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Assessment of Prothrombin Time and Activated Partial Thrombin Time among Sudanese Patients with Falciparum Malaria Infection

Khalid Abdelsamea Mohamedahmed[1,2]*Zainab Omer Mohammed[1]; Khansaa Mohammed Gaberallah[1]

[1]Department of Hematology and Immunohematology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan.

[2]Department of Immunology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan

Abstract: Background: *Malaria remains the one of major health problems globally with increased morbidity and mortality, especially P. falciparum that can cause various complications involving various systems of the body and complications. Coagulopathy is one of these complications.* ***Objectives:*** *we aimed to evaluate the coagulation profiles (PT, INR, and APTT) among patients with falciparum malaria infection.* ***Methodology:*** *A case-control laboratory-based study was conducted in Wad Medani Teaching Hospital, Gezira State, Sudan among 200 subjects (100 patients with falciparum malaria [mean age 20.96 ± 17.65 years; 36% male] and 100 as healthy control [mean age 21.62 ± 12.79 years; 49% male]) in the period from November 2017 to August 2018. 1.8 ml of venous blood was collected. The degree of parasitemia was determined directly from thick blood film. The platelet-poor plasma was obtained by centrifugation of blood at 1200 – 2000 rpm for 15minutes. PT, INR, and APTT were measured by using Coatron M4 coagulometer. SPSS software (V 20.0) was used for data analysis.* ***Results:*** *61 patients (61%) had high PT level where 39 patients (39%) had normal PT with a mean (15.8 ± 1.82 sec); while 77 patients (77%) had high INR compared to 23 patients (23%) had normal INR with a mean (1.19 ± 0.22). Furthermore, 74 patients (74%) had a normal APTT compared to 26 patients (26%) with prolonged APTT, with a mean (37.9 ± 4.67 sec). Significant results in the PT, INR, and APTT in falciparum malaria patients were detected when compared with control (P-value = 0.000, 0.000, and 0.000 respectively).* ***Conclusion:*** *Falciparum malaria causes significant derangement in the coagulation process (intrinsic, extrinsic, and common pathways) so causes prolongation of PT, INR, and APTT. So evaluation of coagulation profiles may be helpful in the management and outcome of patients.*

Keywords: Falciparum malaria, Sudan, PT, INR, APTT, Degree of parasitemia.

Introduction:

Malaria is a human intracellular protozoan parasitic disease caused by feeding female anopheline mosquitoes inoculated genus *Plasmodium* parasite in infected erythrocytes [(1)]. There are five species of plasmodium known to infect humans: *P. falciparum*, *P.* vivax, *P. malariae*, *P. ovale*, and *P. knowlesi* [(2)]. The disease is one of the leading causes of mortality and morbidity in the world especially in Africa [(3)]. In 2017, an estimated 219 million cases of malaria occurred and 435, 000 deaths globally. Children aged under 5 years are the most vulnerable group affected by malaria and accounted for 61% (266 000) of all malaria deaths. Most malaria cases in 2017 were in the WHO African Region (200 million or 92%) and accounted for 93% of all malaria death, 80% of the global malaria burden occurs in countries in sub-Saharan Africa and India. *Plasmodium falciparum* is the most prevalent malaria parasite in the WHO African Region, accounting for 99.7% [(4)] and about 87.6% of malaria cases in Sudan [(5-6)]. It is the most virulent and pathogenic form, causing the most morbidity and mortality in humans [(2)].

Falciparum Malaria can be categorized into two groups: uncomplicated or complicated (severe). The classical uncomplicated malaria (UM) has three stages (cold stage, hot stage, and sweating stage) [(7)]. If falciparum malaria is not treated properly may occur infection red blood cells with malaria parasite; the cells stick to the walls of blood vessels, as the blood vessels become blocked, the blood supply to vital organs stops so malaria is associated with life threading complications and result in severe disease. The following complications: cerebral malaria, severe anemia, hemoglobinuria, pulmonary edema, thrombocytopenia, cardiovascular collapse, shock, kidney failure, hyperparasitemia, metabolic acidosis, and hypoglycemia [(8-9)].

Numerous studies have shown that there is undoubtedly an increase in coagulation activity in malaria, circulating immune complex and inflammatory mediators (e.g. TNF-α, IL-6, and Interferon) has an important role in coagulation activation all this depend on the individual immune response to disease, malaria parasite density, type of Plasmodium spp and clinical status. Also, an increase of neutrophil elastase causes derogation of the fibrin stabilizing factor XIII. At last, the hemostatic tests on these patients will be varied from one patient to another and important to evaluate [(10)]. Common hematological changes that have been reported among falciparum malaria are anemia, thrombocytopenia, atypical lymphocytosis, and infrequently disseminated intravascular coagulation (DIC), leucopenia or leukocytosis, neutropenia, neutrophilia, eosinophilia, and monocytosis [(11-13)].

There is a direct interaction between the parasites and the endothelium of the microcirculation causes endothelial cell injury and sets up a series of reactions characterized by the release of a large variety of cytokines and inflammatory mediators by the endothelium, these, in turn, activate the coagulation pathway leading to widespread thrombin deposition in small arteries and arterioles (disseminated intravascular coagulation) [(14)].

During severe and mild malarial infection, the activation of the coagulation system leading to in vivo thrombin generation. The stimulation of the coagulation system is caused by various procoagulants present during malarial infection. Furthermore, certain substances that are released during malarial infection as TNF α and histamine are additional factors that promote fibrin formation. The degree of activation is proportional to disease severity. In addition; the plasma anti-thrombin (AT) activity decreased within increased concentrations of thrombin anti-thrombin (TAT) complexes, which were proportional to disease severity. Also, defects in inhibitors of coagulation Protein C, protein S, and AT levels were found to be low in P. falciparum, particularly in complicated cases. Tissue factor expression it has been shown that *falciparum*-infected erythrocytes induce TF expression in endothelial cells [(15)].

Patients and Methods:

Participants and setting

The study was a case-control hospital-based study, conducted in Wad Medani Teaching Hospital, Gezira State, Sudan among 200 subjects in the period from November 2017 to August 2018. 100 patients (mean age 20.96 ± 17.65 years; 36% male) previously diagnosed as patients with falciparum malaria infection and 100 normal healthy controls (mean age 21.62 ± 12.79 years; 49% male) according to inclusion and exclusion criteria.

The study included patients with falciparum malaria, from both genders, and residing in Gezira State who were admitted to Wad Medani Teaching Hospital.

The study excluded patients with mixed malaria or vivax malaria, those residing outside Gezira State, those suffering from a recent infection, malignancy, liver diseases, vitamin K deficiency, DIC, and thrombosis, and those on anticoagulant and anti-inflammatory medication.

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

Data were collected on the patient's history of the disease and everything related to personal data during staying in the hospital using a good design questionnaire.

Sampling and investigations

A 1.8 ml venous blood samples were obtained by clean venipuncture in Trisodium citrate containers at the time of admission. Thin and thick films were prepared immediately. Thick blood film was stained with Giemsa's stain for 10 minutes. Thin blood film was stained by leishmen's stain for 3 minutes and a buffer for 7 minutes. Blood films were examined by a microscope using an oil emersion lens to confirmed the presence of malaria parasites (thick film) and to determine the falciparum parasites stage. Parasitemia was determined from thick blood films using plus system [(9)] as shown in Table 1.

Table 1. Plus system for parasite density:

Degree of parasitemia	Parasite estimate
+	1 – 10 parasite per 100 fields
++	11 – 100 parasite per 100 fields
+++	1 – 10 parasite per single field
++++	More than 10 parasites per single field

The platelets poor plasma was obtained by centrifugation of blood at 1200 – 2000 rpm for 15 minutes. Prothrombin Time (PT), International Normalize Ratio (INR), and Activated Partial Thrombin Time (APTT) were measured using a Coatron M4 coagulometer.

Statistical analysis

Comparison of coagulation profiles between study group, between severe and mild falciparum malaria and between gender were analyzed using T-test. comparison of coagulation profiles between malaria parasitemia was analyzed using One Away ANOVA. The data were analyzed using IBM® SPSS software (V 20.0) and Statdisk software (V 13.0).

Results:

This study was included 200 subjects (100 patients with *falciparum* malaria infection and 100 normal healthy controls from different age groups and both gender. Among 100 *falciparum* malaria patients, 64% were female and 36% were male (mean age 20.96 ± 17.65 years). The age group between (1 – 10) years was the most frequent age group (36%). Most of the study population were from urban areas 60 (60%). 66 (66%) of the study population at the primary level of education. Headache was the most frequent symptom distributed in this population of 41 (41%). The most malaria infection frequency was less than 1 year (71%). 91% of patients have mild falciparum malaria. Most of the patients were attended to the hospital after 2 days of the presence of symptoms (73%). Most of the patients diagnosed by the finding of the ring-stage of *P. falciparum* was 99 (99%) (Table 2). The most frequent parasite density was (+) (51%) (Figure1).

Table 2. Demographic and clinical characteristics of study population.

Factors	Patients	Controls

	N = 100	N= 100
Age (years)		
Mean ± SD	20.96 ± 17.65	21.62 ± 12.79
Age group (years)		
Less than 10 years	36 (36%)	19 (19%)
11 – 20 years	20 (20%)	39 (39%)
21 – 30 years	14 (14%)	22 (22%)
31 – 40 years	11 (11%)	9 (9%)
More than 40 years	19 (19%)	11 (11%)
Gender		
Male	36 (36%)	49 (49%)
Female	64 (64%)	51 (51%)
Residence		
Rural	60 (60%)	60 (60%)
Urban	40 (40%)	40 (40%)
Clinical status		
Mild malaria	91 (91%)	
Severe malaria	9 (9%)	
P. falciparum stage		
Ring stage	99 (99%)	
Gametocyte	1 (1%)	
Frequency of disease		
Less than 1 year	71 (71%)	
1 – 2 years	20 (20%)	
More than 2 years	9 (9%)	
Symptoms duration		
Less than 2 days	27 (27%)	
2 – 4 days	43 (43%)	
More than 4 days	30 (30%)	

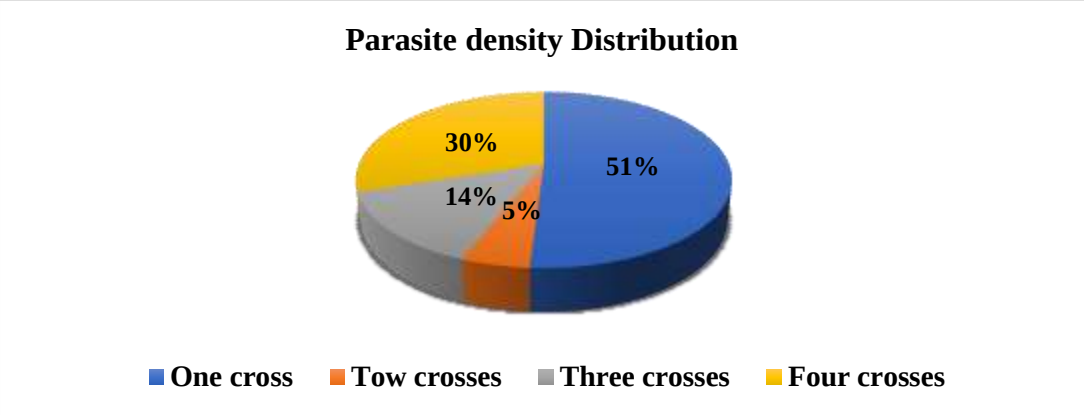

Figure 1. Distribution of parasite density among patients.

Most of the patients have prolonged PT (61%; mean = 15.8 ± 1.82 sec); prolonged INR (77%; mean = 1.19 ± 0.22) and normal APTT (74%; mean = 37.9 ± 4.67 sec) (Table 3).

Table 3. frequency of PT, INR and APTT among patients.

Parameters	Normal	Prolonged
PT (sec)	39 (39%)	61 (61%)
INR	23 (23%)	77 (77%)
APTT (sec)	74 (74%)	26 (26%)

The mean of PT, INR, and APTT was 15.8 ± 1.82 sec, 1.19 ± 0.22, and 37.9 ± 4.67 sec respectively among patients compared to 14.3 ± 1.54 sec, 1.11 ± 0.25 and 34.3 ± 3.00 sec respectively among healthy control giving highly significant statistical differences between them (P value = 0.000, 0.000, and 0.000 respectively) (Table 4).

Table 4. Comparison of PT, INR and APTT between patients and controls.

Parameters	Patients (N = 100)	Controls (N = 100)	P value *
PT (sec)	15.8 ± 1.8	14.3 ± 1.5	**0.000**
INR	1.19 ± 0.22	1.11 ± 0.25	**0.000**
APTT (sec)	37.9 ± 4.67	34.3 ± 3.00	**0.000**

* P value > 0.05

There were no significant differences in the mean of PT, INR, and APTT between patients with mild malaria and patients with severe malaria (P value = 0.090, 0.055, and 0.336 respectively) (Table 5).

Table 5. Comparison of PT, INR and APTT between clinical status.

Parameters	Mild malaria (N = 91)	Severe malaria (N = 9)	P value *
PT (sec)	15.7 ± 1.3	16.7 ± 2.2	0.090
INR	1.19 ± 0.10	1.24 ± 0.29	0.055
APTT (sec)	37.9 ± 4.8	39.2 ± 3.00	0.336

* P value > 0.05

There were no significant differences in the mean of PT, INR, and APTT of *falciparum* malaria patients between degrees of parasitemia (P value = 0.508, 0.087 and 0.424 respectively) (Table 6).

Table 6. Comparison of PT, INR and APTT between degree of parasitemia.

Parameters	+ (N = 51)	++ (N = 5)	+++ (N = 14)	++++ (N = 30)	P value *
PT (sec)	15.6 ± 1.8	15.3 ± 1.0	16.2 ± 2.1	16.0 ± 1.9	0.508
INR	1.15 ± 0.11	1.14 ± 0.07	1.22 ± 0.26	1.27 ± 0.31	0.087
APTT (sec)	37.8 ± 5.2	37.0 ± 8.9	37.2 ± 3.0	38.6 ± 3.5	0.424

* P value > 0.05

There were significant differences in mean of PT and INR between males and females (*P value* = 0.001 and 0.022 respectively) (Table 7).

Table 7. Comparison of PT, INR and APTT between male and female.

Parameters	Male (N = 36)	Female (N = 64)	P value *
PT (sec)	16.1 ± 2.4	15.6 ± 1.4	**0.001**
INR	1.20 ± 0.27	1.10 ± 0.18	**0.022**
APTT (sec)	37.3 ± 4.2	38.3 ± 4.93	0.336

* P value > 0.05

Discussion:

Falciparum malaria accounting for up to 80% of malaria cases globally and is associated with more intense parasitemia [16]. It is the most virulent and pathogenic form, causing the most morbidity and mortality in humans [2], particularly those who are high risk namely young children, pregnant women, and non-immune adults in endemic areas [17].

Coagulation abnormalities are frequently found in patients with severe malaria. Clinically apparent bleeding or disseminated intravascular coagulation (DIC) is associated with very severe disease and high mortality. Bleeding in severe malaria results from several pathological processes such as thrombocytopenia, consumptive coagulopathy, and impaired clotting factor synthesis [15].

The study was a case-control study, 100 cases, and 100 controls were participated according to inclusion and exclusion criteria. From 100 falciparum malaria patients; 36 (36%) were males and 64 (64%) were females. The age groups of the study population were 38 (38%) aged 1-10 years, 19 (19%) aged between 11-20 years, 13 (13%) aged between 21-30 years, 11 (11%) aged between 31-40, and 19 (19%) more than 40 years.

The means of PT (prolonged in 61% of patients), INR (prolonged in 77% of patients), and APTT (prolonged in 26% of patients) were prolonged in cases compared to control (*P value* = 0.000, 0.000, and 0.000 respectively). This finding was consistent with similar previous studies [10, 14, 15, 18,19].

During the bite of an infected female mosquito, sporozoites are released into the circulating blood of the host. Following inoculation, the sporozoites rapidly leave the blood (within 30 minutes to 8 h) and enter liver cells (hepatocytes). Within 6 – 15 days they develop into liver

schizonts and are referred to as pre-erythrocytic (PE) schizonts [(20)], so the life cycle of p. falciparum may disrupt the liver function lead to problems in the synthesis of coagulation factors. This finding may be because 71% of patients have less than one-year frequency of malaria and 73% of patients were attended to hospital after 2 days of the presence of symptoms. The malaria infection can cause activation of the coagulation system leading to in vivo thrombin generation, the release of tissue factor that causes prolongation of PT. During falciparum malaria, the stimulation of the coagulation system is caused by various procoagulants present during the disease. In addition, TNF α and histamine are promoting fibrin formation. Furthermore; the decreasing levels of coagulation inhibitors as Protein C, protein S, and AT-III activity were found to be low in P. falciparum, particularly in complicated cases proportional to disease severity [(15)].

The means of PT, INR, and APTT were not significantly different between degrees of parasitemia (*P value* = 0.508, 0.087 and 0.424 respectively) and malaria severity (*P value* = 0.090, 0.055 and 0.336 respectively). This finding was agreeing with Bharat et al., study [(21)], and disagree with Sahoo and Das study [(10)].

Prothrombin time (PT) and International Normalized Ratio (INR) were significantly prolonged in males compared to females (*P value* = 0.01 and 0.022 respectively); most probably due to physiological changes between males and females.

Coagulation factors once activated are used up. Depleted levels of various factors either in the common pathway or both in intrinsic and extrinsic pathways lead to prolonged PT, INR, and PTT.

Conclusion:

Falciparum malaria causes significant derangement in the coagulation process (intrinsic, extrinsic and common pathways) so causes prolongation of PT, INR, and APTT. So evaluation of coagulation profiles may be helpful in the management and outcome of patients and prevent the occurrence of complications of malaria as coagulopathy, DIC, and abnormal liver function.

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Author roles:

Zainab O. M: Data Curation, Formal Analysis, Investigation, Methodology, Validation, Writing – Original Draft Preparation. **Khansaa M. G:** Data Curation, Formal Analysis, Investigation, Methodology, Validation, Writing – Original Draft Preparation . **Mai S. M:** Supervision, Writing – Review & Editing. **Khalid A. M:** Supervision, Methodology, Writing – Review & Editing.

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