Sudan University of Science and Technology

College of Graduate Studies

Evaluation of Platelets Count and Their Indices as Prognostic Markers for *Falciparum Malaria* among Sudanese Patients in Khartoum North

تقييم عدد الصفائح الدمويه و دلالتها كعلامات تنبؤيه لملاريا الفالسيبرم لدي المرضي السودانين في الخرطوم بحري

A Thesis Submitted in Partial Fulfilment for Requirement of M.Sc. Degree in Haematology and Immune Haematology

By:
Mazin Mohi eldein Dafa Alseed Abd Elgadir
B.Sc.-College of Medical Laboratory Science
Omdurman Islamic University (2013).

Supervisor:
Prof: ShadiaAbdAlatti
Sudan University of Science and Technology

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الاية

قَالَ تَعَالَىٰ:

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الذاريات: ٢١

Dedication

To my beloved parents, my brothers and sister who are everything for me.

To my supervisor;Prof. ShadiaAbdAlatti.

To my teachers for showing me the excitement and joy of research.

To my dear friends and colleagues who supported me.

To those who are interested in hematology all around the world.

I dedicate this work.

Acknowledgement

My gratitude and prayers to almighty Allah for the mercy which accompanied me during the long path of this research.

I owe so much to my supervisor Prof. ShadiaAbdAlatti for her immense effort not only to accomplish this work but to inspire the research's soul on me and to push me to reach my full potential.

I would like to thank the family I found in the Hematology Department for their valuable assistance and encouragement.

For all those whom I haven't mentioned, my deepest gratitude.

Contents

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Abbreviation:

ACTs:Artemisinin-based combination therapies

ADP: Adenine di phosphate

ATP: Adenine tri phosphate

BSS: Bernard-Soulier syndrome

CBC: Complete blood count

DC: Deferential channel

DGs: Dense granules

DIC: Disseminated intravascular coagulation

EDTA: Ethylene diamine tetra acetic acid

ET: Essential thrombocythemia

Fcm: Flow cytometeric

Fg: Fibrinogen

GP: Glycoprotein

GT: Glanzmannthrombothenia

HCT: Haemtocrit

HLA: Human leucocyte antigen

HMS: Hyper-reactive malarial splenomegaly

IgG: Immunoglobulin G

IgM: Immunoglobulin M

LAMP: Loop-mediated isothermal amplification

MCV: Mean cell volume

MPNS: Mylopoliferative neoplasms

MPV: Mean platelets volume

MS: Massspectrometry

P.F: *Plasmodium falciparum*

P.V: *Plasmodium vivax*

PCR: Polymerase chain reaction

PCT: Plateletscrit

PDW: Platelets distribution width

RBCS: Red blood cells

RDW: Red cell distribution width

TNF: Tumor necrosis factor

TSS: Tropical splenomegaly syndrome

VWF: Von willebrand factor

WHO: World Health Organization

Abstract

This is a case control study carried out in Khartoum North Teaching Hospital-Khartoum State, from February to April 2017 to evaluate platelets count, Mean Platelets Volume, Plateletcrit, and Platelet Distribution Width as prognostic markers for *Falciparum malaria*.

Forty five *falciparum malaria* patients (13males and 32 females) and fifty five healthy individuals were enrolled in this study. The patients were divided into three groups according to the duration of the disease that is (1-3 days), (4-6 days) and (7-10 days). Blood samples were drawn under aseptic conditions using EDTA as an anticoagulant and the analysis was performed by an automated sysmex. The obtained data were analyzed by SPSS version 16 and expressed as means and SD in cases versus control. The results revealed that malaria patients had significantly ($P \leq 0.05$) lower values than the healthy individual with regard to the platelets count 10^9/L (250±81 versus 306±59) and plateletcrit (PCT) % (0.19±0.06 versus 0.24±0.05) and a significant ($P \leq 0.05$) increase in mean platelets volume (MPV) fl (8±0.91 versus7.50±0.87) and platelets distribution width (PDW) (16.7±1.39 versus15.4±1.38). Insignificant differences in the studied parameters were found between male and female patients. As the malaria duration was increased the platelets' count was significantly decreased and MPV was significantly increased ($P \leq 0.05$). MPV and PDW recorded a significant inverse correlation with the platelets count. Significant ($P \leq 0.05$) positive correlations were recorded between platelets count and PCT and between MPV and PDW. RDW exhibited significant positive correlations with platelets count and PCT ($P \leq 0.05$) and no correlation was detected between the other platelets' indices and RBCs indices ($P > 0.05$).

It is concluded that, *falciparum malaria* affects the platelets count and indices. More studies are needed to verify the use of these parameters as diagnostic markers for *falciparum malaria* infection.

ملخص الدراسه

هذه دراسة حالة مرضيه وحاله ضابطه أجريت فى ولاية الخرطوم فى مستشفي الخرطوم بحري التعليمي ولايه الخرطوم من فبراير الي ابريل 2017 لقياس عدد الصفائح الدمويه ومتوسط حجم الصفائح الدمويه وحجم شغل الصفائح الدمويه ونطاق توزيع الصفائح الدمويه كعلامات تنبؤيه في مرضي ملاريا الفالسيبرم. خمس وأربعين من مرضي ملاريا الفالسيبرم (13 من الذكور و32 من الاناث) وخمس و خمسون من الاشخاص الاصحاء كمجموعه ضبط كانو مشاركين في هذه الدراسه. تم تقسيم المرضي لثلاث مجموعات وفقا لفتره الاصابه حتي تشخيص المرض (1-3 ايام),(4-6 ايام) و (7- 10ايام) عينات الدم سحبت في اجواء معقمه مستخدمين EDTA كمانع للتجلط وتم التحليل بواسطه جهاز السيسميكس ذاتي الحركه .وتم تحليل النتائج التي حصلنا عليها بواسطه برنامج الحزم الاحصائيه للعلوم الاجتماعيه اصدار 16 وتم التعبير عنها بواسطه الوسط الحسابي والانحراف المعياري في المرضي مقابل الاصحاء. النتائج اظهرت ان مرضي الملاريا لديهم إنخفاض ذو دلاله إحصائيه (القيمه المعنويه أقل من 0.05) اقل قيمه من مجموعه الضبط في ما يختص بعدد الصفائح الدمويه 10^9/ليتر (250±81 مقابل 306±59) وحجم شغل الصفائح الدمويه فيمتوليتر (0.19±0.06 مقابل 0.24±0.05) واظهرت ايضا ارتفاع ذو دلاله احصائيه (القيمه المعنويه اقل من0.05) في متوسط حجم الصفيحه الدمويه % (8±0.91 مقابل7.5±0.87) ونطاق توزيع الصفائح الدمويه (16.7±1.39مقابل 15.4±1.38) . ليس لدينا فروقات احصائيه في العوامل الاختباريه بين مرضي الجنسين الذكور والاناث (القيمه المعنويه اكبر من0.05). كلما ذادت فتره حدوث الاصابه بالملاريا انخفض عدد الصفائح الدمويه انخفاض ذو دلاله احصائيه وارتفع حجم الصفائح الدمويه ارتفاع ذو دلاله احصائيه (القيمه المعنويه اقل من 0.05). حجم الصفائح الدمويه ونطاق توزيع الصفائح الدمويه سجلت ارتباط عكسي ذو دلاله احصائيه مع عدد الصفائح الدمويه. ارتباط ايجابي ذو دلاله احصائيه (القيمه المعنويه اقل من 0.05) سجل بين عدد الصفائح الدمويه و حجم شغل الصفائح الدمويه وايضا بين حجم الصفائح الدمويه و نطاق توزيع الصفائح الدمويه. نطاق توزيع كريات الدم الحمراء وجد في ارتباط ايجابي ذو دلاله احصائيه مع عدد الصفائح الدمويه و حجم شغل الصفائح. ليس هناك ارتباط ذو دلاله احصائيه بين الصفائح الدمويه ودلالاتها وبين كريات الدم الحمراء ودلالاتها الاخري(القيمه المعنويه اكبرمن 0.05)

خلصت الدراسه بان ملاريا الفالسيبرم تؤثر علي عدد الصفائح الدمويه ودلالاتها. نحتاج لمزيد من الدراسات لتاكيد إستخدام هذه العوامل كعلامات تشيخيصيه لعدوي ملاريا الفالسيبرم.

Introduction

Haemostasis is a process which causes bleeding to stop, consist of three components which are platelets, blood vessels and coagulation protein act simultaneously to maintain blood in a fluid state inside blood vessel by forming blood clot when blood vessels were injured and fibrinolysis when it's healed.

Platelets are fragmentation of cytoplasm of megakaryocyte, one of the largest cells in the body which are produced in the bone marrow. The main function of platelet is formation of mechanical plugs during the normal haemostatic response to vascular injury.

Malaria is the most important parasitic disease of humankind. It is a global health problem with an annual incidence of about 212 million cases of malaria and an estimated 429000 deaths in 2015.

Once prevalent over much of the world, it is now confined to the tropical and subtropical areas of Africa, Asia, South and Central America. Event so nearly half of the world's population may be exposed to the risk of malaria.

Changes in platelets count and indices during acute malaria are commonly reported in the medical literature, especially in *plasmodium falciparum* infection, in general the underlying mechanism of thrombocytopenia in malaria are peripheral destruction, excessive sequestration of platelet in spleen and coagulation disturbances. Furthermore platelets activation leads to alter the volume of these cells and platelet distribution width and also alter plateletcrit because the mean platelets volume is combined with absolute platelets count.

Chapter One
Literature Review

1.1. Haemostasis:

The normal hemostatic response to vascular damage depends on a closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors. The Hemostatic systems thus represent a delicate balance between procoagulant and anticoagulant mechanism allied to a process for fibrinolysis. The five major components involved are platelet, coagulation factor, coagulation inhibitors, fibrinolysis and platelet vessel. (Hoffbrand *et al* 2010).

1.1.1. Platelets definition:

Platelets are fragmentation of cytoplasm of megakaryocytes one of the largest cells in the body which are produced in the bone marrow (Hoffbrand *et al* 2010).

1.1.2. Platelets structure:

Platelets are extremely small and discoid, 2–3 μm in greatest diameter, with mean volume 7-11fl. The glycoprotein of the surface coat are particularly important in the platelet reaction of adhesion and aggregation which are the initial event leading to platelet plug formation during haemostasis. Adhesion to collagen is facilitated by glycoprotein Ib(defective on Bernard –Soulier syndrome), and glycoprotein IIb / IIIa (defective in Glanzmann'sthrombasthenia) are important in the attachment of platelets to vonWillebrand factor (VWF) and hence to vascular subendothelium where signaling interaction are occur. The binding site for IIb/IIIa is also the receptor for fibrinogen which is important in platelet-platelet aggregation. The membrane phospholipids are particularly important in coagulation cascade activation (Hoffbrand*et al* 2010). The platelet contains three types of storage granules: dense, alpha and lysosome. Alpha granules contain clotting factor, VWF, platelet –derived growth factor and other protein. Dense granules are less common and contain adenosine diphosphate (ADP), adenosine triphosphate (ATP),

serotonin and calcium. Lysosomes contain hydrolytic enzymes (Hoffbrand*et al* 2010).

1.1.3. Platelets functions:

The main function of platelets is formation of mechanical plugs during the normal haemostatic response to vascular injury. Platelet functions fall into three adhesion, aggregation and release reactions (Hoffbrand*et al* 2010).

1.1.3.1. Platelet adhesion:

The binding of glycoprotein (GP)Ibto vonwillebrand factor lead to adhesion to subendotheliem and also expose the GPIIb/IIIa binding sites to fibrinogen and von willebrand factor leading to platelet aggregation. TheGPIa site permits direct adhesion to collagen and also explores the GPIIb/IIIa binding sites (Hoffbrand*et al* 2010).

1.1.3.2. Platelet aggregation:

Platelet aggregation is induced by a variety of stimuli including ADP, thrombin, thromboxane A_2, collagen, and epinephrine.This is characterized by cross- linking of platelet through active GP IIb/IIIa receptors with fibrinogen bridges. Arresting platelet has about 50-80.000 GP IIb/IIIa receptors, which don't bind fibrinogen, VWF or other ligand. Stimulation of platelet leads to increase in GP IIb/IIIa molecules, enabling platelet cross-linking with fibrinogen bridges (Hoffbrand*et al* 2010).

1.1.3.3. Platelet release reaction:

Primary activation by various agonists induces intracellular signaling, leading to release of alpha granule content. These have an important role in platelet aggregate formation and stabilization and, in addition, the ADP release from dense granules has a major positive feedback role in promoting platelet activation (Hoffbrand*et al* 2010).

During circulation, platelets are reactive to various stimuli and release the materials stored in the specific granules. This 'release reaction' is an important step of primary haemostasis. Energy and messengers required for platelet reactivity are provided by mitochondria and the dense tubular system. Each granule population has specific properties concerning both the structure and the role played by the released constituents. Dense granules contain small non-protein molecules that are secreted to recruit other platelets. F-Granules contain large adhesive and healing proteins. Lysosomes contain hydrolases able to eliminate the circulating platelet aggregate. The extrusion of storage granules' content to the platelet's environment occurs according to regulated secretion events: movements of granules, apposition and fusion of granule and plasma membranes. Typical platelet disorders resulting from a storage granule abnormality are referred to as a storage pool defect and are characterized by a prolonged bleeding time (Rendue and Bohn 2009).

1.1.4. Platelets disorders:

Platelets are important for primary hemostasis. When a blood vessel is damaged, platelets adhere to exposed subendothelial connective tissue and form a hemostatic plug. Formation of the plug is contingent upon a series of processes, with adhesion, activation, and aggregation all being involved. Patients with quantitative platelet disorders have reduced numbers of platelets. Patients with qualitative disorders have platelets that exhibit abnormal functioning. Defects that impair function can affect platelet receptors, secretory responses, or intracellular signaling pathways. Examples of qualitative platelet disorders include Glanzmann’sthrombasthenia (GT) and Bernard-Soulier syndrome (BSS) (Gilbert 2005).

1.1.4.1 Quantitative platelet disorders:

The platelets count is normally 140,000 to 440,000/μL. However, the count can vary slightly according to menstrual cycle phase, decrease during near-term

pregnancy (gestational thrombocytopenia), and increase in response to inflammatory cytokines (secondary, or reactive, thrombocytosis). Platelets are eventually destroyed by apoptosis, a process independent of the spleen (Kuter 2017).

Quantitative platelet disorders either decrease or increase in platelets count (thrombocytopenia and Essential thrombocythemia respectively). Thrombocytopenia occurs by many mechanism include decrease in platelet production usually by bone morrow failure, increase splenic sequestration of platelet with normal platelet survival, increase destruction and consumption and dilution of platelet. (Kuter 2017).

Essential thrombocythemia (ET) is one of the chronic myeloproliferative neoplasms (MPNs), which are collectively characterized by clonal proliferation of myeloid cells with variable morphologic maturity and hematopoietic efficiency. ET has also been called essential thrombocytosis and primary thrombocytosis. It is characterized by excessive, clonal platelet production with a tendency for thrombosis and hemorrhage (Rumi *et al* 2014).

1.1.4.2. Qualitative platelet disorders:

Disorders of platelet function are suspected in patients who show skin and mucosal hemorrhage despite a normal platelets count and normal level of VWF. These disorders may be hereditary or acquired. (Hoffbrand*et al* 2010).

Hereditary disorders include Glanzmannthrombathenia in which platelets fail to aggregate due to quantitative or qualitative defects of the αIIbβ3 integrin, upon platelet activation; αIIbβ3 binds Fg while VWF, fibronectin and vitronectin may also contribute to the protein bridges that mediate aggregation. (Nurden*et al* 2012).

Bernard-Soulier syndrome (BSS) is an extremely rare inherited bleeding disorder the understanding of which is still evolving. It is characterized by the absence of a major carbohydrate-containing protein complex on the platelet surface which

results in a severe deficiency of four glycoproteins (GP), GPIbα, GPIbβ, GPIX, and GPV. (Savoia*et al* 2011).

Storage pool diseases associated with Platelet dense granules (DG) its membrane bound compartments that store polyphosphate and small molecules such as ADP, ATP, Ca^{2+}, and serotonin. The release of DG contents plays a central role in platelet aggregation to form a hemostatic plug. Accordingly, congenital deficiencies in the biogenesis of platelet DGs underlie human genetic disorders that cause storage pool disease and manifest with prolonged bleeding. (Andrea and Santiago 2016).The most common acquired disorders are antiplatelet drugs, hyperglobulinaemia and uraemia. Antiplatelet drugs like aspirin therapy is most common cause of defective platelet function. The cause of aspirin defect is inhibition of cyclooxygenase with impaired thromboxane A_2 synthesis, Hyperglobulinaemia associated with multiple myeloma interference with platelet adherence, release and aggregation (Hoffbrand*et al* 2010).

1.1.5. Platelet indices:

Platelet indices are biomarkers of platelet activation. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of settings without bringing extra costs. Among these platelet indices, plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) are a group of platelet parameters determined together in automatic CBC profiles; they are related to platelets' morphology and proliferation kinetics (Budak*et al* 2016).

1.1.5.1. Mean platelet volume:

The volume of platelets in the bloodstream is heterogeneous, and their structure and metabolic functions differ. Typically, the average mean cell volume is 7.2–11.7 fL in healthy subjects. In MPV, the analyser-calculated measure of thrombocyte volume is determined directly by analysing the platelet distribution

curve, which is calculated from a log transformation of the platelet volume distribution curve, to yield a geometric mean for this parameter in impedance technology systems. In some optical systems, MPV is the mode of the measured platelet volume MPV is determined in the progenitor cell, the bone marrow megakaryocyte. The platelet volume is found to be associated with cytokines (thrombopoietin, interleukin-6 and interleukin-3) that regulate megakaryocyte ploidy and platelet number and result in the production of larger platelet. When platelet production is decreased, young platelets become bigger and more active, and MPV levels increase. Increased MPV indicates increased platelet diameter, which can be used as a marker of production rate and platelet activation. During activation, platelets' shapes change from biconcave discs to spherical, and a pronounced pseudopod formation occurs that leads to MPV increase during platelet activation (Budak*et al* 2016).

MPV acts as a negative or positive acute phase reactant in different inflammatory conditions. High MPV levels are associated with high-grade inflammation owing to the presence of the large platelets in circulation. MPV might decrease in high-grade inflammation due to the consumption and sequestration of these large platelets in the vascular segments of the inflammatory region. Low MPV is associated with low-grade inflammation, like rheumatoid arthritis and attacks of familial Mediterranean fever. MPV decreases and increases in acute and chronic disorders, respectively (Budak*et al* 2016).

MPV shows the activity of disease in systemic inflammation, acute pancreatitis, unstable angina, and myocardial infarction. MPV can be a modifiable marker in identifying patients with active ankylosing spondylitis and rheumatoid arthritis, which is thought to be due to increased consumption of platelets in the inflammation area and MPV increases with therapy in these patients (Budak*et al* 2016).

1.1.5.2. Platelet distribution width:

PDW is an indicator of volume variability in platelets size and is increased in the presence of platelet anisocytosis. PDW is a distribution curve of platelets measured at the level of 20% relative height in a platelet-size distribution curve, with a total curve height of 100% The PDW reported varies markedly, with reference intervals ranging from 8.3 to 56.6% PDW directly measures variability in platelet size, changes with platelet activation, and reflects the heterogeneity in platelet morphology Under physiological conditions, there is a direct relationship between MPV and PDW; both usually change in the same direction (Budak*et al* 2016).

1.1.5.3. Plateletcrit:

PCT is the volume occupied by platelets in the blood as a percentage and calculated according to the formula PCT = platelets count × MPV / 10,000 (25-27). Under physiological conditions, the amount of platelets in the blood is maintained in an equilibrium state by regeneration and elimination. The normal range for PCT is 0.22–0.24% in healthy subjects, platelet mass is closely regulated to keep it constant, while MPV is inversely related to platelets counts. Genetic and acquired factors, such as race, age, smoking status, alcohol consumption, and physical activity, modify blood platelets count and MPV (Budak*et al* 2016).

1.2. Malaria infection:

Human malaria parasite was first seen in 1880, and their development both in the anopheline mosquito and in human blood stream was well understood in 1990.it is thought by some authorities that the malaria parasite is originated firstly in south Asia and spread into Africa and then into Europe (Arora and Arora 2015).

1.2.1 Epidemiology:

The World Malaria Report, published annually by WHO (2016), tracks progress and trends in malaria control and elimination across the globe. It is developed by

WHO in collaboration with ministries of health and a broad range of partners. The WHO (2016) report draws on data from 91 countries and areas with ongoing malaria transmission.

According to the report, there were 212 million new cases of malaria worldwide in 2015 (range 148–304 million). The WHO (2016) African Region accounted for most global cases of malaria (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%)(WHO 2016).

In 2015, there were an estimated 429 000 malaria deaths (range 235 000–639 000) worldwide. Most of these deaths occurred in the African Region (92%), followed by the South-East Asia Region (6%) and the Eastern Mediterranean Region (2%), between 2010 and 2015, malaria incidence rates (new malaria cases) fell by 21% globally and in the African Region. During this same period, malaria mortality rates fell by an estimated 29% globally and by 31% in the African Region (WHO 2016).

1.2.2. Transmission of malaria:

Malaria can be transmitted by several ways that include; biting of the female anopheles mosquito, infected blood transfusion, and congenital intra-uterine transmission. (Arora and Arora 2015).

1.2.3. Life cycle:

The malaria parasite has a complex, multistage life cycle occurring within two living beings, the vector mosquitoes and the vertebrate hosts. The survival and development of the parasite within the invertebrate and vertebrate hosts (Greenwood *et al* 2008).

1.2.3.1. Mosquito cycle:

Mosquitoes are the definitive hosts for the malaria parasites, wherein the sexual phase of the parasite's life cycle occurs. The sexual phase is called sporogony and

results in the development of innumerable infecting forms of the parasite within the mosquito that induce disease in the human host following their injection with the mosquito bite. (Barillas *et al* 2005).

When the female Anopheles draws a blood meal from an individual infected with malaria, the male and female gametocytes of the parasite find their way into the gut of the mosquito. The molecular and cellular changes in the gametocytes help the parasite to quickly adjust to the insect host from the warm-blooded human host and then to initiate the sporogonic cycle. The male and female gametes fuse in the mosquito gut to form zygotes, which subsequently develop into actively moving ookinetes that burrow into the mosquito midgut wall to develop into oocysts. Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After the sporogonic phase of 8–15 days, the oocyst bursts and releases sporozoites into the body cavity of the mosquito, from where they travel to and invade the mosquito salivary glands. When the mosquito thus loaded with sporozoites takes another blood meal, the sporozoites get injected from its salivary glands into the human bloodstream, causing malaria infection in the human host (Barillas *et al* 2005).

1.2.3.2. Human cycle:

1.2.3.2.1. Hepatic cycle:

Plasmodium sporozoites injected by an infected mosquito migrate to the liver and initiate the hepatic stage of the parasite life cycle by invading hepatocytes within which they multiply and differentiate into schizonts containing thousands of hepatic merozoites. These merozoites are subsequently released into the blood where they initiate the erythrocytic stage by invading and replicating within red blood cells (RBCs) (Soulard*et al* 2015).

1.2.3.2.2. Erythrocytic cycle:

The erythrocytic stage begins when the infected liver cell bursts, releasing merozoites into the bloodstream. Within 1-2 min of release, each merozoite attaches to specific receptors on the RBC membrane via ligands on the surface of the merozoite. Subsequently, the host RBC membrane invaginates so that the merozoite moves into the erythrocyte. Residing in the parasitophorous vacuole, the parasite undergoes development from the early ring stage trophozoite to the late trophozoite and then, after mitotic divisions, to the schizont stage, which contains 6-32 merozoites, depending on the parasite species. When the erythrocyticschizont ruptures, the merozoites spill into the blood and each one continues the life cycle by invading another RBC. During this repeated cycle, some merozoites differentiate into male and female gametocytes, which can be taken up by mosquitoes during a blood meal. Then the infectious cycle of *Plasmodium* can repeat itself. It is the asexual blood stage that is responsible for the symptoms of the disease. There is therefore a significant effort to develop a vaccine against this stage of the life cycle, which could limit parasite growth and consequently prevent or minimize clinical disease. The successful development of an asexual blood stage vaccine is critically dependent upon our understanding of immunity to asexual blood stage parasites (Wipasa*et al* 2002).

1.2.4. Signs and symptoms:

Malaria usually presents with fever, chill with rigor, malaise, headache, myalgia, anorexia, vomiting and enlarged spleen. However it can usually present as coma, convulsion, bleeding manifestation, jaundice, and hypoglycemia. The hematological abnormalities that have been reported with malaria include anaemia, splenomegaly and rarely disseminated intravascular coagulation (DIC) (Agrawal*et al* 2015)

1.2.5. Diagnosis of Malaria:

Malaria is diagnosed using different technique using microscopic detection and identification of plasmodium species in Giemsa stained thick blood film (for screening and detection of malaria parasites) and thin blood film (for species confirmation) (Tangpukdee*et al* 2009).

Rapid diagnostic tests are all based on detect malaria antigen on blood flowing along membrane containing specific anti malaria antibodies and its simple, quick, accurate and cost-effective (Tangpukdee*et al* 2009).

Molecular techniques is new laboratory diagnostic technique that display high sensitivity and high specificity such as PCR, loop-mediated isothermal amplification (LAMP), microarray, massspectrometery (MS) and flow cytometric (FCM) assay technique have permitted extensive characterization of the malaria parasite and generating new strategies for malaria diagnosis (Tangpukdee*et al* 2009).

1.2.6. Treatment:

WHO recommended artemisinin-based combination therapies (ACTs) for treatment uncomplicated malaria caused by *plasmodium falciparum*. Severe malaria should be treated with injectable artesunate (intramuscular or intravenous) for at least 24 hours and followed by a complete 3-day course of an ACT once the patient can tolerate oral medicine (WHO 2016).

1.3. Malaria infection and platelets:

Malaria is a multisystem infection and can be associated with many complications. Thrombocytopenia is the most common hematological complication of malaria, but association of thrombocytopenia with different types of malaria and its prognostic implications in context with severity of low platelets count has not been evaluated in many of the previous studies (Arif*et al* 2017).

1.3.1. Mechanism of thrombocytopenia induced by malaria infection:

The mechanism includes both non-immunological as well as immunological destruction of platelets to be implicated in causing thrombocytopenia. The speculated mechanisms are coagulation disturbances, excessive sequestration in spleen, antibody mediated platelet destruction, oxidative stress, low thrombopoietin synthesis, excessive use of platelets associated disseminated intravascular coagulation and the role of platelets as cofactors in triggering severe malaria. Abnormalities in platelet structure and function have been described as a consequence of malaria, and in rare instances platelets can be invaded by malaria parasites (Gupta *et al* 2013).

1.3.1.1. Immunological causes induced thrombocytopenia:

Immune complexes play a role in peripheral destruction of platelets and red blood cells and it has been demonstrated that specific IgG binds directly to malaria antigen in platelets through Fab terminus. Elevated serum levels of pro- and anti-inflammatory cytokines have been seen in cases of malaria with thrombocytopenia (Aggarwal and Shashiraj 2005).

1.3.1.1.1. The hyper-reactive malarial splenomegaly:

Hyper-reactive malarial splenomegaly (HMS) represents one of the leading causes of massive splenomegaly in malaria-endemic countries which lead mainly to thrombocytopenia. HMS is caused by an aberrant immune response to a chronic antigenic stimulation in subjects long exposed to malaria parasites. Previously defined as tropical splenomegaly syndrome (TSS), HMS has long been considered distinct from a splenomegaly directly resulting from malarial parasitaemia (Leoni*et al* 2015).

The syndrome is characterized by macroglobulinaemia with overproduction of immunoglobulin, especially of the IgM class, which aggregate into high molecular immune complexes and cause persistent splenomegaly because of prolonged clearance from the reticuloendothelial tissue. Cryoglobulins and autoantibodies, such as, for instance, rheumatoid factor, contribute to the macroglobulinaemia. A direct correlation between the spleen size and the IgMtitre has been described .Genetic factors are likely to be involved in the development of HMS. Studies carried out in Papua New Guinea reported a higher incidence in individuals with HLA-DR2 haplotype or with HLA heterozygosity .Moreover a retrospective study carried out in Ghana evidenced that the relatives of patients with HMS were more likely to have splenomegaly than population controls (Leoni*et al* 2015).

1.3.1.2. Thrombocytopenia in malaria related to platelets phagocytosis:

Patient with malaria presented marked thrombocytopenia and platelet-like particle inside the monocytes. Indeed, platelet phagocytosis in malaria was shown more than 20 years ago in a patient report with 80% of circulating monocytes presenting platelets inside.Collectively, studies demonstrate that platelet phagocytosis is associated to thrombocytopenia and correlates with TNF-α, a cytokine normally attributed to severity in malaria. Although there are some evidences of phagocytosis involvement in malaria Thrombocytopenia information regarding the mechanisms responsible for this phenomenon is scarce (Coelho *et al* 2013).

1.3.1.3. Thrombocytopenia due to coagulation disorders:

Malaria infection influences blood coagulation by various interacting pathbiological mechanisms, the most important being the overwhelming response of the host to sepsis resulting in a cytokine storm. In addition, the parasite infects the red cells leading to changes in the red cell phospholipid composition which

supports blood coagulation. Red cells infected with *Plasmodium falciparum* also adhere to deeper tissue capillary endothelium leading to profound damage to endothelial cells leading to further activation. This results in widespread consumption of platelets and activation of blood coagulation which at times culminates in a clinically and pathologically detectable disseminated intravascular coagulation (DIC) (Ghosh and Shetty, 2007).

Monocyte–macrophage system also gets activated in this infection compounding the hypercoagulable state. Heavy parasitemia leading to occlusion of hepatic microcirculation leads to abnormalities in synthesis and secretion of coagulation factors and their inhibitors. Drugs used in the treatment for *falciparum malaria* can cause thrombocytopaenia, bone marrow suppression and haemolyticanaemia, all of which can interfere indirectly with blood coagulation. Microparticle formation from platelets, red cells and macrophages also causes widespread activation of blood coagulation, thus in severe *falciparum malaria*, there is activation of blood coagulation system along with thrombocytopaenia, even before widespread DIC and coagulation failure occur (Ghosh and Shetty 2007).

1.4. Rationale:

Plasmodium falciparum is responsible for more than 95% of malaria cases in Sudan. However, an increase in *P. vivax* 5% cases has been noticed in the latest years, WHO (2014) support has reduced the number of malaria cases from more than seven million in 2000 to 592,383 and about 685 reported deaths in 2014. There are currently 34 million people at risk of malaria across the country. Malaria account for 8.7% of health facility visits, 11% of all hospital admission and is a leading cause of illness and death in children under five years in Sudan.

On the other hand platelets play a role in haemostatic mechanism, defects on it lead to bleeding disorders. Platelet is one of the most parameters which are affected by malaria infections.

In Sudan there is scarcity of data concerning the use of platelets and their indices as potential markers of *Falciparum malaria*. So this work was carried out to study the changes in the platelets profile due to *Falciparum malaria* and their prognostic significance.

1.5. Objectives:

General objectives:

To evaluate the platelets count and indices as prognostic markers in patients with *falciparum malaria* infection.

Specific objectives:

1. Measurement of platelets count, MPV, PCT and RDW in patients with *falciparum malaria* infection.
2. Comparison of platelets count, MPV, PCT and RDW between male and female among patients with *falciparum malaria* infection.
3. Assess the effect of duration of *falciparum malaria* on platelets count and indices.
4. Determine the correlation among the platelet indices and between the latter and parallel red cell parameters.

Chapter two

Materials and methods

2.1 Study design:

This is a case control study.

2.2 Study area:

This study was conducted in Khartoum North Teaching Hospital- Khartoum State during the period February 2017 to April 2017.

2.3 Study population:

Forty five Sudanese malaria patients and fifty five Sudanese individuals were enrolled in the study as cases and control respectively.

2.4 Inclusion criteria:

- Patients with confirmed malaria infection.
- Healthy individuals as control group for comparison.

2.5 Exclusion criteria:

Any patients with malaria infection with any factors which may affect the results such as blood or platelet transfusion, pregnancy, liver diseases, mixed infection etc.

2.6 Participants' personal data collection:

Data collected using pre-coded questionnaire which was specifically designed to obtain information by verbally interviewed that helped in study.

2.7 Sample collection:

Venous blood was collected using sterile disposable plastic syringe after cleaning the vein puncture area with 70% ethanol, the blood was added to the anticoagulant at ratio of 2.5 to 1.5 of 0.1% EDTA solution and gently mixed.

2.8 Methodology:

2.8.1 Blood film for malaria parasite:

A small drop of EDTA anticoagulant blood, about 2mm in diameter was placed, about 1cm from one end of the slide and using for preparation of blood film using the push waged method. The slide was placed on flat surface and quickly the spreader was placed just in front of the drop of blood at 45 angle. The thin film was air dried fixed with methanol and then flooded by Giemsa stain for 10 minutes and washed by running tap water then air dried and examine microscopically.

Classification of the degree of parasitaemia:

The malaria parasite density was graded as follow:

1 parasite field: low density (+)

2-9 parasite field: medium density (++)

>20 parasite field: high density

2.8.2 CBC (Automated sysmex technique):

2.8.2.1 Principle of sysmex:

The coulter principle is based on the following:

Particles suspended in an isotonic diluents, when drawn through an aperture which has an electric current flowing through it will cause a measurable drop in voltage which is proportional to the size of the particle passing through the aperture is constant the particle can be quantified per unit volume. This is also called electrical impedance.

2.8.2.2 Methods of sysmex:

Whole blood mode:

Blood is aspirated from the sample probe into the sample rotor valve:1. 4.0 µl of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 µl of diluents and brought to the mixing chamber as diluted sample (1^{st} step dilution).

2. Out of the 1:500 dilution sample 40 µl is measured by the sample rotor valve, diluted into 1:25000 with 1.960 µl of diluent then transferred to the RBCs/plt transducer chamber (2^{nd} step dilution).

250 µl of the sample in the RBCs/plt transducer chamber is aspirated through the aperture. At this time RBCs and platelet are counted by the DC detection method. At the same time, hematocrit (Hct) value is calculated by RBCs pulse height detection method.

2.8.2.3 Quality control:

Using manufacturing control bring to room temperature before analysis then run the control if all parameters fall within manufactures recommended range, then proceed with patient samples. During each run one control is run every 20 sample using sampler mode. If the machine does not produce WBCs differential or when in doubt of some parameters, make a peripheral blood smear, stain and perform WBC differential count.

2.9 Ethical approval:

Ethical approval for conducting the research was obtained from the college of Medical Laboratory Sciences-SUST also permission was obtained from the administration of Khartoum North Teaching Hospital for the same purpose. A verbal consent was obtained from all the participants after they had been informed about the aim of the study, expected outcome, confidentiality of the results and the procedure of blood collection.

1.10 Data analysis:

The participants' characteristics were analyzed qualitatively. Values were given as mean ± SD. Student T-test was used to test the effect of malaria on platelets count and indices and the variation in these responses with gender of the patients. One way analysis of variance was used to examine the significance of the effect of

duration of malaria on platelets count and indices. Spearman correlation was used to determine correlation among the platelet indices and between the latter and parallel red cell parameters .The significance level was set at $P < 0.05$. All statistical analyses were performed using SPSS version 16.

Chapter Three
Results

3.1. Some characteristic of the study participants:

Frequency of sex was 13 males (28.9%) and 32 females (71.2%). Distribution of the malaria patients according to the duration of the disease, between onset of symptoms and diagnosis of malaria was (1-3 days) 18(40%), (4-6 days) 12(26%) and (7-10 days) 15(33%).

3.2. Effect of *falciparum malaria* in platelets count, MPV, PCT and PDW:

Significant decrease in mean of platelets count and PCT when compared with the control (P. <0.05), and significant increase in mean MPV and PDW when compared with the control (P. <0.05).Table (1).

3.3. Effect of gender on platelets count, MPV, PCT and PDW in *falciparum malaria* patients:

No significant variations (P. >0.05) were observed between male and female patients in the studied parameters. Table (2).

3.4. Effect of *falciparum malaria* duration on platelets count, MPV, PCT and PDW:

Table (3) shows that as the duration was increased platelets count significantly decreased and MPV significantly increased (p. <0.05).

3.5. Correlation of platelets count, MPV, PCT and PDW in *falciparum malaria* patients:

Table (4) shows that MPV and PDW recorded significant inverse correlation with platelets count. Platelets count showed significant positive correlation with PCT (P. <0.05). Also a significant positive correlation was recorded between MPV and PDW (P. <0.05).

3.6. Correlation between RBCs (count, indices) and platelets (count, indices):

Table (5) shows a significant positive correlation between platelets count and RDW and between PCT and RDW (P. <0.05).

Table (1) The effect of *falciparum malaria* on Platelets count, MPV, PCT and RDW:

Parameters	Study population	M ± SD	P.value
Platelets count 10^9/L	Malaria patients	250 ± 81	0.000
	Healthy individuals	306 ± 59	
MPV fl	Malaria patients	8 ± 0.91	0.04
	Healthy individuals	7.5 ± 0.87	
PCT%	Malaria patients	0.19 ±0.06	0.000
	Healthy individuals	0.24 ± 0.05	
PDW	Malaria patients	16.7± 2.3 9	0.02
	Healthy individuals	15.4 ± 2.38	

Significance level at (P <0.05).

Table (2) Comparison of platelets count, MPV, PCT and RDW between male and female *falciparum malaria* patients:

Gender / Parameters	Males M ± SD	Females M ± SD	P.Value
Platelets count 10^9/L	233± 63	264 ± 87	0.420
MPV fl	8.2 ±0.76	8.1±0.75	0.228
PCT%	0.18± 0.06	0.20± 0.06	0.408
PDW	17± 0.63	16.2 ± 2.28	0.07

Significance level at ($P < 0.05$).

Table (3) The effect of *falciparum malaria* duration on platelets count, MPV, PCT and RDW:

Duration of malaria(days) / Parameters	M ± SD (1-3)days	M ± SD (4-6)days	M ± SD (7-10)days
Platelets count $10^9/L$	294± 90.4$^{(b)}$	239 ± 77.3$^{(ab)}$	222 ± 69.7$^{(a)}$
MPV fl	6.7 ± 0.76 $^{(b)}$	8.1 ± 0.76 $^{(ab)}$	8.5 ± 0.83 $^{(a)}$
PCT%	0.21 ± 0.07	0.18 ± 0.06	0.17 ± 0.06
PDW	15.7 ± 1.53	15.7 ± 1.53	15.8 ± 2.31

Significance level at (P <0.05).

Means within the same row followed by different superscript ($^{a,\ b,\ ab}$) are significantly different (p <0.05).

Tables (4) Correlation between platelets count, MPV, PCT and PDW in *falciparum malaria* patients:

parameters	correlation	Platelet count 10^9/L	MPV fl	PCT%	PDW
Platelet count 10^9/L	Pearson	1	-0.283	0.901	-0.194
	P.value	-	0.04	0.000	0.05
MPV fl	Pearson	-0.283	1	-0.125	0.37
	P.value	0.04	-	0.215	0.000
PCT%	Pearson	0.901	-0.125	1	0.148
	P.value	0.000	0.215	-	0.143
PDW	Pearson	-0.194	0.37	0.148	1
	P.value	0.05	0.000	0.143	-

Significance level at ($P < 0.05$).

Table (5) Correlation between RBCs (count, indices) and Platelets count and indices in *falciparum malaria* patients:

parameters	correlation	RBCs count 10^9/L	MCV fl	HCT%	RDW
Platelets count 10^9/L	Pearson	0.119	0.11	0.019	0.349
	P.value	0.43	0.46	0.90	0.01
MPV fl	Pearson	0.08	0.42	0.115	0.13
	P.value	0.60	0.35	0.43	0.39
PCT%	Pearson	0.125	0.07	0.008	0.402
	P.value	0.114	0.6	0.960	0.006
PDW	Pearson	0.106	0.21	0.167	0.237
	P.value	0.48	0.89	0.273	0.116

Significance level at ($P < 0.05$).

Chapter Four

Discussion

This study was conducted to investigate the effect of *falciparum malaria* infection on the platelets count, mean platelets volume, plateletcrit and platelets distribution width.

In the present study the platelets count was significantly decreased when comparing platelets count in infected and healthy individuals; this accords with the findings of De Mast *et al* (2010),Gauri (2014) and Sushma *et al*,. (2014). The former researchers suggested that the decrease in platelets count in malaria patients is associated with Gp1b shedding in absence of platelet activation and coagulopathy and is not at all due to a reduction in megakaryocyte in bone marrow which is usually normal. Sushma*et al* (2014) attributed the Platelets count reduction either by destruction due to hyperactivity and activation of platelets by adhesion to parasitized RBCs and damage to endothelial cell which causes lyses of platelet inside the vessel and release of its content, which can cause DIC. Or due to activation by various aggregating agent like immune complex.

In this study in spite of the observed reduction in the Platelets count they were within the normal range for the Sudanese individuals, this contradicts Gauri (2014) who studied the platelets count and indices in 186 patients with malaria and reported a high frequency of thrombocytopenia. This difference may be due to variation in the duration of the disease, sample size, degree of parastiemia or interlaboratory differences.

The present study revealed that there is a significant increase in MPV and PDW, and a significant decrease in PCT which accords with Gauri (2014). A significant increase in MPV of *falciparum malaria* has been observed by Martinez-Salazar and Castano (2014) ,they showed that when platelet decreased in number the bone

morrow megakaryocytes are stimulated by thrombopoietin and their nucleus become hyper lobulated with higher deoxyribonucleic acid (DNA) content thus stimulating megakaryocyte to produce larger platelets. According to that variation in size between giant and normal platelet the PDW was increased which explains the PDW was always in a positive correlation with MPV. Chandra and Chandra (2013) in India tested the validity of using such an increase in MPV in the diagnosis of acute malaria in suspected cases; they concluded that a MPV exceeding 8 μm^3 exhibits a sensitivity and specificity of 70.8 and 50.4% for the diagnosis of malaria, respectively .

PCT is a measure of platelet mass, it was significantly decreased in *falciparum malaria* patients and there was an inverse relation between platelet size (MPV) and platelets count (PC) and it is proposed that the total platelets mass is regulated by changes on MPV and Platelets count; therefore the presence of giant platelet and platelet aggregates led to a decrease in PCT (Sushma*et al* 2014). Patients with malaria had lower platelets count and plateletcrit values compared with the normal individual and high level of PDW and MPV which reflects the thrombocyte heterogeneity, marks platelet immaturity and platelet activation (Martinez-Salazar and Castano 2014).

This study also showed significant inverse correlations between platelets count and mean platelets volume and platelets distribution width, and a significant positive correlation between platelets count and plateletcrit also there was significant positive correlation between mean platelets volume and platelet distribution width. This result agrees with the result of Aleal Santos *et al* (2013) who showed that PDW is linearly correlated with MPV.

Tangvarasitticha *et al* (2016) found a significant inverse correlation between platelets count with MPV and PDW and significant positive correlation between platelets count and PCT which is on line with the current study.

Although all the platelet parameters were changed by malaria infection but all these parameters were still within the normal range of the Sudanese people as was reported by Abass *et al* (2015) as follow; platelets count (males : 130 -357cell/L, females: 146 -378cell/L), mean platelet volume (male: 8.4 -11.4 fl, females: 8 -11.6 fl), plateletcrit(males: 0.14 -0.24%, females: 0.13 -0.33%) and platelet distribution width (males: 8.7 -15.7, females: 7.8 -16.2) respectively.

This study shows the potentiality of using the alterations in platelets count and indices as prognostic markers in patients with *falciparum malaria* and provides further information about the clinical importance of these parameters in malaria.

Conclusions:

- Platelets count and plateletcrit were decreased in patients with *falciparum malaria* infection.
- The mean platelet volume and platelet distribution width were increase in patients with *falciparum malaria* infection.
- No differences between the studied parameters according to gender.
- Duration of malaria infection decreased the platelets count and increased MPV.
- A positive correlation was observed between platelets count and PCT. and between MPV and PDW.
- Platelets count recorded a significant inverse correlation with MPV and PDW.
- The RDW exhibited significant positive correlations with platelets count and PCT.

Recommendation:

Further studies with a larger sample size are recommended to:

1. Elucidate the utility and clinical importance of these parameters in malaria.
2. Study the hepatic function and thrombopoietin serum level in malaria patients.
3. Ensure that malaria was the most likely cause of the observed alterations by post- treatment evaluation.
4. Correlate the alterations on the platelet parameters with the level of parasitemia.

References

Abass, A; Ismail, I; Yahia, R; Ali, E; Mohammed, R; Alamin, H. and Ali, A. (2015). Reference value of platelets count and indices in Sudanese using sysmex KX-21. International journal oh health sciences. 3(2): 120-125.

Aggarwal, A .and ShaShiraj, R.S. (2005). Plasmodium vivax Malaria Presenting with Severe Thrombocytopenia. Journal of Tropical Pediatrics.51 (2): 120-121.

Agrawall, N; Nath, K; Chandel, K; Singh, M; Agrwall, P. and Gupta, A. (2015). Hematological changes in malaria. Journal of evolution of medical and dental science. 4(65): 11367-11374.

ALeal Santos, A.F; Silva, S.B; Crepaldi, N.P; Martin, A.F.E. and Fontes, E.R. (2013). Altered platelet indices as potential markers of severe and complicated malaria caused by Plasmodium vivax: a cross-sectional descriptive study. Malarial Journal.12 (1): 1-2**.**

Andrea, L.A. and Santiago, M.D. (2016). Storage pool diseases illuminate platelet dense granule biogenesis. Platelets. 28(2): 138-146.

Arif, M; Jelia, S; Meena, S.R; Meena, S; Jain P; Ajmera D; Jatav, V.S. and Agarwal, V. (2017). A study of thrombocytopenia in malaria and its prognostic significance. International Journal of Research in Medical Sciences. 4(6): 2373-2378.

Arora, D.R. and Arora, B. (2015). Medical Parasitology.4th Ed.New Delhi.CBS.

Barillas, C. and Kumar, M.S. (2005). Plasmodium –mosquito interactions: a tale of dangerous liaisons. Cellular Microbiology. 7(11): 1539-1545.

Budak,Y.U; Polat, M.andHuysal, K. (2016). The uses of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non traumatic abdominal surgery: a systematic review. BiochemiaMedica. 26(2): 178-193.

Chandra, S. and Chandra, H. (2013). Role of Haematological Parameters as an Indicator of Acute Malarial Infection in Uttarakhand State of India. Mediterranean journal of haematology and infectious disease 5(1): 313-320.

Coelho, H.C.C; Lopes S.C.P; Pimentel, J.P.D; Nogueira, P.A. and Costa F.T.M. (2013) Thrombocytopenia in Plasmodium vivax Malaria is Related to Platelets Phagocytosis. PLOS ONE 8(5): 1-7.

De Mast, Q; de Groot.P.G; Roestenberg, M; Nieman, A.E. and Sauerwein, R.W. (2010). Thrombocytopenia in early malaria is associated with GP1b shedding in absence of systemic platelet activation and consumptive coagulopathy. British Journal Haematology.151: 495-503.

Ghosh, K. and Shetty, S. (2007) Blood coagulation in *falciparum malaria*-a review. Parasitology Research.102 (4):571.

Gilbert, C. (2005).Congenital and Acquired Platelet Disorders: Current Dilemmas and Treatment Strategies. Seminars in Haematology.43 (8): 37-41.

Greenwood, B.M; Fidock D.A; Kyle D.E; Kappe S.H; Alonso P.L. and Collins F.H. (2008). Duffy Malaria: progress, perils, and prospects for eradication. Journal of Clinical investigation.118: 1266-1276.

Gauri, S. (2014). Malaria and platelets: A well-known association with new perspective. 3(36): 9449-9455.

Gupta, N.K; Bansal, S.B; Jain, U.C. and Sahare, K. (2013). Study of thrombocytopenia in patients of malaria. Tropical Parasitology. 3(1): 58-61.

Hoffbrand, A.V; Pettit, J.E. and Moss, P.A.H. (2010). Essential haematology. 6thED.Oxford, Blackwell Science.

Kuter, J.D. (2017).Merck Manual Professional Version.19th Ed.Over view of Platelet Disorders. Merck Sharp and Dhome. USA.

Leoni, S; Buonfrate, D; Angheben, A; Gobbi, F. and Bisoff, Z. (2015). The hyper-reactive malarial splenomegaly: a systematic review of the literature. Malaria journal.14:185.

Marteniz-Salazar E.L. and castano-Tobon, A. (2014).Platelet profile is associated with clinical complicated in patient with vivax and falciparum malaria in Colombia. Revista da SociedadeBrasileria de Medicina Tropical. 47(3): 341-349.

Nurden, A.T; Freson, K. and Seligsohn, U. (2012).Inherited platelet disorders.Haemophilia. 18(4): 154-160.

Rendue, F. and Bohn, B. (2009). The platelet release reaction: granules' constituents, secretion and function. Platelets. 28 (3): 261-273.

Rumi, E; Harutyunyan.A.S.andPietra.D. (2014). CALR exon 9 mutations are somatically acquired events in familial cases of essential thrombocythemia or primary myelofibrosis. Blood .123:2416.

Savoia, A; Pastore, A; Rocco, D; Civaschi, E; Di Stazio, M; Bottega, R; Melazzini, F; Bozzi, V; Pecci, A; Magrin, S.L; Balduini, C. and Noris P. (2011). Clinical and genetic aspects of Bernard-Soulier syndrome: searching for genotype/phenotype correlations. Haematologica. 96: 417-423.

Soulard, V; Bosson-Vanga, H; Lorthiois, A; Roucher, C; Franetich, J.F; Zanghi, G. and Mazier, D. (2015). Plasmodium *falciparum* full life cycle and Plasmodium ovale liver stages in humanized mice. Nature Communications.6: 7690.

Sushma, N; Reddy, M.M; Vijayashree, R; Padmavathy, F; Begum, R. and Arudra, P. (2014). Haematological parameters including platelet indices in vivax and *falciparum malaria*. Chettinad Health City Medical Journal. 3 (3): 95-100.

Tangpukdee, N; Duangdee.C; Wilairatana, P. and Krudsood.S. (2009).Malaria diagnosis: A Brief Review. Korean journal parasitology.47 (2): 93-102.

Tangvarasitticha, O; Srikong, M. and Tangvarasittichai, S. (2016). Platelets count and platelet indices used as potential markers for first malaria infection diagnosis. International Journal of Pharmaceutical and Clinical Research. 8(10): 1454-1458.

Wipasa, J; Elliott, S; Huji, X. and Good, M.F. (2002). Immunity to asexual blood stage malaria and vaccine approaches. Immunology and Cell Biology.80: 401-414.

World Health Organization (2014).World Malaria Report. Retrieved from:http://www.who.int/malaria/puplications/world_malaria_report_2014/en.(Accessed: 3th May).

World Health Organization (2016).World Malaria Report.186.Retrieved from :http://www.who.int/mediacentre/contacts/en.(Accessed:3th May).

Appendix (1)

Sudan University of Science and Technology

College of Graduate Studies

Measurement of Platelets count, MPV, PCT and PDW in *falciparum malaria* Patients

Questionnaire

Name:……

Age: ( )

Sex: male ( ) female ( )

Weight: ( )

Duration of disease: ( )

Pregnancy: Yes ( ) No ( )

Blood transfusion: Yes ( ) No ( )

Splenomegaly: Yes ( ) No ( )

Bleeding problem: Yes ( ) No ( )

Splenectomy: Yes ( ) No ( )

Investigation:

Platelets count……cell/L MPV…..fl PCT….% PDW….

After understanding the content of this questionnaire and aim of research I agree to collect sample from me.

Signature….. …. .. Date……..

Appendix (2)

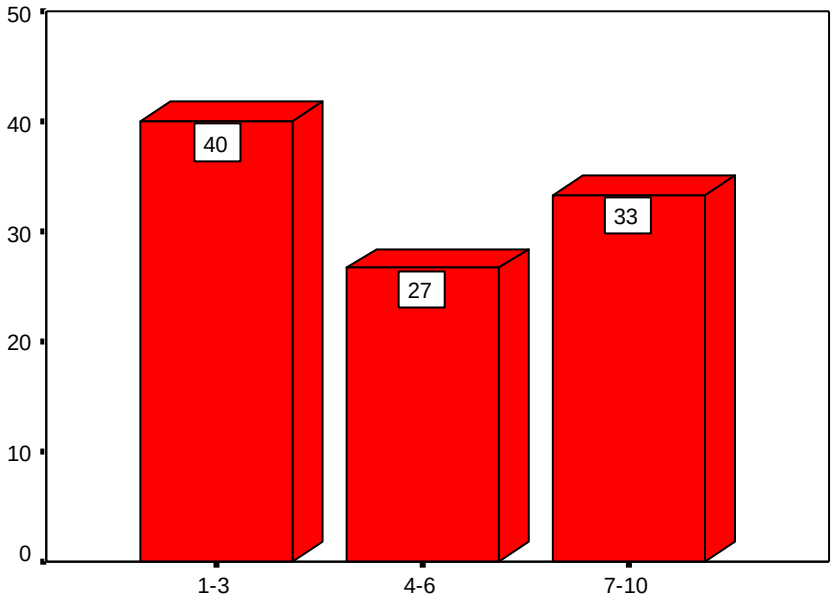

Figure (1): Distribution of *falciparum malaria* patients according to duration of disease.

Appendix (3):

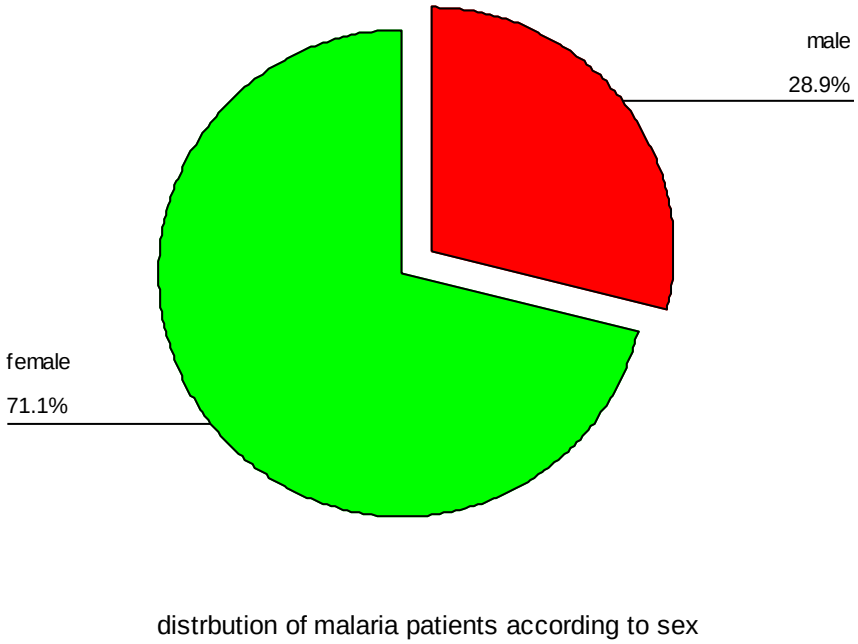

Figure (2): Distribution of *falciparum malaria* patients according to Gender.