

Incidence of thrombocytopenia amongst children with malaria attending Ibrahim Malik Teaching Hospital September 2021 - February 2022

Mohammed Abdein Mohammed Ali ^a, Omer S.M. Suliman ^{a, b}, Hassan I. Osman ^{c, d},
 Lina Mohammed Ahmed Alsheekh ^e

^a hassanismai603@gmail.com

a = Department of Paediatrics and Child Health, Ibrahim Malik Teaching Hospital, Khartoum, Sudan
 b= Assistant Professor, Paediatrics and Child Health, University of Khartoum, Khartoum North, Sudan
 c = Napata Research and Innovation Center (NRIC), Napata College, Khartoum, Sudan
 d = Public Health Institute, Federal Ministry of Health, Khartoum, Sudan
 e = Department of Paediatrics and Child Health Ahmed Gasim Hospital, Khartoum North, Sudan

Abstract

Introduction: Malaria is an issue that has beset humanity for way too long. Many treatments have been proposed for the eradication of the illness. Despite efforts, the issue remains found in our healthcare systems. Recently, the World Health Organization (WHO) has deemed thrombocytopenia not of importance when discussing severe malaria.

Thrombocytopenia (platelet count less than $150 \times 10^9/L$) is a common platelet abnormality and hematological change as well as a common feature of malaria due to all plasmodium species more recently, low platelet counts have been associated with mortality in patients with *P. falciparum* and *P. vivax* infection. However, other studies have not demonstrated an association between thrombocytopenia and significant clinical risk. This analysis was conducted to establish the comparative platelet counts of patients infected by the different Plasmodium species and to define the associated risks of morbidity and mortality. Objectives: General objective: To study the association of malaria with thrombocytopenia in children at Ibrahim Malik Teaching Hospital; Specific

Objectives: The general objective was to determine Thrombocytopenia among children with malaria as well as to determine common symptoms of malaria in children at Ibrahim Malik teaching hospital; to identify the complications of malaria with thrombocytopenia, and finally to identify the outcome(s) of malaria and thrombocytopenia.

Materials and methods: This was an analytical case-control hospital-based study that was conducted in Ibrahim Malik Teaching Hospital. The study was conducted on pediatric patients diagnosed with malaria in Ibrahim Malik Teaching Hospital. This was a multi-stage systematic sampling. A structured data collection sheet was filled by researcher.

Results: A total of 334 cases of malaria were included in this study. Amongst those, the prevalence of thrombocytopenia was 28.4 % (95 cases). The mean platelet count was 247.42 ± 187.89 . The range was between 7 and 704. The mean age of participants was 6 years old ± 5.13 ; the range was between 1 year and 17 years. In other words, 182 of our participants (52%) were under 5 years of age and were the largest group of our participants, followed by participants aged 6-10 years of age at 80 (23%), followed by 11-14 years olds at 63 (18%), with the remainder 25 (7%) being 15-18 years of age. 182 (52%) of our participants were female, while the remainder 48% were male. Thrombocytopenia was most found in males ages 5 and under, more specifically in the age range of 2-3. Of our participants, 68.8% were of urban residence, while the remainder 31.2% were of rural residence. Of our participants, 52% (182) were ill for 4-7 days, while 31% (110) of them were ill for 1-3 days prior to hospitalization. Finally, 17% (59) of our participants were ill for 8 days or more. Mean duration of illness was 6.04 days ± 4.13 . All participants were symptomatic. The most commonly reported symptom was

fever, which was reported in all 334 (100%), followed by vomiting reported in 58.3% (203). The 3rd most commonly reported symptom was diarrhea, which founded in 37.5% (131). The 4th most reported symptom was headache, reported in 20.8% (73). This was followed by fatigability, which affected 12.5% (44).

Finally, the least reported symptom was nausea which founded in 4.2% (15). Of importance, 56.2% (197) of our participants had at least 1 symptom of severe malaria. The parasite count found in this study showed that 66.7% (233) had a parasite count of one cross (+), only 6.3% (22) had a parasite count of two crosses (++), 23% (88) had a parasite count of three crosses (+++), while only 4.2% (15) had a parasite count of four crosses (++++). These are illustrated in figure 4.7 below. In so far as parasite count is concerned, a significant correlation was found between hyperparasitemia (four crosses (++++)) and thrombocytopenia. As per platelets are concerned, 219 (62.5%) had a platelet count of over 150,000 platelets per microliter of blood, 36 (10.4%) had a platelet count of 100,000-149,999 platelets per microliter of blood, 44 (12.5%) of them had a platelet count of 50,000-99,999 platelets per microliter of blood, 36 (10.4%) had a platelet count of 20,000-49,999 platelets per microliter of blood. Finally, only 4.2% (15) had a platelet count of less than 20,000 platelets per microliter of blood. Mild thrombocytopenia (100,000-150,000) was found in 10.4%, moderate thrombocytopenia (50,000-99,999) in 12.5%, while severe thrombocytopenia was found in 14.6%, divided as follows, 10.4% had a platelet count of (20,000-49,999) and 4.2% had a platelet count of under 20,000. The mean total WBC count was 9.94, with an SD of 4.73. 35 participants (10.42%) founded leucopenia, while 202 (60.4%) had leukocytosis. In so far as total WBC count is concerned, 54.2% (190) of our participants had a high total, 35.4% (124) of them were of normal range, and the remainder 10.4% (36) founded a low total WBC count. The mean Hemoglobin count was 8.42 ± 2.91 g/dl. No significant correlation was found between HB levels and thrombocytopenia. The data illustrated a significant correlation between the listed symptoms of severe malaria which founded in our population and thrombocytopenia as well as a significant correlation between high total White Blood Cells (WBCs) and thrombocytopenia. No significant correlation was found between Renal Function Test (RFT) and thrombocytopenia. No significant correlation was found between Liver Function Test (LFT) and thrombocytopenia. A statistically significant correlation was found between rural residence and thrombocytopenia. A significant correlation was found between the youngest age group (under 5 years of age) and thrombocytopenia. A significant correlation was found between the male gender and thrombocytopenia. Our findings showed that 58.3% (204) of our participants were treated using artisunate (injection).

Conclusion: In conclusion, our study found a significant correlation between severe malaria and thrombocytopenia. Additionally, it was found that high total WBC count was significantly correlated with thrombocytopenia in malaria. Furthermore, we found that thrombocytopenia was significantly correlated with rural residence, young age (namely under 5), and male gender. In addition, no correlation was found between thrombocytopenia and Renal Functions and Liver Functions. We, therefore, find it appropriate to conclude that thrombocytopenia was associated with a number of signs and symptoms (as well as laboratory investigations) of malaria.

Recommendation(s): The immediate recognition of thrombocytopenia as a marker of severe malaria

In the context of absence of investigations for malaria (e.g.: Blood Film For Malaria (BFFM)), the above findings can be used to hypothesize the presence of malaria

Seeing to it that pediatricians recognize the importance of thrombocytopenia in the context of malaria

Authorities reminding doctors of the importance of observing platelet counts in malaria patients

The establishment and funding of further research in this topic (thrombocytopenia in malaria)

Keywords: Type your keywords here, separated by semicolons ;

1. Introduction

Malaria is a protozoan disease caused by parasite genus *Plasmodium* (1) transmitted to man by infected female *Anopheles* mosquito of which there are five species *Plasmodium* (*falciparum*, *vivax*, *ovale*, *malariae* and *knowlesi*) (2). Malaria is a major health problem worldwide, there were estimated 243 million per year cases of malaria most of them about 85% were in African region followed by south east Asian region 10% and east Mediterranean 4%. Malaria accounts for an estimated 1-3 million deaths worldwide per year, of which 89% in African region followed by east Mediterranean 6% and south east Asia 5%. Most deaths of malaria caused by *Plasmodium falciparum* infection which causes life threatening (cerebral, respiratory, renal, hepatic, hemodynamic, hematologic) dysfunction.

Malaria is a major health problem in Sudan, it leads to 7.5 million cases and 35,000 deaths every year.

In malaria all blood components are affected. Thrombocytopenia has been associated with malaria and considered as characteristic of malaria and in some places it is used as indicator for malaria in patient presenting with fever.

The normal platelets count amongst Africans is 100,000-300,000/ml (3). Thrombocytopenia is defined as a platelet count < 100,000/ml (3) or <150,000/ml (4) and classified as mild (<150,000 and > 50,000) moderate (<50,000 and > 20,000) and severe (< 20,000) , platelets of < 150,000/ml increase the likelihood of malaria by 12 - 15 times.

Thrombocytopenia (platelet count less than $150 \times 10^9/L$) is a rather common platelets abnormality and hematological change, 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) (3,5). Platelet survival is reduced to 2-4 days in severe *falciparum* malaria. The pathophysiology of malaria associated thrombocytopenia and reduced platelet survival rate are multi factorial. 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). Some, but, as expected, not all, studies have shown that there is strong association between thrombocytopenia and severity of malaria. Severity and mortality of patients with *falciparum* malaria are increased with severe thrombocytopenia.

Thrombocytopenia seems to rarely found in acute malaria (3).

Of interest, children under 5 years of age who found high mortalities that can be attributed to malaria seem to be asymptomatic for COVID-19 (6).

Rationale:

Malaria remains an important threat to public health, particularly in communities with poor resources. Although *Plasmodium falciparum* accounts for the majority of severe malarial disease in sub-Saharan Africa, outside of Africa, non-*falciparum* malarias are responsible for an increasing proportion of infections.

Thrombocytopenia (platelet count less than $150 \times 10^9/L$) is a common platelet abnormality and hematological change as well as a common feature of malaria due to all *Plasmodium* species more recently, low platelet counts have been associated with mortality in patients with *P. falciparum* and *P. vivax* infection. However, other studies have not demonstrated an association between thrombocytopenia and significant clinical risk. This analysis was conducted to establish the comparative platelet counts of patients infected by the different *Plasmodium* species and to define the associated risks of morbidity and mortality.

Objectives:

To study the association of malaria with thrombocytopenia in children at Ibrahim Malik Teaching Hospital

Specific Objectives:

- 1) To determine common symptoms of malaria in children at Ibrahim Malik teaching hospital
- 2) To determine the association between malaria and thrombocytopenia in children at Ibrahim Malik Teaching Hospital.
- 3) To identify the complications of malaria with thrombocytopenia
- 4) To identify the outcome(s) of malaria and thrombocytopenia

Literature review:

Malaria is a life-threatening disease caused by parasite of plasmodium species that transmitted to people through a bite from infected female anopheles mosquito which is malaria vector. there are five malaria species that are known to infect human {p.falciparum, p.vivax, p.ovale, p.malariae and p.knowlesi} of which p. falciparum and vivax are the greatest threat (7) Children under 5 years are the most vulnerable group affected by malaria in 2019 and account for 67% of all malaria deaths worldwide, the WHO African regions carries a disproportionately high share of global malaria prudency, in 2019 the region was whom 94% malaria cases and deaths (7). total funding for malaria control and elimination reached an estimated 3 billion dollars, contribution from government of endemic countries accounted 900 million dollars represented 31% of total funding. according to last world malaria report released in 13, November 2020 there were 229 million cases of malaria in 2019 compared to 228 million in 2018, the estimated number of deaths stood at 409,000 in 2019 compared to 411,000 deaths in 2018 (7)

The life cycle of malaria started by transmission of plasmodium sporozoites via bite from an infected female of anopheles, sporozoites then travel from a salivary glands of mosquito through the blood stream of the host to liver where they invade the hepatocytes, these cells divided many thousands until mature tissue schizonts are formed and each contains thousands of daughter merozoites, this preerythrocytic phase is asymptomatic (8).

The liver schizonts rupture after 6-30 days and release thousands merozoite into the blood stream where they invade red blood cells, when the cell bursts they invade more red cells. clinical symptoms including fever occur in synchrony with rupture of red blood cells. in some red blood cells the merozoite mature successfully from ring form to trophozoite and gametocyte over 24 hours, circulate in blood stream and are ingested during mosquito bite, the ingested gametocytes develop in mosquito to mature sex cells which develop into ookinetes which actively burrow through the mid gut of the mosquito and form oocytes in which developed thousands of active sporozoites which pursue to travel to salivary glands of mosquito and the cycle of human infection begins again (9).

As beautifully articulated by Milner:

'Pathogenesis, the manner of development of a disease, for a human malaria clinical illness is a complex story that has many players, settings, and potential outcomes. As with any truly successful parasite, the observed outcome of evolution in malaria is the undisturbed transition from mosquito to human to mosquito with little impact on the vector and host. Although impact of malaria can be seen at the individual, community, country, and global level, from the parasite's perspective, a healthy host serving as two blood meals with a bit of fever in between is the norm. In fact, human clinical disease is quite rare relative to the global interaction network of mosquitoes and humans. The biology of Plasmodium falciparum malaria parasites, as measured in vitro, is finite, predictable, and easily experimentally perturbed during the 48-hour life cycle. In the mosquito-human life cycle, however, this parasite, along with the other five species infecting humans (Plasmodium vivax, Plasmodium ovale wallikeri, Plasmodium ovale curtisi, Plasmodium malariae, and Plasmodium knowlesi), undergoes 10 or more morphological states, replicate from a single to 10,000 cells, and vary in total population from one to many more than 10⁶ organisms.' (8) As per a 2012 paper: 'Disruption of the endothelial cell (EC) barrier leads to pathology via edema and inflammation. During infections, pathogens are known to invade the EC barrier and modulate vascular permeability. However, ECs are semi-professional antigen-presenting cells, triggering T-cell costimulation and specific immune-cell activation. This in turn leads to the release of inflammatory mediators and the destruction of infected cells by effectors such as CD8⁺ T-cells. During malaria, transfer of parasite antigens to the EC surface is now established. At the same time, CD8 activation seems to play a major role in cerebral malaria' (10).

According to a 2011 paper:

'Data on the real burden of thrombocytopenia associated with malaria is contradictory in the literature and it is not usually considered when conducting patient selection.

A study based on 31 American soldiers in Vietnam with chloroquine-resistant falciparum malaria noted the following changes in the acute phase of the disease using the same patients as their own controls during convalescence: decrease in the platelet count and prothrombin activation time, increase in the activated thromboplastin time, and reduction in factors V, VII and VIII with normal fibrinogen. This report suggested that thrombocytopenia was simply a consequence of the coagulation disorders presented by these patients, an idea that persisted for many decades in the literature. In another series of 21 patients with falciparum malaria, six had developed disseminated intravascular coagulation (DIC). The authors noted that the patients with more severe thrombocytopenia also had DIC and that there was correlation between platelet count and C3 protein levels. However, the reduction in C3 was proportional to that in parasitaemia, suggesting that thrombocytopenia was not independently associated with C3. In Manaus, 2004, a study with falciparum and vivax patients demonstrated a negative correlation between platelet counts, thrombin-anti-thrombin complex and D-dimers, suggesting that the activation of coagulation could be partially responsible for thrombocytopenia. The spleen in malaria has played a crucial role in the immune response against the parasite, as well as controlling parasitaemia due to the phagocytosis of parasitised red blood cells (RBCs). Some data suggested that platelets were sequestered in the spleen during the acute infection. In the experimental model with *Plasmodium chabaudi*,

thrombocytopenia was absent in splenectomised mice, showing that the spleen was essential for thrombocytopenia. The term hypersplenism was proposed to describe the clinical picture of the enlarged spleen followed by the decrease in one or more peripheral blood lineages (usually reverted after splenectomy), probably due to sequestration or destruction of cells inside the spleen.

The relationship between malaria and auto-immunity has been discussed in the literature and the first epidemiological association was made based on the presence of fewer auto-immune diseases in malarigenous areas. The formation of circulating immune complexes (CIC) in vivo in malaria, as well as in other infectious diseases, is a continuous process from antigens and antibodies and/or complement elements. CIC seems to modulate the immune response to several antigens that remain sequestered in B lymphocyte or dendritic cell-rich follicles for a longer time, which contributes to the formation of B-cell immunological memory, as seen in vaccine studies. During acute malaria, thrombocytopenia is most probably associated with the binding of parasite antigens to the surface of platelets to which antimalarial antibodies also bind, leading to the *in situ* formation of immune complexes (ICs).

Platelets from patients with acute malaria are highly sensitive to adenosine diphosphate (ADP) addition in vitro and it is believed that ADP release following hemolysis could contribute to higher platelet aggregation. Actually, the incubation of platelets with *P. falciparum*-parasitized RBCs also increases platelet aggregation *per se* in vitro, especially after ADP and thromboxane A₂ addition even electron microscopic examination of non-stimulated, fresh platelets from malarial patients show centralization of dense granules, glycogen depletion and microaggregates and phlopopoids as a sign of *in vivo* activation, which could be responsible for a pseudo-thrombocytopenia due to sequestration of these activated particles in the interior of the vessels.

Free radicals may play an important role in the Platelet destruction in malarial infection. There is evidence that the decrease in total cholesterol in vivax malaria is due to lipidic peroxidation.

The finding of a *P. vivax* trophozoite inside a human platelet suggested that thrombocytopenia could be the result of invasion of these particles by the parasites themselves, similar to what was classically proposed for RBCs'. (11)

Severe malaria, on the other hand, is, for the most part, caused by *Plasmodium falciparum* (12). This is believed to be because of its ability to induce infected (RBC) cytoadherence to the vascular endothelium and consequent end-organ dysfunction (12). Other plasmodium species can cause severe disease and AKI (12). Their ability to cause coma is rather questionable for the time being (12).

The most characteristic symptom of malaria is fever. Other common symptoms include chills, headache, myalgias, nausea, and vomiting. Diarrhea, abdominal pain, and cough are occasionally seen. As the disease progresses, some patients may develop the classic malaria paroxysm with bouts of illness alternating with symptom-free periods. The malaria paroxysm comprises three successive stages. The first is a 15-to-60-minute cold stage characterized by shivering and a feeling of cold. Next comes the 2-to-6-hour hot stage, in which there is fever, sometimes reaching 41°C, flushed, dry skin, and often headache, nausea, and vomiting. Finally, there is the 2-to-4 hour sweating stage during which the fever drops rapidly and the patient sweats. In all types of malaria, the periodic febrile response is caused by rupture of mature schizonts. In *P. vivax* and *P. ovale* malaria, a brood of schizonts matures every 48 hr, so the periodicity of fever is tertian ("tertian malaria"), whereas in *P. malariae* disease, fever occurs every 72 hours ("quartan malaria"). The fever in *falciparum* malaria may occur every 48 hr, but is usually irregular, showing no distinct periodicity. These classic fever patterns are usually not seen early in the course of malaria, and therefore the absence of periodic, synchronized fevers does not rule out a diagnosis of malaria.

Physical findings in malaria are nonspecific and offer little aid in diagnosis. In many cases there may be no positive findings other than fever. Splenomegaly is common but may not be apparent early in disease. Hepatomegaly, jaundice, hypotension and abdominal tenderness may also be seen. Malaria does not cause lymphadenopathy and is not associated with a rash.

Many of the deaths from malaria are the result of delayed diagnosis and treatment because the health care provider did not suspect malaria. The diagnosis of malaria requires a high index of suspicion; malaria should be considered in any individual who has a fever and has visited an endemic area for malaria, received a blood transfusion, or used intravenous drugs. Although 95 percent of individuals infected with malaria develop their primary illness within 6 weeks of exposure, some may have primary attacks up to a year after exposure, and relapses of malaria can occur up to 2–3 years after exposure. Therefore, individuals having a febrile illness and a history of exposure in the last 2–3 years should be evaluated for malaria.

Definitive diagnosis of malaria generally requires direct observation of malaria parasites in Giemsa-stained thick and thin blood smears. Thick blood smears are more difficult to interpret than thin blood smears but they are much more sensitive, as more blood is examined. Thin blood smears, in which parasites are seen within erythrocytes, are used to determine the species of the infecting parasite. The presence of diagnostic forms can vary markedly with the stage of the life cycle, especially early in disease. In *falciparum* malaria, most organisms are not present in the peripheral blood because they are sequestered in the microvascular tissue of internal organs. If malaria is suspected, blood smears should be examined every 6 to 12 hr for at least 2 days. New diagnostic methods include a rapid antigen-capture dipstick test.

and a technique for detecting parasites with a fluorescent stain. Both of these tests are fast, easy to perform and are highly sensitive and specific

Other diagnostic methods include assays to detect malaria antibodies and antigens, and polymerase chain reaction/DNA and RNA probe techniques. These techniques are used primarily in epidemiologic studies and immunization trials and rarely in the diagnosis of individual patients. (13)

Thrombocytopenia (platelet count less than $150 \times 10^9/L$) is common platelets abnormalities and hematological changes 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) Platelet survival is reduced to 2-4 days in severe falciparum malaria 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. Some but not all studies have shown that there is strong association between thrombocytopenia and severity of malaria Severity and mortality of patients with falciparum malaria are increased with severe thrombocytopenia (14)

Ideally malaria treatment should not be initiated until the diagnosis has been established by laboratory testing.

“Presumptive treatment”, i.e., without prior laboratory confirmation, should be reserved for extreme circumstances, such as strong clinical suspicion of severe disease in a setting where prompt laboratory diagnosis is not available. Once the diagnosis of malaria has been made, appropriate antimalarial treatment must be initiated immediately

For pediatric patients, the treatment options are the same as for adults except the drug dose is adjusted by patient weight, and artemether-lumefantrine (Coartem™) and atovaquone-proguanil (Malarone™) can only be used in children ≥ 5 kg. The pediatric dose should never exceed the recommended adult dose. Pediatric dosing with quinine may be difficult due to unavailability of non-capsule forms of this antimalarial. If using a quinine-based regimen for children less than 8 years old, doxycycline and tetracycline are generally not recommended; therefore, quinine can be given in combination with clindamycin as recommended above. In rare instances, doxycycline or tetracycline can be used in combination with quinine in children less than 8 years old if other treatment options are not available or are not tolerated, and the benefit of adding doxycycline or tetracycline is judged to outweigh the risk. For infections attributed to “species not identified” in areas with chloroquine resistance that are subsequently diagnosed as being due to *P. vivax* or *P. ovale*, additional treatment with primaquine or tafenoquine should be administered (see *P. vivax* and *P. ovale*, section below). *P. falciparum* or Species Not Identified — Acquired in Areas Without Chloroquine Resistance for *P. falciparum* infections acquired in areas without chloroquine-resistant strains, which include Central America west of the Panama Canal, Haiti, and the Dominican Republic, patients can be treated with oral chloroquine, or, alternatively, hydroxychloroquine at recommended doses. In addition, any of the regimens listed for the treatment of chloroquine-resistant malaria may be used for the treatment of chloroquine-sensitive *P. falciparum* malaria. Prompt initiation of an effective regimen is vitally important, so using any one of the effective regimens that is readily available would be the preferred strategy. Due to the risk of progression to severe disease in patients with *P. falciparum* infection, patients should be hospitalized to monitor clinical response, and check parasite density every 12–24 hours until clinical presentation improves and a decrease in parasite density becomes apparent. Then, treating clinicians can consider outpatient completion of treatment for patients with improved clinical symptoms and decreasing parasite density. If infections initially attributed to “species not identified” are subsequently diagnosed as being due to *P. vivax* or *P. ovale*, additional treatment with primaquine or tafenoquine should be administered

Patients with any foundations of severe malaria, e.g. impaired consciousness/coma, hemoglobin $<5g/dL$, acute kidney injury, acute respiratory distress syndrome, circulatory collapse/shock, acidosis, jaundice (with other signs of severe malaria), disseminated intravascular coagulation, and/or parasite density of $\geq 5\%$ should be treated promptly and aggressively with parenteral antimalarial therapy, regardless of the species of malaria seen on the blood smear. If severe malaria is strongly suspected but a laboratory diagnosis cannot be made at that time, blood should be collected for diagnostic testing to be done as soon as it becomes available and parenteral antimalarial drugs should be started. All patients with severe malaria, regardless of infecting species, should be treated with intravenous (IV) artesunate. Clinicians caring for patients with severe malaria at a hospital where commercially-available IV artesunate cannot be obtained within 24 hours, should call CDC to obtain IV artesunate, which is available through CDC as part of an expanded-use investigational new drug (IND) protocol.

Severe malaria can progress to a fatal outcome rapidly, so its treatment should be initiated as soon as possible.

Clinicians at hospitals where IV artesunate is not in stock should consider interim treatment with an effective oral antimalarial while obtaining IV artesunate.

Whichever is fastest. If the patient is unable to tolerate oral medications, clinicians will need to consider alternative ways to administer oral medications while awaiting IV artesunate. For example, for patients with nausea and vomiting, an anti-emetic preceding the antimalarial may help, and, for comatose patients, a nasogastric tube can be considered (15).

Plasmodium vivax and *Plasmodium falciparum* frequently found thrombocytopenia as a hematological alteration in their finding (16,17).

Furthermore, it seems as if thrombocytopenia is rather found in individuals with splenomegaly and non-palpable spleens (18,19)

Published data indicates an association between life-threatening malaria requiring endotracheal intubation and thrombocytopenia (20). Which is rather frightening.

Peculiar Associations:

A major point illustrated by researchers from Ghana and the UK is that the fight against malaria seems to have hindered the efforts set forth in the fight against malaria (6).

Data seems to suggest that there exists an association between ABO blood groups and *P. falciparum* infections (21)

Similar Studies:

Despite a rather small data-base, evidence is suggestive of a strong association between *P. vivax* infection and severe thrombocytopenia (22).

Data collected in Sudanese children concluded that severe thrombocytopenia is associated with *falciparum* malaria infection and malarial hyperparasitemia (23).

Another study from Sudan illustrated that most (69.4%) of infections were with *P. falciparum* as well as that severe malaria was associated with hyperparasitemia and *P. vivax*, the same study also showed that AKI was the only significant mortality risk factor (24).

A third study from Sudan illustrated that 22.2% of their participants (all of whom had severe *P. vivax* infections) suffered from thrombocytopenia (25). A study conducted in White Nile State in Sudan reported that 'The median platelet count was significantly lower in *P. falciparum*-infected patients, with thrombocytopenia being significantly associated with *falciparum* malaria' (26).

In 2011, a paper from India showed a significant association between *P. vivax* infection and thrombocytopenia (27), so much so that the authors clearly advocated for the insertion of thrombocytopenia in the criteria of severe malaria diagnosis.

Authors from a 2003 study wrote the following in regards to the topic 'Initial thrombocytopenia was present in 43–58% of children with *P. falciparum* malaria but was not more frequent in severe outcome or cerebral malaria' (28).

In neighboring Ethiopia, a study strongly suggested a correlation between 'platelet indices' and the predictability of severe malaria (29).

Platelet count and parasite count were negatively correlated in another study (30).

Research Methodology:

Study design

This is an analytical case-control hospital-based study

Study area

The study was conducted in Ibrahim Malik Teaching Hospital, El-Sahafa, Khartoum, Khartoum, Sudan. The hospital is approximately 2.5 Kilometers South of Khartoum International Airport (GHQ2+RV Khartoum, Sudan). It is surrounded by a neighborhood of middle socioeconomic status. The hospital is public and contains an Emergency Room, Internal Medicine Department, Surgery Department, Pediatrics Department, and Obstetrics and Gynecology Department.

Study population

The study was conducted on pediatric patients diagnosed with malaria in Ibrahim Malik Teaching Hospital

Inclusion and exclusion criteria

Inclusion criteria:

All pediatric patients investigated for malaria and thrombocytopenia presented to study area during study period

Exclusion criteria:

Patients with malaria and thrombocytopenia due to congenital or other acquired causes were excluded from the study and those who or their co-patient refuses to participate in study

Sampling methodology:
This was a multi-stage systematic sampling targeting the ward and ER of Ibrahim Malik Teaching Hospital. The systematic sampling will take place in the following manner:

i) We will give the participants numbers (so as to assure confidentiality) (on basis of 2)

ii) These numbers were placed into an online random number generator

iii) The numbers picked by the generator were sampled

iv) The sample size was calculated based on the equation below.

For example, the numbers 8, 17, 22, 25, 60, 99, 77, and 32 were inserted into the generator; the generator will pick one half of them (e.g.: 8, 25, 77, 32); following this, participants with these numbers were sampled.

Equation: $n = N1 + N(D)2$

n = Sample size

D = Degree of Precision

N = Sample

n = $7501 + 750 (0.04)^2$

n = 334

Study variables:

Independent variables:

Age, gender, residence

Constant variables:

1) Symptoms and signs of malaria

2) Platelet count

3.6 Data collection tools and methods:

A structured data collection sheet was filled by researcher

The data was collected from patients or co- patients who met the inclusion criteria by interview questionnaire, after explanation in a simple language, about research plans, objectives, and the accepted outcome. All patients or co- patients participated in the study were consented, identified through reviewing of their medical report. This took place in the aforementioned hospital's ER and ward.

3.7 Ethical consideration

First, Ethical clearance was obtained from ethical clearance committee of Sudan Medical Specialized Board Council. Then, Ethical clearance was obtained from EDC. Following this, the Khartoum State Ministry of Health (MOH)'s ethical committee was contacted for ethical clearance. Finally, the Hospital administration's approval was obtained. Written Informed Consent was obtained from all participants. Privacy of collected data was considered [no name, data coded and interpreted in form of statements, tables, and figures].

Results

A total of 334 cases of malaria were included in this study. Amongst those, the prevalence of thrombocytopenia was 28.4 % (95 cases). The mean platelet count was 247.42 ± 187.89 . The range was between 7 and 704.

The mean age of participants was 6 years old ± 5.13 ; the range was between 1 year and 17 years. In other words, 182 of our participants (52%) were under 5 years of age and were the largest group of our participants, followed by participants aged 6-10 years of age at 80 (23%), followed by 11-14 years olds at 63 (18%), with the remainder 25 (7%) being 15-18 years of age. These are illustrated in figure 4.1 and table 4.1 below. 182 (52%) of our participants were female, while the remainder 48% were male (illustrated in figure 4.2 and table 4.2). Thrombocytopenia was most found in males ages 5 and under, more specifically in the age range of 2-3.

Of our participants, 68.8% were of urban residence, while the remainder 31.2% were of rural residence (Figure 4.3).

Of our participants, 52% (182) were ill for 4-7 days, while 31% (110) of them were ill for 1-3 days prior to hospitalization. Finally, 17% (59) of our participants were ill for 8 days or more. Mean duration of illness was 6.04 days ± 4.13 (Figure 4.4) reported in all 334 (100%), followed by vomiting reported in 58.3% (203). The 3rd most commonly reported symptom was diarrhea, which founded in 37.5% (131). The 4th most reported symptom was headache, reported in 20.8% (73). This was followed by fatigability, which affected 12.5% (44). Finally, the least reported symptom was nausea which founded in 4.2% (15) (Figure 4.5).

Illustrated in figure 4.6 are the features of severe malaria that founded in our participants. Of importance, 56.2% (197) of our participants had at least 1 symptom of severe malaria.

The parasite count found in this study showed that 66.7% (233) had a parasite count of one cross (+), only 6.3% (22) had a parasite count of two crosses (++), 23% (88) had a parasite count of three crosses (+++), while only 4.2% (15) had a parasite count of four crosses (++++) . These are illustrated in figure 4.7 below. In so far as parasite count is concerned, a significant correlation was found to exist between hyperparasitemia (four crosses (++++)) and thrombocytopenia (table 4.2).

As per platelets are concerned, 219 (62.5%) had a platelet count of over 150,000 platelets per microliter of blood, 36 (10.4%) had a platelet count of 100,000-149,999 platelets per microliter of blood, 44 (12.5%) of them had a platelet count of 50,000-99,999 platelets per microliter of blood, 36 (10.4%) had a platelet count of 20,000-49,999 platelets per microliter of blood. Finally, only 4.2% (15) had a platelet count of less than 20,000 platelets per microliter of blood.

Figure 4.8 illustrates the platelet count, mild thrombocytopenia (100,000-150,000) was found in 10.4%, moderate thrombocytopenia (50,000-99,999) in 12.5%, while severe thrombocytopenia was found in 14.6%, divided as follows, 10.4% had a platelet count of (20,000-49,999) and 4.2% had a platelet count of under 20,000.

The mean total WBC count was 9.94, with an SD of 4.73. 35 participants (10.42%) founded leucopenia, while 202 (60.4%) had leukocytosis. In so far as total WBC count is concerned, 54.2% (190) of our participants had a high total, 35.4% (124) of them were of normal range, and the remainder 10.4% (36) founded a low total WBC count (Figure 4.9). The mean Hemoglobin count was 8.42 ± 2.91 g/dl. No significant correlation was found between HB levels and thrombocytopenia (table 4.3).

The data illustrated a significant correlation between the listed symptoms of severe malaria which founded in our population and thrombocytopenia (table 4.1) as well as a significant correlation between high total White Blood Cells (WBCs) and thrombocytopenia (in the context of malaria) (table 4.1).

Fever, vomiting, diarrhea, headache, nausea and fatigability were run through the same test, but were not found to be significant (p-value of over 0.05), these are also illustrated in table 4.1. No significant correlation was found between Renal Function Test (RFT) and thrombocytopenia (table 4.4).

No significant correlation was found between Liver Function Test (LFT) and thrombocytopenia (table 4.5).

A statistically significant correlation was found between rural residence and thrombocytopenia (table 4.6).

A significant correlation was found between the youngest age group (under 5 years of age) and thrombocytopenia (table 4.7)

A significant correlation was found between the male gender and thrombocytopenia (table 4.8)

Our findings showed that 58.3% (204) of our participants were treated using artisunate (injection), followed by quinine injection at 27.1% (95), while only 14.6% (51) received coartem tabs. 100% of our participants fully recovered and had normal range platelet counts by discharge (Figure 4.10).

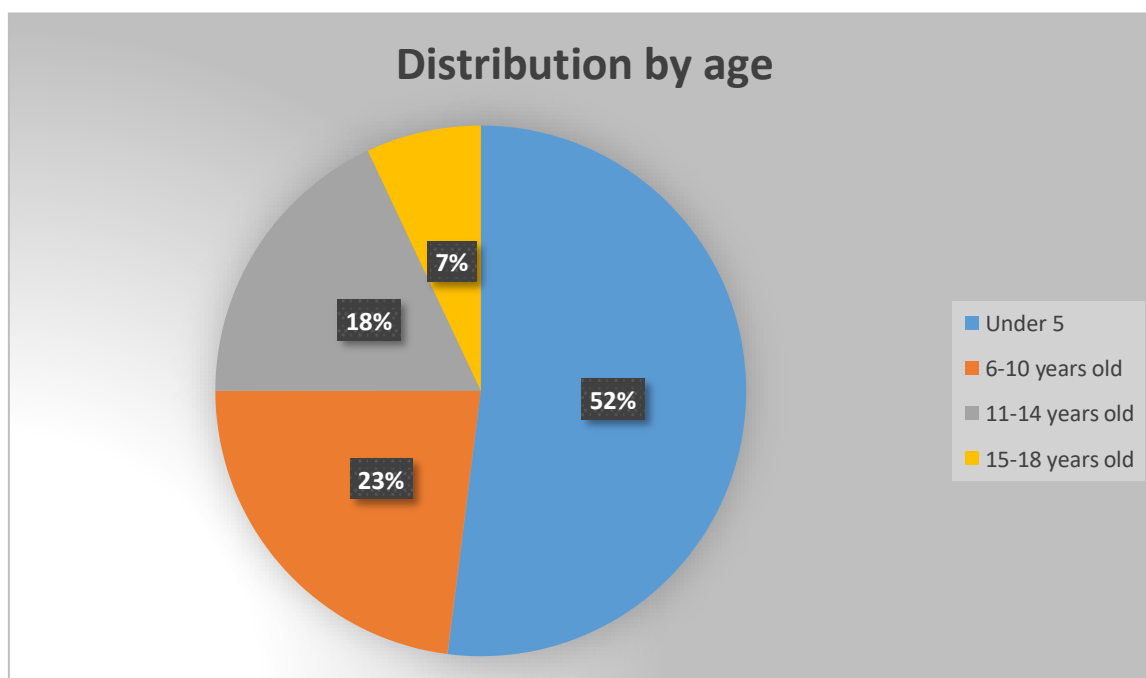


Figure 1: Distribution of participants by age

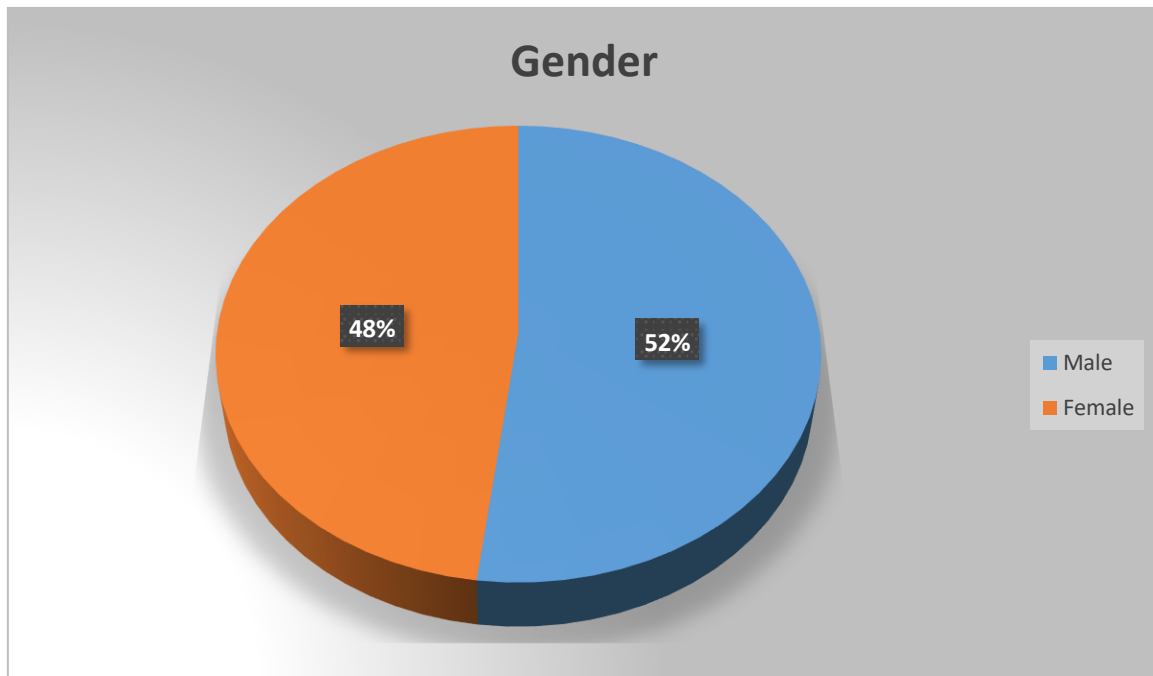


Figure 2: Distribution of participants by age

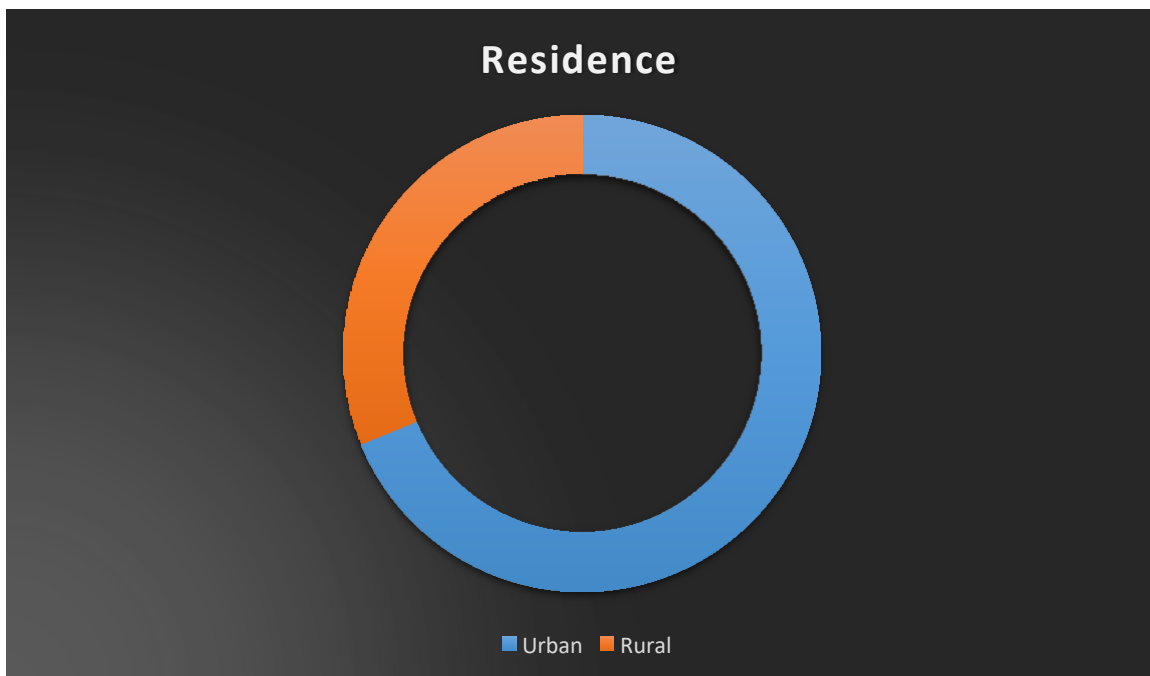


Figure 3: Distribution of participants by residence

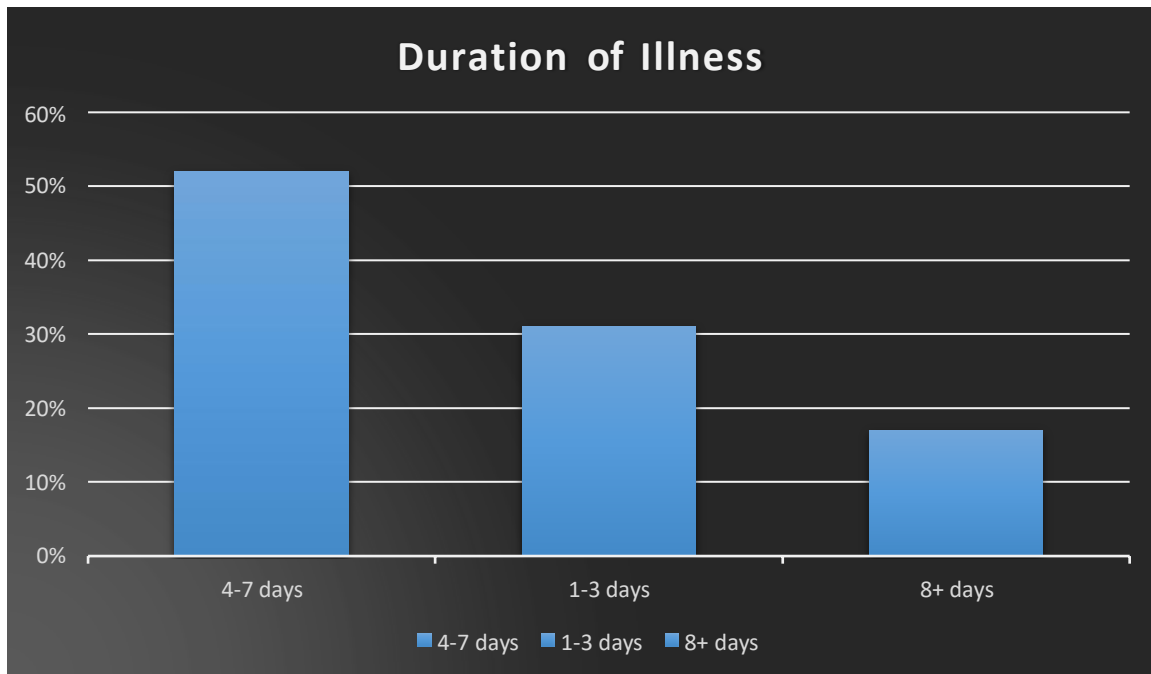


Figure 4: Distribution of participants by duration of illness

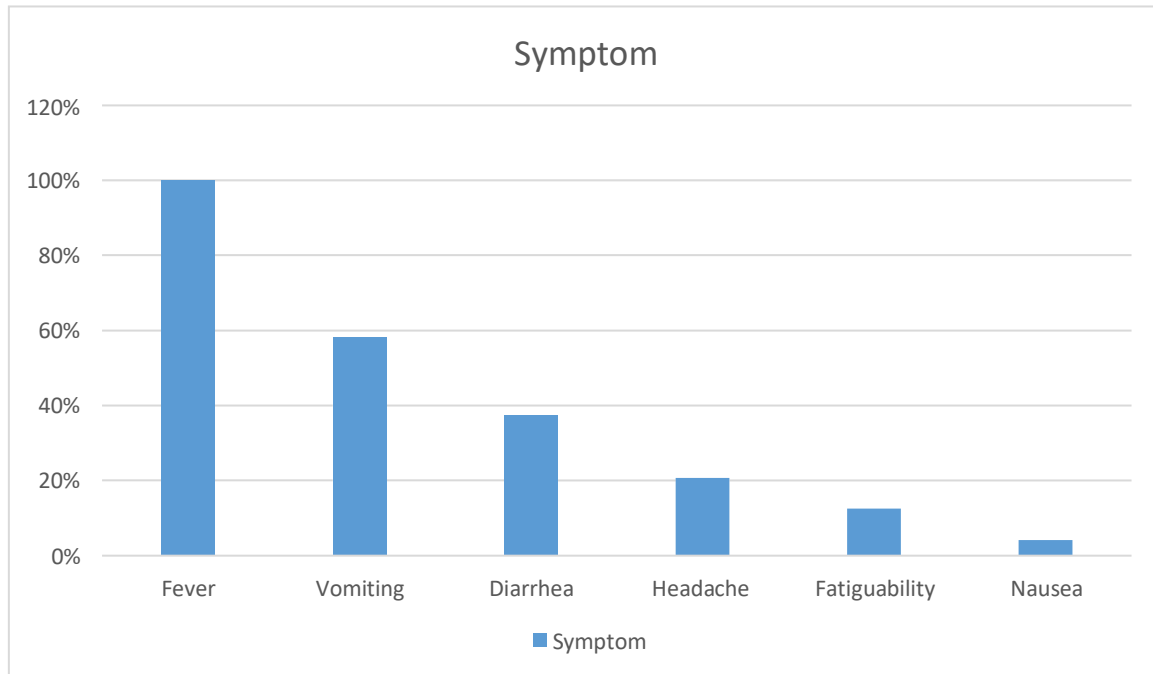


Figure 5: Symptoms

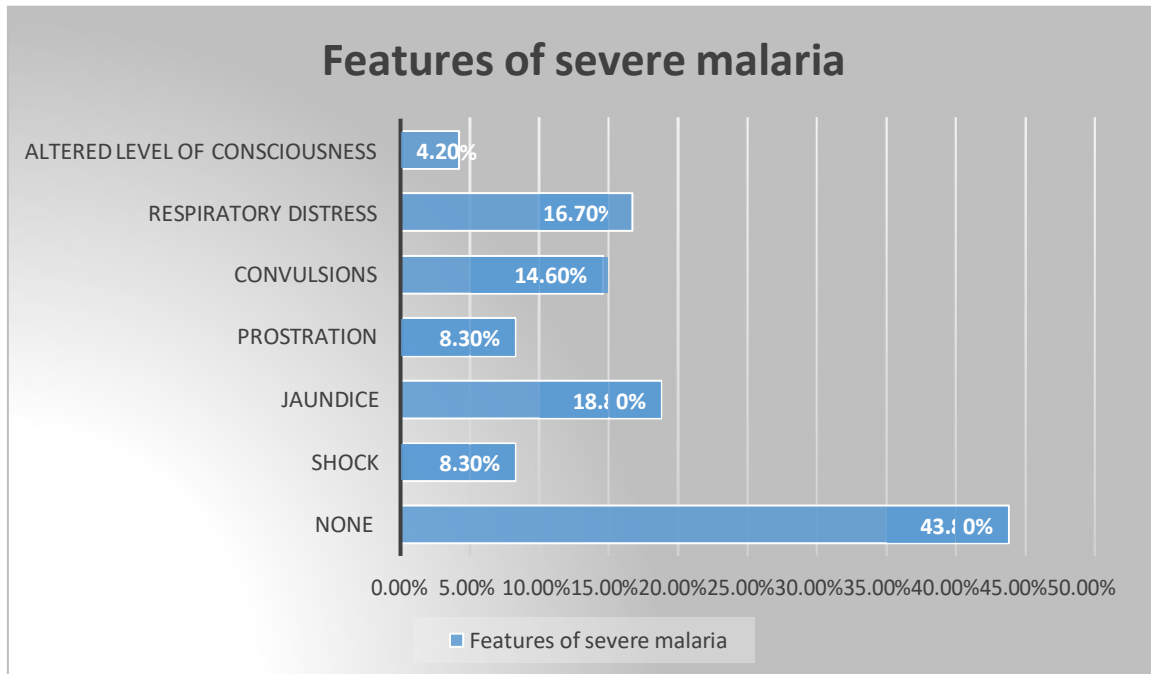


Figure 6: Features of severe malaria

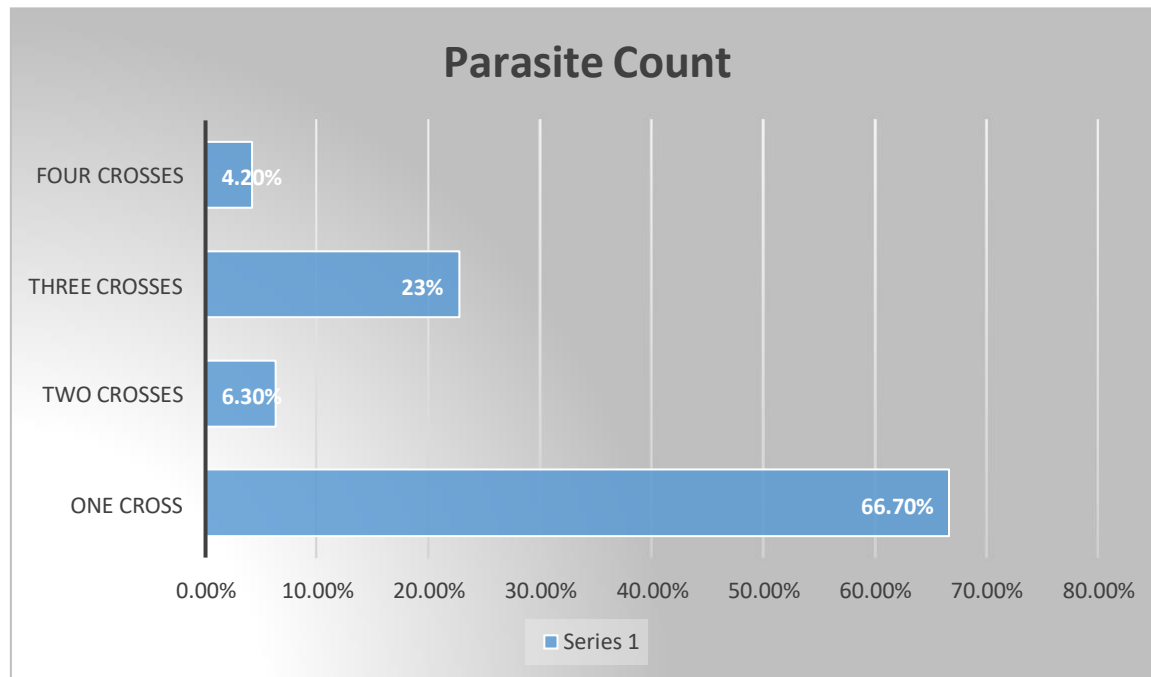


Figure 7: Parasite count

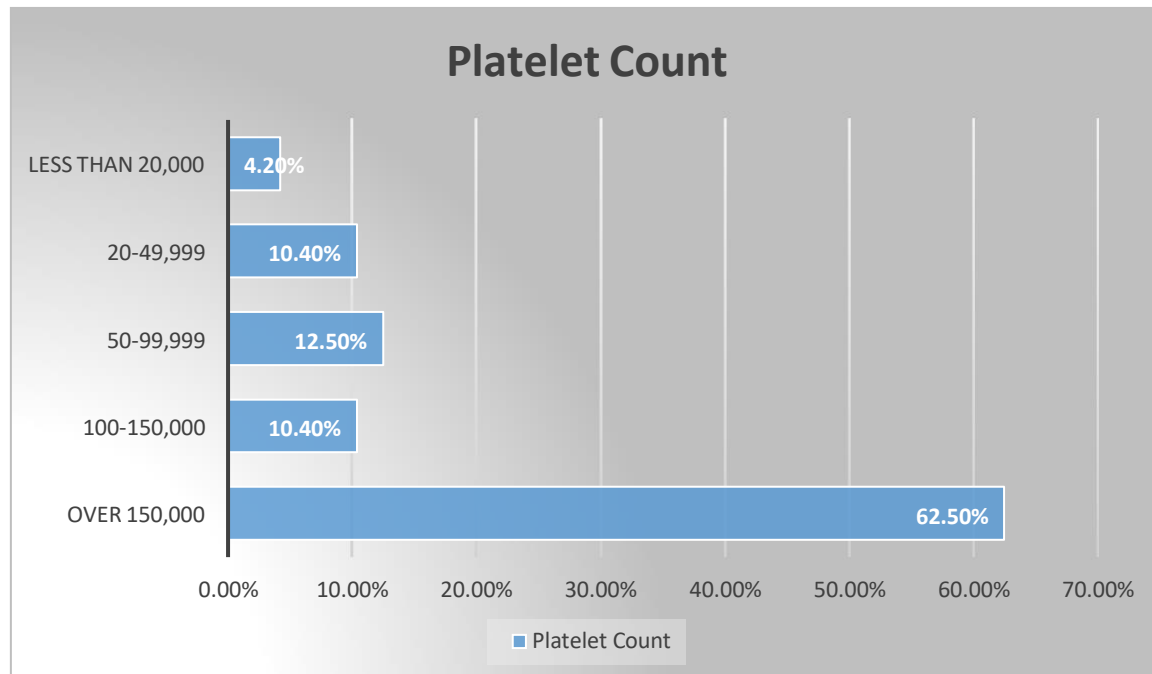


Figure 8: PLT count

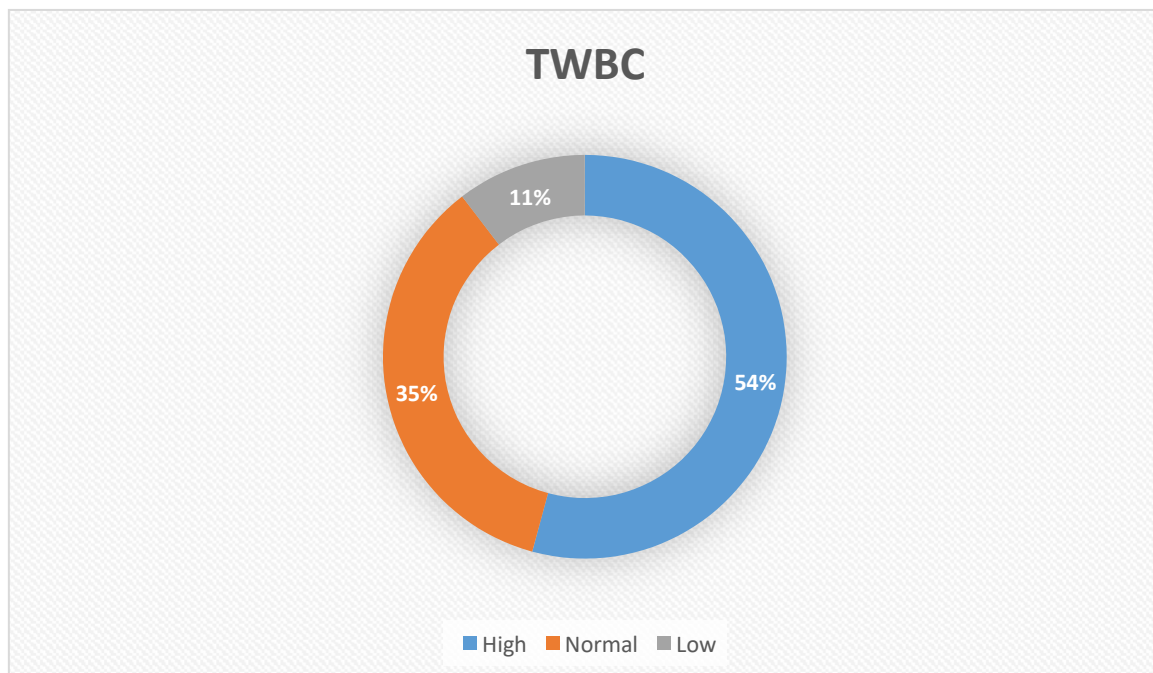


Figure 9: TWBC count

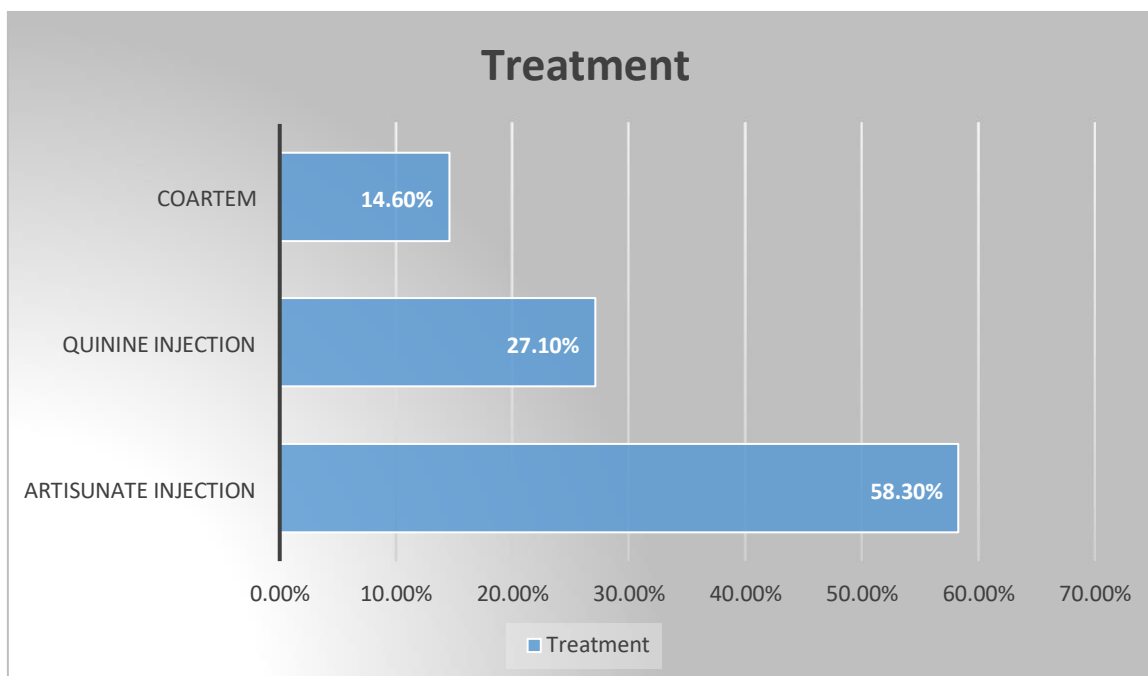


Figure 10: Treatment

Table 1: Correlation between Thrombocytopenia signs of severe malaria

Sign	Thrombocytopenia	P-value
Jaundice	Present	0.02
	Absent	
Leukocytosis	Present	0.04
	Absent	
TWBC count (normal)	Present	0.42
	Absent	
Leukopenia	Present	1.22
	Absent	
Altered LOC	Present	0.03

	Absent	
Respiratory distress	Present	0.04
	Absent	
Convulsions	Present	0.04
	Absent	
Prostration	Present	0.03
	Absent	
Shock	Present	0.04
	Absent	

Table 2: Correlation between Thrombocytopenia and Parasite count

Parasite count	Thrombocytopenia	P-value
One-cross (+)	Present	0.25
	Absent	
Two crosses (++)	Present	0.32
	Absent	
Three crosses (+++)	Present	0.06
	Absent	
Four crosses (++++)	Present	0.02
	Absent	

Table 3: Correlation between Thrombocytopenia and HB level

HB level	Thrombocytopenia	P-value
Normal	Present	0.25

High	Absent	
	Present	2.01
Low	Absent	
	Present	1.35
	Absent	

Table 4: Correlation between Thrombocytopenia and Renal Function Tests

RFT test	Thrombocytopenia	P-value
Raised Blood Urea	Present	0.12
	Absent	
Raised Serum Creatinine	Present	0.35
	Absent	
Abnormal Serum Sodium	Present	1.23
	Absent	
Abnormal Serum Potassium	Present	2.12
	Absent	

Table 5: Correlation between Thrombocytopenia and Liver Function Tests

LFT test	Thrombocytopenia	P-value
Raised ASP	Present	0.87
	Absent	
Raised AST	Present	0.69
	Absent	
Total Hyperbilirubinemia	Present	0.86

Direct Hyperbilirubinemia	Absent	1.54
	Present	
Low Serum Albumin	Absent	4.33
	Present	
	Absent	

Table 6: Correlation between Thrombocytopenia and Residence

Residence	Thrombocytopenia	P-value
Urban	Present	0.98
	Absent	
Rural	Present	0.47
	Absent	

Table 7: Correlation between Thrombocytopenia and Age

Age	Thrombocytopenia	P-value
Less than 5	Present	0.45
	Absent	
6-10	Present	0.61
	Absent	
11-14	Present	0.69
	Absent	
15-18	Present	0.78
	Absent	

Table 8: Correlation between Thrombocytopenia and Gender

Gender	Thrombocytopenia	P-value
Male	Present	0.35
	Absent	
Female	Present	0.98
	Absent	

Discussion

Our results were highly similar to those reported by other studies. This research clearly illustrated a significant correlation between severe malaria and thrombocytopenia as well as a significant correlation between leukocytosis and thrombocytopenia (in the context of malaria). These are highly similar to those by Mohamedahmed and colleagues (23).

Just like our study, data collected in Sudanese children concluded that severe thrombocytopenia is associated with falciparum malaria infection and hyperparasitemia (23).

Another study from Sudan illustrated that most (69.4%) of infections were with *P. falciparum* as well as that severe malaria was associated with hyperparasitemia and *P. vivax*, the same study also showed that AKI was the only significant mortality risk factor (24).

In agreement with our study, third study from Sudan illustrated that 22.2% of their participants (all of whom had severe *P. vivax* infections) suffered from thrombocytopenia (25).

A study conducted in White Nile State in Sudan reported that 'The median platelet count was significantly lower in *P. falciparum*-infected patients, with thrombocytopenia being significantly associated with falciparum malaria' (26).

In 2011, a paper from India showed a significant association between *P. vivax* infection and thrombocytopenia (27), so much so that the authors clearly advocated for the insertion of thrombocytopenia in the criteria of severe malaria diagnosis. These results are in agreement with the above mentioned findings reported in this study.

Authors from a 2003 study wrote the following in regards to the topic 'Initial thrombocytopenia was present in 43–58% of children with *P. falciparum* malaria but was not more frequent in severe outcome or cerebral malaria' (28). This is in agreement with the results we just reported.

A study from Ethiopia strongly suggested a correlation between 'platelet indices' and the predictability of severe malaria (29). This study is compatible with our reported findings.

However, in another study, unlike our study, platelet count and parasite count were negatively correlated (30).

A study from Nigeria reported the following: 'While thrombocytopenia was the most common haematological finding and may be of diagnostic importance, anaemia and leucocytosis were more common in the under fives' (31).

A 2021 paper out of Ghana reported a lower mean platelet level amongst urban residents suffering from malaria (32). This is not in agreement with our findings reported earlier.

In 2021, a paper out of Nigeria reported no significant association between gender and platelet count in malaria patients (33). These do not agree with our findings above.

Malarial infection was found to cause low platelets, leukopenia and low lymphocyte counts amongst patients living in the Thailand-Myanmar border (34). These are rather similar to our findings.

Conclusion:

In conclusion, our study found a significant correlation between severe malaria (founded as positive BFFM and symptoms of severe malaria) and thrombocytopenia. Additionally, it was found that high total WBC count was significantly correlated with thrombocytopenia in malaria.

Furthermore, we found that thrombocytopenia was significantly correlated with rural residence, young age (namely under 5), and male gender.

In addition, no correlation was found between thrombocytopenia and Renal Functions and Liver Functions.

We, therefore, find it appropriate to conclude that thrombocytopenia was associated with a number of signs and symptoms (as well as laboratory investigations) of malaria.

Recommendation(s):

- 1) The immediate recognition of thrombocytopenia as a marker of severe malaria
- 2) In the context of absence of investigations for malaria (e.g.: Blood Film For Malaria (BFFM)), the above findings can be used to hypothesize the presence of malaria
- 3) Seeing to it that pediatricians recognize the importance of thrombocytopenia in the context of malaria
- 4) Authorities reminding doctors of the importance of observing platelet counts in malaria patients

- 5) The establishment and funding of further research in this finding (thrombocytopenia in malaria)

List of Abbreviations (in alphabetical order):

BFFM = Blood Film For Malaria

DIC = Disseminated Intravascular Coagulopathy

EC = Endothelial Cell

ER = Emergency Room

LFT = Liver Function Test

LOC = Level Of Consciousness

PLT = Platelet

RBC = Red Blood Cell

RFT = Renal Function Test

SD = Standard Deviation

WBC = White Blood Cell

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