Assessment of Complete Blood Count and D-dimer among Patients with \textit{Plasmodium falciparum} Malaria

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

\textbf{Background:} The most virulent of the human malaria parasites is \textit{P. falciparum} which is responsible for the bulk of the malaria-related morbidity and mortality. Changes in blood cell parameters are already a well-known feature of malarial infections.

\textbf{Aim:} The purpose of this research was to assess the total blood count and D-dimer levels in patients with \textit{P. falciparum} malaria in Khartoum State, Sudan.

\textbf{Study Design:} It is case-control study was conducted on blood cells parameters and plasma D-dimer for patients infected with \textit{Plasmodium falciparum} malaria.

\textbf{Place and Duration of Study:} study was carried out in Yastabshiroon hospital, Khartoum state, from October 2020 to January 2021.

\textbf{Methodology:} The study comprised fifty people who tested positive for \textit{P. falciparum} using the National Public Health Laboratory's malaria diagnostic standard. The control group consisted of fifty healthy Khartoum locals with about equal gender and age distribution. To collect data from the study group's personal and medical information, such as name, gender, age, and medical condition, a structured questionnaire was developed.

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Keywords: D-dimer; Plasmodium falciparum; Malaria; hematological parameters.

1. INTRODUCTION

Malaria remains an important source of morbidity and death in more than 90 countries, responsible for at least one million deaths and 241 million illness episodes each year [1]. The majority of malaria-related disease and mortality is caused by P. falciparum, the most aggressive of the human malaria parasites. P. falciparum is responsible for 91% of malaria cases worldwide, with the majority (86%) occurring in Africa [2]. Africa had the greatest fatality rate (90%), followed by Southern East Asia (7%) and the eastern Mediterranean (7%) [3]. Malaria is a major public health issue in Sudan, accounting for 37% of all maternal death [4]. Plasmodium pathogenesis has been linked to endothelial activation and damage in both simple and severe instances of malaria [5]. Platelets are important in the pathogenesis of malaria infection [6]. A common clinical finding in P. falciparum malaria infection is thrombocytopenia [7]. Thrombocytopenia is another prevalent characteristic of Plasmodium-caused malaria [8]. Thrombocytopenia is unrelated to bleeding and does not necessitate therapy, with platelet counts soon returning to normal when the malarial episode is properly managed. In prenatal and newborns seem to be at a higher danger of thrombocytopenia, while the repercussions remain unknown [9]. Thrombocytopenia is seen in 60–80% of malaria cases and is more common and severe in complex P. falciparum [10]. In complex falciparum infection, it is more common and more severe. Thrombocytopenia itself rarely causes bleeding unless it is coupled by coagulopathy, which is only seen in severe complex falciparum infection. Decreased platelet survivability from peripheral destruction (by immunological, consumptive, or other mechanisms), increased splenic uptake or sequestration, and decreased platelet synthesis are all possible reasons [11]. D-dimer, the end product of plasma-mediated breakdown of fibrin-rich thrombi, has evolved into a screening test that may be used in diagnostic methods to rule out venous thromboembolism. D-dimer levels offer certain advantages over other thrombin generation assays since they are resistant to ex vivo activation, are relatively constant, and have a long half-life [12]. Elevated serum D-dimer levels may indicate endothelial activation, which in malaria could be related to parasite density and illness intensity [13]. The goal of this study was to evaluate the effect of P. falciparum malaria on total blood count and D-dimer levels in plasma of infected patients in Khartoum State, Sudan.

2. METHODOLOGY

2.1 Study Design

This study was intended to be an analytical case control study. The study was carried out in Yastabshiroon hospital, Khartoum state, from October 2020 to January 2021. The study included fifty adult individuals (35 male and 15 females their age ranging between 20 and 45 years) who tested positive for P. falciparum malaria using the National Public Health Laboratory standard for diagnosing malaria. Fifty healthy Khartoum residents with roughly identical gender and age distribution were enrolled as the control group. A structured questionnaire was created to collect data from the study group's personal and medical information, such as name, gender, age, and medical condition.

2.1.1 Inclusion criteria

All patients with positive malaria P. falciparum were enrolled in the study.

**Result:** D-dimer levels were considerably greater in P. falciparum malaria cases compared to non-falciparum malaria cases, neutrophil and lymphocyte counts were significantly lower, although white blood cells (WBCs) count was significantly lower in P. falciparum infection patients compared to non-falciparum (p≤0.05). Hemoglobin concentration and platelet count were similarly significantly lower (p≤0.05). Platelet counts and mean cell hemoglobin concentration (MCHC), on the other hand, were significantly lower in individuals with severe parasitemia compared to those with low and moderate parasitemia. **Conclusion:** The study concluded that P. falciparum infection causes considerable hematological alterations. The results of the current investigation revealed a significant increase in the D-dimer mean level, as well as a significant decrease in the count of hemoglobin, total white blood cells (TWBCs), lymphocytes, neutrophils, and platelets. It was also discovered that patients with high parasitemia had considerably lower platelet counts and mean cell hemoglobin concentration (MCHC) levels than those with low and moderate parasitemia.

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**References:**
2.1.2 Exclusion criteria

All patients with other malaria species, Red cells and White blood cells disorder, inherited or acquired coagulation disorders were excluded from this study.

2.2 Sampling

5 ml of blood in ethylene diamine tetra acetic acid (EDTA) and one ml of blood in 3.2 % sodium citrate were taken from the superficial vein in the antecubital fossa of the study participants in aseptic condition.

2.3 Statistical Analysis

Data were analyzed using software of SPSS version 20. Qualitative date was presented as mean ±1 SD. Association between qualitative variable was tested using person Chi square (x2) and Fishers exact test. P ≥ 0.05 is significant.

2.4 Methods

2.4.1 Complete blood count analysis

The Sysmex KX-21N is a quantitative automated hematology analyzer has been used to determine 17 hematological parameters in vitro. Anemias, leukemias, allergic reactions, viral, bacterial, and parasite diseases can all be diagnosed using the numerical and/or morphologic findings of a complete blood count. The Sysmex KX-21N analyzer analyzes white blood cell (WBC), red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), platelet (PLT), lymphocyte %, mixed %, and neutrophil % directly. The remaining parameters, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean platelet volume (MPV), red cell distribution width - coefficient of variation (RDW-CV) and red cell distribution width -standard deviation (RDW-SD),and differential percentages lymphocyte %, mixed %, and neutrophil %, are calculated or derived.

2.4.2 Procedure

Under sterile conditions, 5 ml of blood were collected in EDTA from the superficial vein in the antecubital fossa of the study population, gently mixed for three minutes, transferred to the sample probe, and analyzed using an Sysmex KX-21N following the manufacturer's protocol [14].

2.4.3 D-dimer

The Maglumi 800 Chemiluminescence Immunoassay Analyzer was used to measure D-dimer. In the presence of a complementary antigen and antibody, the antibody's paratope attaches to the antigen's epitope to form an antigen-antibody or immunological complex. Chemiluminescence Immunoassay (CLIA) is based on estimating the amount of such immune complexes using tagged antibodies. It entails the employment of stationary solid particles coated with the desired antigen or antibody. Substrate is provided after incubation to ensure the formation of intact immune complexes. These causes light to be generated, the intensity of which is directly proportional to the quantity of labeled complexes present, and thus indirectly contributes in the quantification of the analyte of interest. Light intensity is measured in units of Relative Light Units (RLU).

2.4.4 Procedure

The D-Dimers assay was done using a reagent kit and following the manufacturer's protocol [15]. Under sterile conditions, one ml of blood was collected in 3.2 % sodium citrate from the superficial vein in the antecubital fossa of the research population. Within one hour of collection, the samples were centrifuged for 10 minutes at 5000 RPM before being delivered to the sample area of the MAGLUMI 800. The results were then evaluated and expressed in mcg/ml.

3. RESULTS

3.1 Demographic Analysis

In this study, there were 100 people: 50 cases (with positive P. falciparum malaria) and 50 controls (Healthy individual). In terms of age, the mean was (32.86 ±19.9 and 35.16 ±20.1 SD) for the case and control groups, as shown in Table 1.

3.2 Mean Complete Blood Count and D-Dimer among patients with P. falciparum Malaria among Cases and Controls

The current investigation discovered a significant increase in the mean of D-Dimer (2.27 ±1.7) for patients compared to controls (0.47± 0.1), (p=0.000), and a significant decrease in the
mean of TWBCs (5.05 ±1.7) for cases compared to controls (6.75 ±1.8), (p=0.000). As shown in Table 2, HB was significantly lower in the case group (13.14 ±1.76), compared to the control group (13.71 ±1.8) (p=0.000), Platelets were significantly lower in the case group (132.48 ±66.7) compared to the control group (275.52 ±69.7) (p=0.000), and Lymphocytes were significantly lower in the case group (1.046 ±0.6SD) compared to the control group (1.784 ±0.87) (p=0.000).

3.3 Hematological Parameter at Different Parasitemia Level

The findings of the current study D-dimer levels were observed to be considerably greater in patients with high parasitemia than in those with low or moderate parasitemia (p=0.005). Patients with severe parasitemia had a significantly older mean age than those with low and moderate parasitemia (P= 0.005). Patients with severe parasitemia had significantly lower MCHC than those with mild or moderate parasitemia (p=0.019). Platelets count was also significantly lower in patients with high parasitemia as compared to patients with low and moderate parasitemia p (0.008). It is possible that the association between malarial parasitemia and several hematological indicators is influenced by age. As indicated in Table 2, age is a factor that causes a significant increase in malarial parasitemia and hematological variables. Fig. 1 represented the percentage of Parasitemia severity in cases, which was 64 percent in Mild, 32% in Moderate, and 2% in Severe.

Table 1. Mean Complete Blood Count and D-Dimer among patients with *P. falciprum* malaria among cases and controls

| Parameter     | Status | Mean± SD     | P- value |
|---------------|--------|--------------|----------|
| Age           | Case   | 32.86 ± 19.9 | 0.567    |
|               | Control| 35.16 ± 20.1 |          |
| D-dimer       | Case   | 2.27 ±1.7    | 0.000**  |
|               | Control| 0.47 ± 0.1   |          |
| WBCs          | Case   | 5.05 ±1.7    | 0.000**  |
|               | Control| 6.74±2.4     |          |
| RBCs          | Case   | 4.76 ±0.6    | 0.227    |
|               | Control| 4.91 ±0.5    |          |
| HB            | Case   | 13.14 ±1.7   | 0.000**  |
|               | Control| 13.71±1.8    |          |
| HCT           | Case   | 41.11 ± 6.9  | 0.356    |
|               | Control| 42.42 ±7.0   |          |
| MCV           | Case   | 84.6 ±6.5    | 0.272    |
|               | Control| 85.94±5.7    |          |
| MCH           | Case   | 27.53 ±2.4   | 0.466    |
|               | Control| 27.89 ±2.38  |          |
| MCHC          | Case   | 32.83 ± 1.7  | 0.352    |
|               | Control| 32.56 ± 0.99 |          |
| Platelets     | Case   | 132.48 ± 66.7| 0.000**  |
|               | Control| 275.52 ± 69.7|          |
| Lymphocytes   | Case   | 1.0480 ± 0.6 | 0.000**  |
|               | Control| 1.784 ± 0.87 |          |
| Neutrophils   | Case   | 3.7380 ± 1.5 | 0.027*   |
|               | Control| 4.620 ± 2.35 |          |
| RDW-CV        | Case   | 12.478 ± 0.82| 0.472    |
|               | Control| 12.354 ± 0.95|          |

*P≤0.05 significant association, **P≤ 0.001 is highly significant association
Table 2. Hematological Parameter at Different Parasitemia Level

| Parameter | Severity of parasitemia | P- value |
|-----------|-------------------------|----------|
| Age       | Mild: 34.0, Moderate: 25.8, Severe: 72.5 | 0.005*   |
| D-dimer   | Mild: 1.997, Moderate: 2.507, Severe: 4.918 | 0.050*   |
| WBCs      | Mild: 5.159, Moderate: 4.650, Severe: 6.650 | 0.293    |
| RBCs      | Mild: 4.81, Moderate: 4.67, Severe: 4.80 | 0.785    |
| HB        | Mild: 13.2, Moderate: 13.0, Severe: 12.7 | 0.883    |
| HCT       | Mild: 40.61, Moderate: 41.99, Severe: 42.30 | 0.796    |
| MCV       | Mild: 84.20, Moderate: 84.84, Severe: 88.65 | 0.642    |
| MCH       | Mild: 27.5, Moderate: 27.9, Severe: 26.3 | 0.630    |
| MCHC      | Mild: 33.02, Moderate: 32.87, Severe: 29.50 | 0.019*   |
| Platelets | Mild: 152.3, Moderate: 103.13, Severe: 49.00 | 0.008*   |
| Lymphocytes | Mild: 1.10, Moderate: 0.95, Severe: 0.95 | 0.650    |
| Neutrophils | Mild: 3.82, Moderate: 3.35, Severe: 5.50 | 0.138    |
| RDW-CV    | Mild: 12.55, Moderate: 12.50, Severe: 11.60 | 0.309    |

*P≤0.05 is significant association

**Fig. 1.** Shows frequency and degree of parasitemia severity among cases

4. DISCUSSION

Blood coagulation changes are hypothesized to have a role in malaria pathogenesis. The protozoan parasite *P. falciparum* is responsible for the vast majority of fatal cases [1].

This study aimed at evaluating complete blood counts and D-dimer levels in patients with *P. falciparum* in Sudan. It was a case control study design conducted in Khartoum state in 100 individuals, 50 as case and 50 as control. There were 35 (35%) males and 15 (15%) females among them.

The study found a substantial increase in D-dimer levels in all 50 cases, which was consistent with a recent study done in Sudan by Bashir and Ahmed 2020 [16], which found a significant increase in D-Dimer levels in 37.5% of patients indicating that *P.falciparum* has potential effect in blood components and patient may at high risk of thrombosis. The study found a substantial drop in TWBCs, Neutrophils, and Lymphocytes in the case group, with a P value of 0.000. This finding was consistent with a previous study done in south eastern Nigeria by Irole-Eze *et al* 2017 [17], which concluded that Total white blood count and two of its differentials (lymphocytes and granulocytes) were significantly lower among malaria infected subjects compared to non-infected subjects, and the study showed a significant decrease in Hemoglobin level among case group P value.
0.000, which was consistent with a previous study done in by Omarine et al 2020 [18].

This study concluded that there was a statistically significant decrease in hemoglobin levels (HB < 12 g/dL) in individuals with malaria compared to those without malaria. The study found a significant drop in platelet count among the case group, which was consistent with the findings of Ansari et al 2009 [19], who concluded that there is a high prevalence of mild to moderate thrombocytopenia in P. falciparum malaria. The study also revealed that the majority of cases had low platelet counts at various parasitemia levels of P. falciparum infections. In the instance of P. falciparum infection, the p-value revealed a substantial correlation. This finding was consistent with a prior study conducted in Sudan by Hamid et al in 2016 [20]. It revealed that the majority of P. falciparum had lower levels of platelets, hemoglobin, leukocytes, lymphocytes, and neutrophils. P. falciparum and these factors had a statistically significant connection (p-value > 0.05). These findings were consistent with a recent study conducted in Thailand by Kotepui et al. 2015 [21], which found substantial relationships between hemoglobin levels, leukocytes, and P. falciparum differential counts.

Potential effect of P. falciparum on blood components and patient may at risk of thrombosis especially significant increasing in D-dimer

5. CONCLUSION

This study concluded that P. falciparum has a potential effect on hematological parameters; moreover, an increase in P. falciparum parasitemia significantly increases the concentration of D-dimmers and causes significant thrombocytopenia; and finally, this study revealed that the association between P. falciparum parasitemia and hematological parameters is influenced by age. Further research needs to be done on blood count indices in malaria patients due to limited sample size utilized in this study. Molecular research could potentially add to our understanding of how P. falciparum affects blood cell parameters.

CONSENT AND ETHICAL APPROVAL

The experiment was authorized by the Ethical Committee of the College of Medical Laboratory Sciences at National University, and verbal consent was obtained from each participant.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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