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# Urine Abnormality in Association with Hematological Parameters in Ambulant Patients with Malaria, River Nile State - SUDAN

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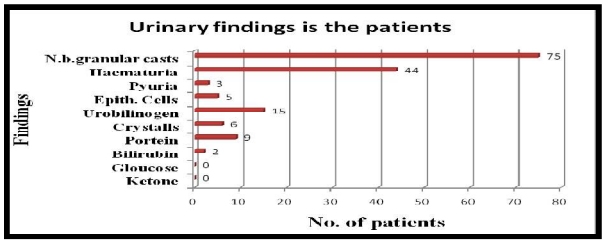
## ABSTRACT

*Malaria can be diagnosed clinically and is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests. Modern techniques that use the polymerase chain reaction to detect the parasite's DNA have also been developed, but these are not widely used in malaria-endemic areas due to their cost and complexity. The disease is widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas. The World Health Organization estimates that in 2010, there were 219 million documented cases of malaria, that year, the disease killed between 660,000 and 1.2 million people, many of whom were children in Africa. According to reports of Ministry of health, River Nile state, there were 2865 cases and about 8 deaths were occurred in 2013.Changes in platelet counts during acute malaria are commonly reported in the medical literature, especially in P. falciparum infections; such changes are a major cause of concern to clinicians because such cases are more likely to evolve into serious and complicated disease cases. However, many recent studies have also found thrombocytopaenia associated with P. vivax.*

## Graphical Abstract




Urinary findings in the patients

**Keywords:** Thrombocytopenia– Platelet Distribution Width (PDW)– Platelet Mean Volume (PMV) - Narrow bore granular cast -Sudan.

## INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a type of unicellular microorganism) of the genus Plasmodium. Commonly, the disease is transmitted by a bite from an infected female Anopheles mosquito, which introduces the organisms from its saliva into a person's circulatory system. In the blood, the parasites travel to the liver to mature and reproduce [1]. Five species of Plasmodium can infect humans. The vast majority of deaths are caused by *Plasmodium falciparum* and *Plasmodium vivax*, while *Plasmodium oval*, and *Plasmodium malaria* cause a generally milder form of malaria that is rarely fatal. The zoonotic species *Plasmodium knowlesi*, prevalent in Southeast Asia, causes malaria in macaques but can also cause severe infections in humans. Malaria is common in tropical and subtropical regions because rainfall, warm temperatures, and stagnant waters provide an environment ideal for mosquito larvae [2]. Malaria can be diagnosed clinically and is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests [3-4]. Modern techniques that use the polymerase chain reaction to detect the parasite's DNA have also been developed, but these are not widely used in malaria-endemic areas due to their cost and complexity [5-7].The disease is widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas. The World Health Organization estimates that in 2010, there were 219 million documented cases of malaria, that year, the disease killed between 660,000 and 1.2 million people, many of whom were children in Africa [8]. According to estimates from the World Health Organization, over 207 million cases and about 627,000 deaths were occurred in 2012. *Plasmodium falciparum* and *Plasmodium vivax* are the most common. *Plasmodium falciparum* is the deadliest [9].

In this study, Full urine analysis were done for all cases, however some studies have found reversible kidney dysfunction. Also, Platelet count and platelet Indices were determined. Changes in platelet counts during acute malaria are commonly reported in the medical literature, especially in *P. falciparum* infections; such changes are a major cause of concern to clinicians because such cases are more likely to evolve into serious and complicated disease cases [10, 11]. However, many recent studies have also found thrombo cytopaenia associated with *P. vivax* [12]. The aim of this study was to evaluate the effect of malaria on selected Hematological parameters and urinary findings in Sudanese ambulant patients with malaria River- Nile state- Sudan.

## MATERIALS AND METHODS

**Study design:** A prospective study of platelets count, platelet indices and urinary findings in 153 Sudanese ambulant patients with severe malaria from health centers and some hospitals in Shendi locality River Nile State.

**Sampling:** Venous blood was collected from participants by clean venipuncture in EDTAblood tubes. Each patient was requested to pass urine in a clean plastic container.

**Ethical consideration:** Ethical consideration was taken verbally from the mothers of the babies. This study posed no physical risk to participants though an interview of 10 min might have been convenient to some participants. It is a convenient study, thus neither the participants name nor his institution in use in any of the study materials and each participant was assigned a unique identification number. Collected data will be secured in a computer protected by password.

**Detection of Malaria parasite:** Thick and thin blood films were done. After drying the thin films were fixed with absolute methanol for 30 sec. The films were stained with freshly prepared 10 % Giemsa stain by mixing 1ml of tock solution with 9ml of buffer pH: 7.2. The slides were flushed with tap water and lift in the upright position to dry. The blood films were examined using100 oil immersion lens. A positive smear was included with each new batch of working Giemsa stain for

quality control. Parasite densities were assessed as parasite/thick film field. All slides were double-checked and only considered negative if no parasites were detected in 100 oil immersion lens.
Parasites were counted and Parasitemia graded as follow:

1-10 parasite\ 100 field (+)
1-10 parasite\ 10 field (++)
1- 10 parasite\ 1 field (+++)
>10 parasite\ 1 field (++++).

Thin blood films were used for the identification of the plasmodium species. Mindray Hematology Analyzer (Mindray bc-3000):

**Principle:** Blood cells can be broadly divided into three categories. Red blood cells, White blood cells and platelets. The analyzer measures the number of cells and distinguishing between their types according to size using sheath flow DC detection. Electrical current is passed through a solution; this method measures the changes in electrical resistance that occurs when blood cells pass through detection aperture.

**Method:** The EDTA blood samples were aspirated into analyzer through a sample probe, and the counting was started automatically, the results were displayed on the screen within (20) second, the print key was pressed to print out the results.

**Urine Examination (Dipstick):** The urine dipstick is a plastic strip to which paper tabs impregnated with chemical reagents have been affixed. The reagents in each are chromogenic. After timed development, the color on the paper segment is compared with a chart. Some reactions are highly specific. Others are sensitive to the presence of interfering substance or extremes of pH. Discoloration of the urine bilirubin or blood may obscure the color [13]. Fresh urine samples were examined with test strips Camphor 10 from Healgen-USA for pH, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, Specific Gravity, leukocyte and blood.

## RESULTS AND DISCUSSION

**Statistical analysis:** All data was analyzed using statistical package for the social sciences (SPSS) version [15]. The mean was obtained and cross-tabulated test, independent T- test, and Spearman correlation were used for comparison, and presented in the form of figures and tables. The *P. value* and odd ratio was obtained to assess the significant of the result.

**Table 1.** Mean platelet count, *MPV* and *PDW* in patients and Controls

| No | Test | Values of patients *Mean ± SD* | Values of Control *Mean+SD* | *P. Value* |
|---|---|---|---|---|
| 1 | Platelet count | 134.000 ± 83.6 | 275 ± 65.7 | 0.000 |
| 2 | Mean platelet volume | 8.5± 0.99 | 10.2 ± 0.94 | 0.000 |
| 3 | Platelet Distribution width | 16.0 ± 0.53 | 15.2 ± 0.34 | 0.000 |

**Table 2.** Mean of platelet count in patients according to type of *plasmodium species*

| No | *Plasmodium Species* | Platelet count$\mu l^{-1}$ | *P. value* |
|---|---|---|---|
| 1 | *P. Falciparum* | 139.400± 86 | 0.03 |
| 2 | *P. Vivax* | 128.900± 69.2 | |

**Table 3.** Mean of *MPV* in patients according to type of *plasmodium species*

| No | *Plasmodium Species* | MPV | *P. value* |
|---|---|---|---|
| 1 | *P. Falciparum* | 8.4±0.8 | 0.23 |
| 2 | *P. Vivax* | 8.3± 0.91 | |

**Table 4.** Mean of *PDW* in patients according to type of *plasmodium species*

| No | *Plasmodium Species* | *PDW* | *P. value* |
|---|---|---|---|
| 1 | *P. Falciparum* | 16.02± 0.6 | 0.34 |
| 2 | *P. Vivax* | 16.12± 0.54 | |

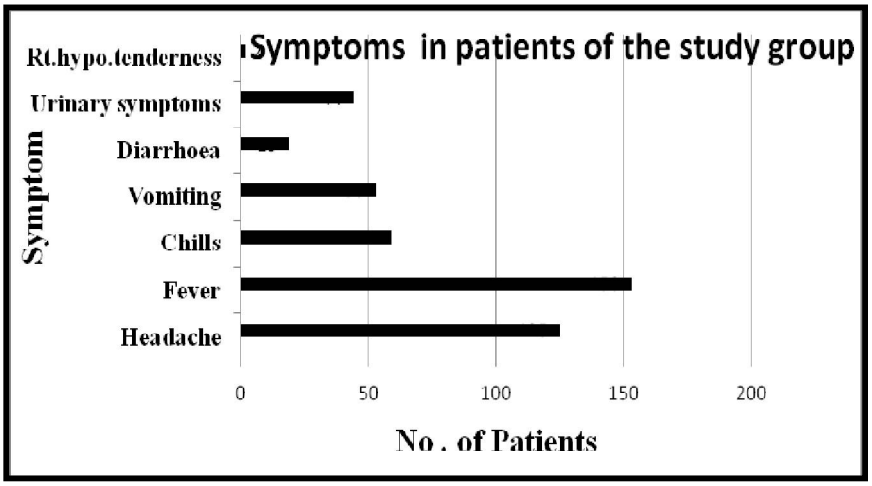



**Figure 1.** Symptoms in patients of the study group

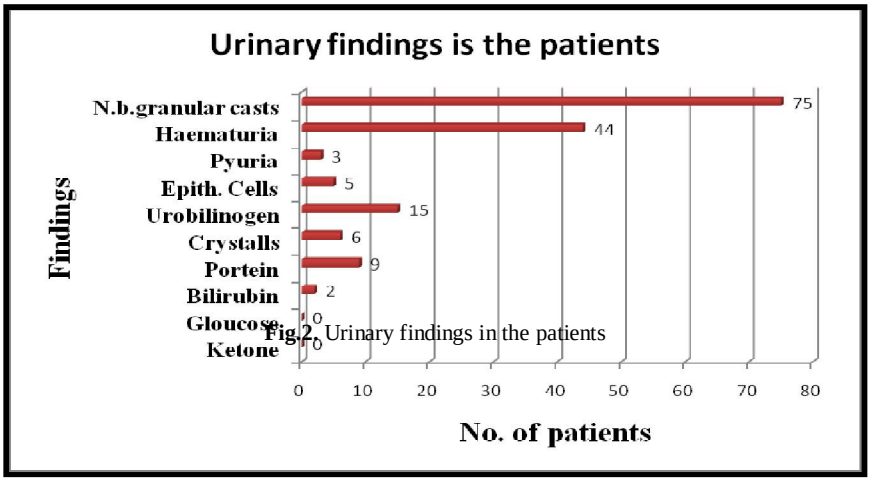



**Fig.2.** Urinary findings in the patients

Urinary findings of the patients

Proteinuria was found in only9 patients ranging from 30– 500 mgdL$^{-1}$. It was statistically correlated to fever, but not to duration of symptoms or plasmodium species. Fever is a known contributing factor

in proteinuria, a rise in urine protein 150 – 1500 $mgday^{-1}$ was found in 12 patients out of 27 patients with *P.falciparum* infection [14].

**Table 5.**Thrombocytopaenia according to degree of parasitemia

| Parasitaemia | Frequency | Percentage | Mean of platelet count/µL | *P. value* |
|---|---|---|---|---|
| + | 21 | 13.7% | 143.000 | 0.007 |
| ++ | 82 | 53.6% | 136.000 | |
| +++ | 38 | 24.9 % | 133.000 | |
| ++++ | 12 | 7.8 % | 117.000 | |

Granular casts were found in 75 patients in this study. They were of the narrow-bore type and their number differed from one sample to another but they were usually of low number. There was no significant statistical correlation between these narrow – bore granular cast and age, sex, parasitemia or plasmodium species. Increased urobilinogen was found in 15 patients while bilirubinuria was found in two patients. The fifteen patients mentioned above had elevated unconjugated hyperbilirubinemia, range of serum bilirubin from 1.1 – 4.0 $mgdL^{-1}$ and the range of unconjugated bilirubin from 0.8 – 3.8 $mgdL^{-1}$. It has been suggested that destruction of red cells may result from the operation of different pathophysiological mechanism:

1. The mechanical destruction of red cells by the malaria trophozoite forms growing inside them [15]. The trophozoite divides repeatedly forming the merozoites and ultimately causing rupture of the red cells release of the merozoites into the circulation in order to attack further red blood cells. The high degree of hemolysis resulting from this mechanism had been attributed to the ability of *P. falciparum* to enter red cells of all ages with special predilection for young red cells [15]. In addition, the membranes of infected erythrocytes showed major alterations in their components which might be responsible for the increased osmotic fragility, membrane fluidity and passive permeability [16].

2. A role for immunity in red blood cells destruction in *P. falciparum* malaria was postulated. Opsonization and erythrophagocytosis following red blood cell sensitization by either the adherence of immune complexes or by *IgG* and or complement components [17]. This may occur in the absence of parasitemia. Autoimmune mechanisms were also suggested for the red blood cell destruction in malaria[18],in which *IgM* antibodies are formed against normal erythrocytes resulting in their increased destruction. It was suggested that these *IgM* antibodies occur in individuals in endemic areas exposed to malaria since birth [18].

As shown in table 1 there is a significant variation in mean of platelet count, mean platelet volume and platelet distribution width. The results revealed a high frequency of thrombocytopenia and changes in *MPV* and *PDW*. In this study, platelet counts were significantly reduced in malarial infected subjects. Thrombocytopaenia occurred in 67.5 % of malarial cases in comparison to study done in Pakistan which had high percent 85.5 %. Effect of *P. falciparum* on platelet count showed thrombocytopaenia with mean of platelet count of $139400\mu L^{-1}$, *P. vivax* were thrombocytopaenia with mean of platelet count of $128,900\mu L^{-1}$ in comparison to study achieved in India showed the mean of platelet count in *P. falciparum* infection was $100,900\mu L^{-1}$ and in *P. vivax* was $115,390\mu L^{-1}$ and *P. malairae* were thrombocytopenia with mean of platelet count of $103,000\mu L^{-1}$ [19].The trend of decreasing platelet counts with increasing levels of parasitemia observed in this study has been previously noted by Eze Evelyn M *et al*. 2012 [20]

In general, the underlying mechanisms of thrombocytopenia in malaria are peripheral destruction, excessive sequestration of platelets in spleen, and excessive use of platelets associated with the disseminated intravascular coagulation phenomenon In addition to the reduction in the number of

platelets, platelet function is also compromised in these patients; this is generally evidenced by changes in the volume and other features of platelet cells [21].

## APPLICATION

Urine examination showed the presence of proteinuria in 9 febrile patients. The incidence of proteinuria was directly correlated with degree of parasitemia. Narrow bore granular cast were found in 75 patients and were not statistically correlated with plasmodium species, degree of parasitemia or age of the patients. There is a significant variation in mean of platelet count, mean platelet volume and platelet distribution width. The results revealed a high frequency of thrombocytopenia and changes in MPV and PDW. There were presumptive evidences that malaria infection cause significant thrombocytopenia and marked changes in platelet Indices.

## CONCLUSIONS

They were a narrow-bore type of urinary casts associated with moderate proteinuria in febrile patients. Platelet count in this study was shown to be significantly reduced in patients compared to controls, and thrombocytopaenia is directly proportional to degree of parasitaemia. *P. vivax* seems to altered platelet count more than *p. falciparum*.

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