endemic areas in Africa (23-25).

In the present study male: female ratio was higher in Group 2 (1.56: 1) than Group 1 (1: 1.122) (P value = 0.023). Furthermore 81 (40.5%) patients from Urban; while 119 (59.5%) patients from rural (P value = 0.002). Similarly, survey was done in Sudan in 21,988 individuals to show the prevalence of malaria and results showed infection was higher in male more than female, and also higher in ruler area compare to urban (26).

The hyperparasitemia was most common Group 2 (93%) compared to Group 1 (32 %) (P value = 0.000).

Malaria thrombocytopenia was common in SM (62%) compared to UM (25%). The mild, moderate and severe thrombocytopenia were account (31%, 30% and 3% respectively). Platelet survival is reduced to 2 – 4 days in severe falciparum malaria (13). Gérardin et al reported that thrombocytopenia most common in SM (74.6%) compared to UM (25.4%) (17). Multiple studies in endemic area reported thrombocytopenia were observed in 73.6% (27), 70% (11), 85.5% of SM (28) of falciparum malaria children. The prospective study was conducted on 200 diagnosed cases of malaria. The data showed that 171 (85.5%) patients were having low platelet count; 141 (70.5%) had mild, 21 (10.5%) moderate, and 9 (4.5%) had severe thrombocytopenia. Twenty-nine (14.5%) patients had normal platelet count (29). Study done in Ethiopia observed that the thrombocytopenia was found in 56.8%, while mild, moderate, and severe thrombocytopenia accounted for 41.6%, 45.3% and 13.1% (23). Similar studies in endemic area found the severe thrombocytopenia was 8.8% in 45.6% of thrombocytopenia in France, 14.1% in 43.6% of thrombocytopenia in Senegal, 14.8% and in 58% of thrombocytopenia in Libreville (Moulin *et al*., 2003), 5% in 49% of thrombocytopenia in Kenya (30), 1.5% in 48.6% of thrombocytopenia in Colombia (31), and 12.9% in 68.7% of thrombocytopenia in India (32). Similar previous studies observed that the thrombocytopenia was significantly more frequent and more profound in those with SM than in those with mild forms (31, 33).

Thrombocytopenia had strong negative correlation with parasitemia (r – 0.335, P value 0.000), and parasite count (r – 0.268, P value 0.000). Previous studies concluded that the thrombocytopenia is associated with peripheral parasitemia levels (14, 30, 32, 34-36). The average of platelets count among Group 2 was lower than Group 2 (P value 0.000). The study done in Kenya showed the platelets count were significantly lower in malaria-infected children (30).

Children with thrombocytopenia were 24.4 times (odds ratio) more likely to have falciparum malaria. Maina et al., reported that children with platelets count of <150,000/uL were 13.8 times (odds ratio) more likely to have malaria. Similar previous study concluded that Individuals with platelet counts < 150,000/µL are 12-15 times more likely to have malaria infection than persons with platelet counts > 150,000/µL. The risk of thrombocytopenia was 36.0% higher for SM (2.44 times) compared to UM, and 38.9% higher for hyperparasitemia (3.08 times) compared to non hyperparasitemia. The risk of severe thrombocytopenia is 14.0% higher for SM (4.50 times) compared to UM, and 16.2% higher for hyperparasitemia compared to non hyperparasitemia. Previous study demonstrated that malaria parasite has a direct lytic effect on the platelets (33).

Thrombocytopenia is a frequent finding in falciparum malaria and possible causes are due to decreased platelets production, increased levels of cytokines and immunological destruction due to antibody and cellular immune responses, Oxidative stress damage of platelets, removal of platelets from circulation through enhanced splenic uptake and sequestration or by of fibrin deposition. In addition may result from antibodies, produced against the antimalarial drugs quinine and quinidine, interacting with platelets (7, 13, 15).

5. Conclusion

The study concluded that the thrombocytopenia associated commonly with severe falciparum malaria and falciparum malaria hyperparasitemia particularly severe thrombocytopenia (PLTs > 50×10^9/µl). So thrombocytopenia and their severity may help to assess the disease severity and to improve the management of falciparum malaria among patients.

Compliance with ethical standards

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