Sudan University of Sciences and Technology

College of Graduates

Evaluation of Platelet Count and Indices in Malaria Patients in Khartoum State

تقويم تعداد الصفائح الدموية ومؤشراتها لدي مرضي الملاريا بولاية الخرطوم

A Thesis Submitted in Partial Fulfillment of the Requirement for MSc. Degree in Hematology and Immunohematology

Submitted by

Amal Alfadil Abdallah Ali

Medical Iaboratory Sciences

(B.Sc. USH 2017)

Supervisor

Dr. Abdalla Musa Abdalla Mohammed

January 2021

الآيـة

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

قال تعآلي:

وَاللَّهُ أَخْرَجَكُمْ مِنْ بُطُونِ أُمَّهَاتِكُمْ لَا تَعْلَمُونَ شَيْئًا وَجَعَلَ لَكُمُ السَّمْعَ وَالْأَبْصَارَ وَالْأَفْئِدَةَ لَعَلَّكُمْ تَشْكُرُونَ

صدق الله العظيم

سورة النحل الآية 78

DEDICATION

To my **… Dear Husband**

Who has been a constant source of support and encouragement during all the way and made sure that I give it all it takes to finish that which I have started. I am truly thankful for having you in my life.

To my **… Loving Parents**

Who have always loved me unconditionally and whose good examples have taught me to work hard forthe things that I aspire to achieve. your prayers have been answered.

To my **… All Friends**

Who have supported me throughout the process. I will always appreciate all you have done.

ACKNOWLEGMENTS

In the Name of Allah, the Most Merciful, the Most Compassionate all praise be to Allah, the Lord of the worlds; and prayers and peace be upon Mohamed His servant and messenger.

First and foremost, I must acknowledge my limitless thanks to Allah, the Ever-Magnificent; the Ever-Thankful, for His help and bless. I am totally sure that this research studywould have never become truth, without His guidance.

I am forever indebted to my supervisor **Dr.Abdalla Musa**,for his countless hours of reflecting, reading, encouraging, patienceand excellent guidance throughout the entire research.

Finally, I am grateful to malaria patients, who provided the blood sample. **the laboratory staff In Amal National Hospital**for their assistance in collection of specimen. For printing facility computer technical aids and A4 papers for print out.

Abstract

Malaria remains one of the major health problem in the tropical and sub tropical countries particularly Africa and Asia, with increased morbidity and mortality rate. This study is conducted to analyze the effects of malaria on platelets count and indices.

This is a case control study carried out in Khartoum State, to estimate platelets count and platelets indices in malaria patients in Khartoum State. Fifty patients and fifty healthy individual were enrolled in the study after taking their verbal consent. Venous blood (2.5ml) was collected from the participants in EDTA containers and gently mixed. Platelets count and Platelets indices were determined by automated Sysmex (KX-21) technique. The obtained data were analyzed by both Student's Independent T test using SPSS version 23.0 computer program. The platelet count C/cmm was decreased among case group 108.7± 22.2 and increased among control group 286.6 ± 32.4 (P.value =0.00), increase of mean MPV in the cases(10.9±0.90) compared with the control (9.4 ±0.85); (*P.*value =0.00), increase of mean PDW in the cases (13.4±1.9) compared with the control (11.2 ±1.5); (P.value=0.00). The Patients recorded significantly (*P*.value =0.00) higher values for P-LCR (29.1±8.6%) than the control (21.2%±6.0%).

It is concluded that the platelets count and indices are affected in patients with Malaria.

المستخلص

تمثل الملاريا إحدى المشكلات الصحيه في البلدان الاستوائية وشبه الاستوائية، ولا سيما في افريقيا وآسيا، مع زيادة معدلات الاعتلال والوفيات. تم إعداد هذه الدراسة لتحليل آثار الملاريا على عدد الصفائح الدموية ومؤشراتها.

دراسة حالة تم إجراؤها في ولاية الخرطوم لتقدير عدد الصفائح الدموية ومؤشرات الصفائح الدموية في مرضى الملاريا بولاية الخرطوم. تم تسجيل خمسين مريضًا وخمسين فردًا سليمًا في الدراسة بعد أخذ موافقتهم الشفهية. تم جمع الدم الوريدي (2.5 مل) من المشاركين في حاويات EDTA وخلطها بلطف. تم تعداد الصفائح الدموية ومؤشرات الصفائح الدموية بواسطة جهاز (KX-21-Sysmex) الآلية. تم تحليل البيانات التي تم الحصول عليها من خلال اختبار T المستقل للطالب باستخدام برنامج الكمبيوتر SSPS الإصدار 23.0. انخفض عدد الصفائح الدموية C / cmm بين مجموعة الحالة 108.7 ± 22.2 وزاد بين المجموعة الضابطة 286.6 ± 32.4 (قيمة P.value = 0.00)، زيادة في متوسط MPV في الحالات (10.9±0.90) مقارنة مع الضابطة (9.4 ±0.85)، (Pvalue = 0.00)، ارتفاع في متوسط PDW في الحالات (13.4 ± 1.9) مقارنة بالضابطة (11.2 ± 1.5)، (Pvalue = 0.00). سجل المرضى قيمًا أعلى بشكل ملحوظ (Pvalue = 0.00) لـ (P-LCR 29.1 ± 8.6٪) من المجموعة الضابطة (21.2٪ ± 6.0٪).

خلصت الدراسة إلى أن تعداد ومؤشرات الصفائح الدموية تأثرت لدى مرضى الملاريا.

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Lists of Abbreviation

ADP	Adenosine diphosphate
CD39	Cluster differentiation 39
CDC	Centers for Disease Control and Prevention
CTD	Connective tissue disease
CLL	Chronic lymphocytic leukemia
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EMP1	Erythrocyte Membrane Protein 1
GPVI	Glycoprotein VI
HMS	Hyper-reactive malarial splenomegaly
HRP-2	Histidine rich protein-2
ICT	Immunochromatographic test
IDA	Iron deficiency anemia
IL-1	Interleukin-1
IL-11	Interleukin-11
IL-6	Interleukin-6
ITP	Idiopathic thrombocytopenic purpura
ITP	Immune thrombocytopenias
MPD	Myeloproliferative disorder
MPV	Mean platelet volume
MT	Malignant tertian

PCT	Plateletcrit
PDW	Platelet distribution width
PLCR	Platelet large cell ratio
PLDH	Plasmodium lactate dehydrogenase
PLT	Platelets
QBC	Quantitative buffy coat
RDTs	Rapid diagnostic tests
SCD	Sickle cell disease
SLE	Systemic lupus erythematosus
TB	Tuberculosis
TNF	Tumor necrosis factor
TPO	Thrombopoietin
TSS	Tropical splenomegaly syndrome
VWF	Vonwillebrand factor
WBCs	White blood cells
WHO	World Health Organization

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CHAPTER I

Introduction

1.1 Introduction

Malaria has been one of the most prominent and ancient diseases which has been profiled and studied. It has been one of the greatest burdens to mankind, with a mortality rate that is unmatched by any other modern disease other than tuberculosis. This dreadful disease, caused by four different agents (Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale) of the same genus, is a major health problem in most of the countries in the tropics (Sudhakar and Subramani, 2017).

Over the years, as per statistical records, it has been estimated that there may be three hundred to five hundred million new infections and one to three million infection related deaths annually caused by malaria, and it has also been found that more than 90% of these deaths occur in the regions in and around Sub-Saharan Africa (Sudhakar and Subramani, 2017). Malaria kills in 1 year what AIDS killed in 15 years. It accounts for 2.6 percent of the total disease burden of the world. It is responsible for the loss of more than 35 million disability-adjusted life-years each year (Sudhakar and Subramani,2017). The clinical diagnosis of malaria is challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other febrile illnesses common in tropical regions (Jairajpuri*et al.,* 2014). This impairs diagnostic specificity and often promotes the indiscriminate use of antimalarials (Jairajpuri, *et al.,* 2014). As parasites of the blood for the majority of their complex life cycle, they expectedly induce haematological alterations (Jairajpuri*et al.,* 2014).229 million cases and 409,000 deaths from severe malaria were estimated in 2019 worldwide with the African region accounted for 94.0% of cases(WHO, 2020). In Sudan over 85.0% of the population are at high risk of malaria. Malaria incidence in Sudan was estimated to be about 9 million episodes in 2002 and the number of deaths due to malaria was about 44,000. 2,877,000 DALYs were lost in Sudan in 2002 due to malaria mortality, episodes, anaemia and neurological sequelae. Males had the highest incidence and mortality, but females lost more DALYs (Abdalla*etal,* 2007).

Based on RBM objectives and elements with the contribution from different partners Sudan RPM strategic plan was developed in 2002. The plan aims to halve malaria allover Sudan by 2010 in terms of morbidity and mortality. Adopted strategies include: Early diagnosis and prompt treatment, multiple prevention (vector control, insecticide treated nets and intermittent preventive treatment), forecasting, early detection and containment of epidemics and building capacity (Malik *etal*, 2004).

In 2019 malaria cases was estimated to be about 1.7 million and 1,663 malaria related deaths being reported in Sudan (WHO, 2020).

1.2 Rationale

Approximately, 3.2 billion people were at risk of malaria in 2015worlwide. Most malaria cases and deaths occur in sub-Saharan Africa. The effect of malaria parasites on hematological parameters has attracted much interest among scientists. The hematological changes include anemia, thrombocytopenia and disseminated intravascular coagulation (DIC). An understanding of these changes will help in diagnosis and treatment and may also serve to predict and prevent various complications. Therefore, this study was carried out to assess the effect of malaria parasites on platelets count and platelets indices in Khartoum state.

1.3 Objectives

General objective

To assess the effect of malaria on platelet count and platelets indices.

Specific objectives

- To measure platelet count and their indices in the study population.
- To compare between case and control according to agegroups, gender, PLT count and PLT indices.

CHAPTER II

Literature Review

2.1 Haemostasis

Haemostasis is one of a number of protective processes that have evolved in order to maintain a stable physiology. It has many features in common with (and to some extent interacts with) other defense mechanisms in the body, such as the immune system and the inflammatory response (Hoffbrand*et al*, 2016).

2.2 Haemostatic response

Vasoconstriction isan immediate vasoconstriction of the injured vessel and reflex constriction of adjacent small arteries and arterioles is responsible for an initial slowing of blood flow to the area of injury. When there is a wide spread damage this vascular reaction prevents exsanguination. The reduced blood flow allows contact activation of platelets and coagulation factors. The vasoactive amines and thromboxane A2 liberated from platelets, and the fibrin peptides liberated during fibrin formation, also have vasoconstrictive activity (Hoffbrand*et al*, 2016).

2.3 Platelet reactions and primary hemostatic plug formation

Platelets are small cell fragments (average size 3–4 μm) that are important forhemostasis and coagulation. The normal platelet count is between 150,000 and450,000/μL. Platelets are derived from megakaryocytes, which are very large cellswith a large, multi-lobulated nucleus. The mean DNA content of megakaryocytesis at least eight times that of other somatic cells. One megakaryocyte can produceat least several thousands of platelets. The formation and release of platelets isrelated to a preformed structure in the cytoplasm of megakaryocytes, the so-calleddemarcation membrane system. Megakaryocytes are derived from megakaryocyteprogenitors, which in turn originate in the hematopoietic stem cell.Megakaryocytes are mainly found in the bone marrow but can transit to manyorgans, including the lung, where part of the platelet release occurs. Thematuration of megakaryocytes and the production of platelets occur under theinfluence of thrombopoietin (TPO).

TPO acts, together with certain other cytokineslike IL-6 and IL-11, on early megakaryocyte progenitors as well as maturemegakaryocytes. Under physiological conditions, the serum levels of TPO are lowat normal or elevated platelet counts and high in individuals with low

plateletcounts (Wintrobe, Lippincott, and Wilkins,2009).Following a break in the endothelial lining, there is an initial adherence of plateletsto exposed connective tissue, potentiated by VWF. Collagen exposure andthrombin produced at the site of injury cause the adherent platelet to release their granule contents and also activate platelet prostaglandin synthesis leading to theformation of thromboxane . Released ADP causes platelets to swell and aggregate. Additional platelets from the circulating blood are drawn to the area ofinjury. This continuing platelet aggregation promotes the growth of the haemostaticplug which soon covers the exposed collective tissue. The unstable primaryhaemostatic plug produced by these platelet reactions in the first minute or sofollowing injury is usually sufficient to provide temporary control of bleeding. Andsmooth muscle cells in the vessel wall adjacent to the area of damage, is importantin limiting the extent of the initial platelet plug (Wintrobe *et al*, 2009).

2.4 Platelets

Platelets are dynamic blood particles whose primary function, along with the coagulation factors is haemostasis, or the prevention of bleeding. Platelets interact with each other, as well as with leukocyte and endothelial cells, searching the vascular bed for sites of injury, where they become activated. When stimulated, platelets undergo a shape change, increasing their surface area and bioactive molecules stored within their alpha and dense granules‟ molecules are rapidly secreted (Lopez *et al*, 2015). In addition to their important role in haemostasis and thrombosis, accumulating evidence demonstrates that platelets contribute to the inflammatory process, microbial host defense, wound healing, angiogenesis, and remodeling (Golbiewska and Poole, 2015). Platelets plays an important role in physiological and pathological processes such as coagulation, thrombosis, inflammation and maintenance the integrity of vascular endothelial cells (Gardiner and Andrews, 2014).

Platelets (PLT) are membrane bound discoid structures that play a Central role in haemostasis. Normal platelet count ranges from 1,50000/mm3 to 4,50000/mm3 (Drew *et al*, 2007) Platelets are the first line of defense in preventing blood loss due to micro and macro vascular injury. Abnormal platelet function and counts can result in bleeding typically characterized by mucocutaneous hemorrhage (Hackner, 2011). Platelet are versatile fragments of cytoplasm whose major function is to arrest bleeding (Zucker and Nachmias, 2015).

2.4.1Platelets structure

Structurally platelet can be divided into four zone, from peripheral to innermost peripheral zone – is rich in glycoproteins required for platelet adhesion, activation, and aggregation. For example, GPIb/IX/X; GPVI; GPIIb/IIIa, sol – gel zone – is rich in microtubules and microfilaments, allowing the platelets to maintain their discoid shape., organelle zone – is rich in platelet granules. Alpha granules contain clotting mediators such as factor V, factor VIII, fibrinogen, fibronectin, platelet-derived growth factor, and chemotactic agents. Delta granules, or dense bodies, contain ADP, calcium, serotonin, which are platelet-activatingmediators, membranous zone – contain membranes derived from megakaryocytic smooth endoplasmic reticulum organized into a dense tubular system which is responsible for thromboxane A2 synthesis. This dense tubular system is connected to the surface platelet membrane to aidrelease of thromboxane A2 (Machlus*et al*, 2014).

Platelet structure is classified into four general areasThe platelet surface, The membranous structure, The cytoskeleton (sol-gel-zone) and the granules (Greer, 2014).

2.4.2 Platelets Production

Platelets are produced in the bone marrow, the same as the red cells and most of the white blood cells. Platelets are produced from very large bone marrow cells called megakaryocyte which develop into giant cells; they undergo a process of fragmentation that results in the release of over 1,000 platelets per megakaryocyte. The dominant hormone controlling megakaryocyte development is thrombopoietin (often abbreviated as TPO). Production of platelets depends on the proliferation and differentiation of a hemopoietic stem and progenitor cells to a cell committed to the large megakaryocyte lineage, its maturation to a large, polyploidy megakaryocyte , and its final fragmentation into platelet (Bonnefoy*et al*, 2011) The external influences that impact mega-karyopoiesis and thrombosis are asupportive marrow stroma consisting of endothelial and other cells, matrix, glucoseaminoglycans, and a family of protein hormones and cytokines, including thrombopoietin, stem cell factor, interleukin-6, interleukin-11 and stromal-cell derived factor-1 (Bennett *et al*,2011).

Megakaryopoiesis is mainly controlled by thrombopoietin (TPO). *In vitro*, TPO is essential to differentiate hematopoietic progenitors into megakaryocytes, a differentiation enhanced by cytokines such as IL-6, IL-1β, IL-3, IL-9 and IL-11. *In vivo*, megakaryopoiesis occurs in the bone marrow (BM), a complex environment in which innate and adaptive immune cells

produce cytokines regulating this process, some positively (such as IL-6, TNF-α and IL-1β), others negatively (IL-10, IL-4 and TGF-β). This influence is exemplified by reactive thrombocytosis, attributed to infections or inflammatory diseases[7] and largely mediated by IL-6. More recently, IL-1α was shown to enhance thrombopoiesis. Whether other cytokines are involved in the regulation of megakaryopoiesis requires further investigation in order to better understand inflammatory thrombocytosis.(Benbarche, Salima*et al*,2017).

2.4.3 Platelet Function

Primarily, platelet activity is associated with the initiation of coagulation cascades. Damage in blood vessel makes the sub endothelial surface the primary target site of platelet action, where it establishes the haemostasis. Various proaggregatory stimuli also known as platelet agonists promote the action of platelet adhesion to the sub endothelial surfaces. During this process, platelet changes its shape, release its granule contents, and gradually forms aggregates by adhering with each other. Thus, its primary activity remains associated with minimizing blood loss (Bennett *et al,* 2011).

The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets spontaneous leakage of blood through small vessels may occur. Central to their function are platelets activation, adhesion, secretion, aggregation, fusion and procoagulant activity (Hoffbrand et al, 2016). Platelets cause leucocytes to accumulate around the platelet plug; that is, they may release chemotactic substances, release vasoactive amines, release hydrolyticand proteolytic enzymes directly into the intimal and subintimalstructures provoking changes that mayeventually lead to atheroma, act to transport serotonin from sites of synthesis to other sites of function,also play important role in innate immunity as well as regulation of tumor growth and extravasation in the vessel (Holinstat,2017).and play important role in inflammatory and proliferative events. Also have critical role for tissue remodeling and wound healing (Platelet Research Laboratory, 2015).

Thrombus formation on an intact endothelium is prevented by nitric oxide, prostacyclin and CD39.platelet cells are attached to the subendothelial collagen by von Willebrand factor (vWF) which these cells produce vWF and stored in the Weibel-Palade bodies of the endothelial cells and secreted constitutively into the blood. Platelets store vWF in their alpha granules, when the endothelial layer is disrupted collagen and vWF anchor platelets

to the sub endothelium and platelet GpIb-IX-V receptor binds with vWF and GPVI receptor and integrin α2β1 bind with collagen (Wang *et al*, 2016).

The intact endothelial lining inhibits platelet activation by production nitric oxide, endothelial-ADPase, and PGI2, endothelial-ADPase degrades the platelet activator ADP, resting platelets maintain active calcium efflux via a cyclic AMP activated calcium pump, intracellular Platelet activation begin seconds after adhesion occurs, it is triggered when collagen from the sub endothelium binds with its receptors on the platelet. GPVI is associated with the Fc receptor γ- chain and leads via the activation of a tyrosine kinase cascade finally to the activation of PLC-γ2 PLCG2 and more calcium release, tissue factor also binds to factor VII in the blood which initiates the extrinsic coagulation cascade to increase thrombin production, thrombin is a potent platelet activator acting through Gq and G12, these are G protein coupled receptors and they turn on calcium mediated signaling pathways within the platelet, overcoming the baseline calcium efflux, families of three G proteins (Gq, Gi, G12) operate together for full activation, thrombin also promotes secondary fibrin-reinforcement of the platelet plug (Gupta et al, 2010).calcium concentration determines platelet activation status as it is the second messenger that drives platelet conformational change and degranulation (Hegazai*et al*, 2010).

Aggregation begins minutes after activation and occur as aresult of turning on the GPIIb/IIIa receptor allowing these receptors to bind with vWF or fibrinogen, when any one or more of at least nine different plt surface receptors are turned on during activation,intraplatelet signaling pathways cause existing GpIIb/IIIa receptors to change shape and thus capable to binding. Since fibrinogen is a rod- like protein with nodules on either end capable of binding GpIIb/IIIa can bind fibrinogen to aggregate. GpIIb/IIIa may also further anchor the plt to subendothelial vWF for additional clot structural stabilization (Hegazai*et al*, 2010).

2.5Disorder of platelet

2.5.1 Thrombocytopenia

Causes ofdecrease platelet numbers can be classified by mechanism and include: decreased platelet production (Aplastic anemia,Drug and Deficiency of folate or vitamin B12) increased splenic sequestration of platelets with normal survival,increased platelets destruction or consumption both immunologic(ITP,SLE,CLL,CTD)non immunologic (DIC) and platelets dilution. (Al-Samkari*et al*, 2020)

2.5.2 Thrombocytosis

Causes of increase platelet numbers include: MPD, IDA associated with active bleeding, Carcinoma, Chronic inflammatory disease (TB) and SCD associated with a non functioning spleen or after Splenectomy.(Hegazai*et al*, 2010).

2.5.3 Platelet Dysfunction

Platelet dysfunction may stem from an intrinsic platelet defect or from an extrinsic factor that alters the function of normal plateletsDysfuction may be hereditary or acquired.Hereditary disorders of platelet function consist of von willebrand disease,the most common hereditary hemorrhagic disease, and hereditary intrinsic platelet disorders, which are much less common. Acquired disorders of platelet dysfunction are commonly due to diseases (renal failure) as well as to aspirin and other drugs. (Al-Samkari*et al*, 2020)

2.6Platelet indices

Definition of platelet indices: PLT indices are a group of parameters that are used to measure the total amount of PLTs, PLTs morphology and proliferation kinetics. The commonly used PLT indices include PLT count, mean platelet volume (MPV), platelet distribution widelth (PDW), and plateletcrit (PCT) (Zhang *et al*, 2014). The MPV refers to the ratio of PCT to PLT count. PDW is numerically equal to the coefficient of PLT volume variation, which is used to describe the dispersion of PLTs volume (Zhang *et al*, 2014).

2.6.1 Platelet Count

A platelet count is a diagnostic test that determines the number of platelets in the patient blood. The normal count of platelet is between 150,000 – 450,000 platelets in each microliter of blood. The primary function of platelet count is to assist in the diagnosis of bleeding disorder and to monitor patient who are being treated for any disease involving bone marrow failure, patient who have leukemia, polycythemia vera or aplastic anemia are give periodic platelet count test to monitor their health (Platelet Definition Online, 2013).

2.6.2 Plateletcrit (PCT)

Plateletcrit is a measure of total platelet mass, values vary depending on mean platelet volume resulting in overlap between normal platelets, thrombocytopenia and thrombocytosis, plateletcrit is an effective screening tool for detecting platelet quantitative abnormalities. Normal platelet count has aplateletcritwithin the range of 0.20 – 0.36% (Zhang *et al*, 2014).

2.6.3 Mean Platelet Volume (MPV)

MPV is a reflection of megakaryocyte ploidy, it's increased in conditions associated with increased platelet turnover. The platelet mass remains constant in normal individuals (Zhang *et al*, 2014).

2.6.4 Platelet Distribution Width (PDW)

PDW is a marker of differences in the platelet size, which can be an indicator of active platelet release. These platelet parameters are estimated routinely by automated blood counters (Zhang *et al*, 2014). Is usefulness for distinguishing between reactive thrombocytosis and thrombocytosis associated with the myeloproliferative disorder (Bhutta*et al*, 2013).

2.6.5. Platelet Large Cell Ratio (P-LCR)

Means platelet large cell ratio with normal range (13.0 -43.0%) and it's calculated in automated blood analyzers. Increased percentage of large platelets (P-LCR) is observed in patients with hyper-lipidemia and suggest possible riskthrombosis .an increase in P-LCR+MPV+PDW has been observed in autoimmune thrombocytopenic purpura(Bhutta*et al*, 2013).

2.7Malaria

Malaria is caused by a protozoan parasite of the genus Plasmodium. The most common species are *P. falciparum*, *P. ovale*, *P. vivax*and *P.malariae*(Svenson*et al*., 1995). The most severe forms of malaria are caused by *P. falciparum*, with other species rarely producing serious complications, debilitating relapses, and even death (Svenson*et al.*, 1995). Malaria is an important cause of death and illness, especially in tropical countries (Trampuz*et al*., 2003). Malaria affects more than 2400 million people, over 40% of the world's population, in more than 100 countries in the tropics from South America to the Indian peninsula (Sudhakar and Subramani, 2017). The tropics provide ideal breeding and living conditions for the Anopheles mosquito, and hence this distribution (Sudhakar and Subramani, 2017). The World Health Organization estimates that in 2015 malaria caused 214 million clinical episodes, and 438,000 deaths (WHO, 2015).

2.7.1 Transmission

Principal mode of spread of malaria is by the bites of female Anopheles (from Greek hurtful, harmful) mosquito. Of more than 480 species of Anopheles, only about 50 species transmit malaria, with every continent having its own species of these mosquitoes

(Srinivas, 2015). The habits of most of the Anopheline mosquitoes have been characterized as anthropophilic (prefer human blood meal), endophagic (bite indoors), and nocturnal (bite at night) with peak biting at midnight, between 11 pm and 2 am (CDC, 2015). The blood meal from a vertebrate host is essential for the female mosquitoes to nourish their eggs (CDC, 2015).

The mosquitoes find their host by seeking visual, thermal, and olfactory stimuli and of these; carbon dioxide, lactic acid, skin temperature, and moisture are more important mosquito attractants (CDC, 2015). Depending on the strength of these stimuli, the attractiveness of different persons varies, with adults, men, and larger persons being more attractive than others (Carolina and Sanjeev, 2005 and Mark, 1998).

2.7.2 Other modes of transmission

Rarely malaria can spread by the inoculation of blood from an infected person to a healthy person. In this type of malaria, asexual forms are directly inoculated into the blood and pre-erythrocytic development of the parasite in the liver does not occur (Srinivas, 2015). Therefore, this type of malaria has a shorter incubation period and relapses due to persisting exo-erythrocytic forms do not occur (Srinivas, 2015).

2.7.3 Mother to the growing fetus (Congenital malaria)

Transfer of parasitized red cells from infected mother to the child either transplacentally or during labor can lead to in malaria in the newborn, called as congenital malaria (Neena et al., 2017).Congenital malaria seems to be rarely reported and has always been considered to be more frequent in the non-immune population than in the endemic areas (Srinivas, 2015).

Transfusion Malaria: Malaria can be transmitted by transfusion of blood from infected donors. First reported in 1911, transfusion malaria is one of the most common transfusion-transmitted infections today (Srinivas, 2015).

Needle stick injury: Cases of malaria transmission through needle-stick injuries, accidentally among health care professionals (some even fatal) or due to needle sharing among drug addicts, have also been reported (Chauhan *et al*., 2009, Slinger *et al*., 2011 and Weir, 2017).

2.7.4 Life cycle

When a mosquito bites an infected individual, it sucks the gametocytes, the sexual forms of the parasite, along with blood (Srinivas, 2015). These gametocytes continue the sexual phase of the cycle within the mosquito gut and the sporozoites that develop then fill the

salivary glands of the infested mosquito (Srinivas, 2015). When this female mosquito bites another man for a blood meal, the sporozoitesare inoculated into the blood stream of the fresh victim, thus spreading the infection (Srinivas, 2015).The natural ecology of malaria involves malaria parasites infecting successively two types of hosts: humans and female Anopheles mosquitoes. In humans, the parasites grow and multiply first in the liver cells and then in the red cells of the blood (CDC, 2016).

In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites "merozoites" that continue the cycle by invading other red cells. The blood stage parasites are those that cause the symptoms of malaria (CDC, 2016). When certain forms of blood stage parasites "gametocytes" are picked up by a female Anopheles mosquito during a blood meal, they start another, different cycle of growth and multiplication in the mosquito (CDC, 2016). After 10-18 days, the parasites are found as "sporozoites" in the mosquito's salivary glands. When the Anopheles mosquito takes a blood meal on another human, the sporozoitesare injected with the mosquito's saliva and start another human infection when they parasitize the liver cells (CDC, 2016). Thus, the mosquito carries the disease from one human to another (acting as a "vector"). Differently from the human host, the mosquito vector does not suffer from the presence of the parasites. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female Anopheles mosquito inoculates sporozoites into the human host (CDC, 2016). Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. Note that in*P. vivax*and *P. ovale* a dormant stage (hypnozoites) can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later. After this initial replication in the liver (exo-erythrocyticschizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocyticschizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they

develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (figure 2.1) (CDC, 2016).

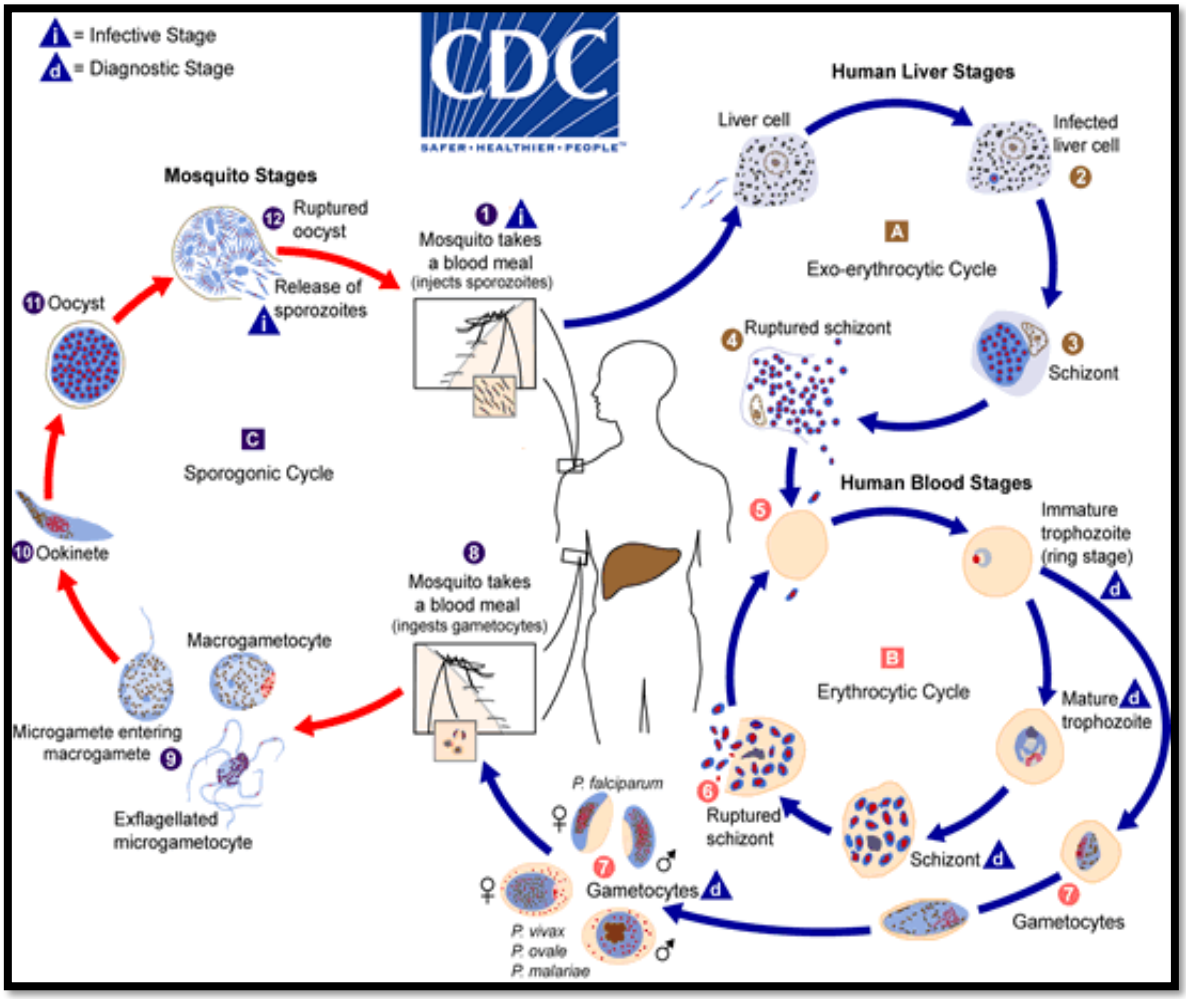

Figure 2.1: Malaria life cycle (CDC, 2016).

2.7.5 Pathogenesis and clinical picture

The incubation period varies usually from 8 to 40 days, being shortest in *P. falciparum* and longest in *P.malariae* infections. The average incubation periods are 8-11 days for *P. falciparum*, 10 to 12 days for *P. vivax* and *P. ovale* and 18 to 40 days for quartan malaria. However, very much longer incubation periods, up to 9 months have been recorded with some strains of *P. vivax* (*P. vivax*hibernans) (Paniker, 2017).

The incubation period is to be distinguished from the prepatent period, which is the interval between the entry of the parasites into the host and the time when they first become detectable in blood. The minimum level of parasitaemia for their microscopic detection is called the microscopic threshold. This is about 20 to 25 parasites per cu/mm. Clinical disease develops only later,when after a number of further cycles of multiplication, the level of parasitaemia rises high enough to cause fever, the so-called fever threshold or pyrogenic density (Paniker, 2017).

The first clinical illness marking the end of the incubation period is called the primary attack. The typical picture of malaria consists of periodic bouts of fever with rigor, followed by anemia and splenomegaly. True rigor is typically present in *P. vivax*malaria and is less common in *P. falciparum* infection. The febrile paroxysm comprises three successive stages (Paniker, 2017).

In the cold stage, lasting for 15 to 60 minutes, the patient experiences intense cold and uncontrollable shivering. This is followed by the hot stage, lasting for 2 to 6 hours, when the patient feels intensely hot. The fever mounts to 41°C or higher. Severe headache, nausea and vomiting are common. Afterwards, comes the sweating stage, when the patient is drenched in profuse sweat. The temperature drops rapidly and the patient usually falls into deep sleep, to wake up refreshed. The paroxysm usually begins in the early afternoon and lasts for 8 to 12 hours. The periodicity of the attack varies with the species of the infecting parasite. The periodicity is approximately 48 hours in tertian and 72 hours in quartan malaria. Quotidian periodicity, with the fever occurring at 24-hour intervals may be due to two broods of tertian parasites maturing on successive days, or due to mixed infection. Regular periodicity is seldom seen in the primary attack, but is established usually only after a few days of continuous, remittent or intermittent fever. All clinical manifestations in malaria are due to the products of erythrocyticschizogony and the host's

reactions to them. The exo-erythrocytic liver cycle and gametogony do not appear to contribute to clinical illness (Paniker, 2017).

The febrile paroxysms follow the completion of erythrocyticschizogony, when the mature schizont ruptures, releasing red cell fragments, merozoites, malarial pigment and other parasitic debris. Macrophages andpolymorphs phagocytose these and release large quantities of endogenous pyrogens, leading to elevation of temperature. Cytokines such as tumor necrosis factor (TNF) and interleukin-l (IL-1) may play a pivotal role in the pathogenesis of malarial fever (Paniker, 2017).

2.7.6 Recrudescence and relapse

After a number of paroxysms, the primary attack subsides with the development of partial immunity in the host. This is followed by a period of latency during which there is no clinical illness or sometimes evenparasitaemia (Paniker, 2017).

The parasites are not, however, eliminated at this stage, but persist in some erythrocytes, though the level of parasitaemia is below the fever threshold, or sometimes even below the microscopic threshold. Erythrocyticschizogony continues in the body at low levels and gradually the numbers of parasites build up to cross the fever threshold. Fresh malarial attacks then develop (Paniker, 2017).

These new malarial attacks that appear after a period of latency usually within eight weeks after the culmination of the primary attack and resulting from persistence of the erythrocytic cycle of the parasites are called recrudescences. Recrudescence may be due to waning immunity of the host or possibly to antigenic variations in the parasite. There may be several such recrudescence, which are generally milder than the primary attack. After a varying number of such attacks, the infection is eliminated in *P. falciparum* and *P.malariae* infections (Paniker, 2017).

In *P. vivax*and *P.ovale* infections the parasites may survive for long periods in a dormant exoerythrocytic stage as hypnozoites in liver cells. Reactivation of hypnozoites leads to initiation of fresh erythrocytic cycles and new attacks of malarial fever. Such new attacks of malaria caused by the dormant exoerythrocytic forms being reactivated after long periods, usually from 24 weeks to 5 years after the primary attack are called relapses. The term recurrence has been used to refer to bothrecrudescence and relapse, and so carries no specific meaning. Several factors including stress, intercurrent infection, pregnancy and alcoholism have been proposed as precipitating causes for recurrences (Paniker, 2017).

2.7.7 Malignant tertian malaria

The most serious and fatal type of malaria is malignant tertian (MT) malaria caused by *P. falciparum*. When not treated promptly and adequately, dangerous complications develop. The term pernicious malaria has been applied to a complex of life-threatening complications that sometimes supervenes in acute *P. falciparum* malaria. These may present in various forms, the most important of which are the cerebral, algid and septicaemic varieties. These occur following heavy parasitisation of red cells. The parasitized red cells become deformed, sticky and adhere on the capillary endothelium in internal organs causing anoxic damage, oedema and inflammatory reaction (Paniker, 2017). Cerebral malaria is characterized by hyperpyrexia, coma and paralysis.

Algid malaria resembles surgical shock, with cold clammy skin, peripheral circulatory failure and profound hypotension. Gastrointestinal symptoms such as vomiting, dysenteric or choleraicdiarrhoea may occur. Some cases develop severe hiccup, with profuse bilious vomiting, a condition formerly called bilious remittent fever. In septicaemic malaria, characterized by a high degree of prostration, there is high continuous fever with involvement of various organs. Acute renal failure and acute pulmonary oedema are other serious complications (Paniker, 2017).

2.7.7.1 Blackwater fever

A syndrome called black water fever (malarial hemoglobinuria) is sometimes seen in *P. falciparum* malaria, particularly in patients who have experienced repeated infections and inadequate treatment with quinine. Patients with Glucose 6 phosphate dehydrogenase deficiency may develop this condition aftertaking oxidant drugs, even in the absence of malaria. Clinical manifestations include bilious vomiting and prostration, with passage of dark red or blackish urine (black water). The pathogenesis is believed to be massive intravascular hemolysis caused by anti erythrocyte autoantibodies, leading to hemoglobinaemia and hemoglobinuria (Paniker, 2017).

2.7.7.2 Anemia

Anemia occurs in all types of malaria, but is most pronounced in falciparum infections. The type of anemia is hemolytic, normocytic, normochromic. The degree of anemia is greater than what could be explained by the destruction of parasitized red cells. In addition, there occurs increased destruction of red cells possibly by autoimmune mechanisms, and decreased erythropoiesis (Paniker, 2017).

2.7.7.3 Splenomegaly

The spleen is invariably affected, being always enlarged in malaria. The initial change is congestion, leading to a soft enlargement. Later, it becomes dark due to accumulated malarial pigment. Diffuse cellular hyperplasia, dilated sinusoids and accumulation of macrophages accentuate the enlargement of spleen, which become shard due to fibrosis (Paniker, 2017).

2.7.7.4 Tropical splenomegaly syndrome

Tropical splenomegaly syndrome (TSS) also known as hyper-reactive malarial splenomegaly (HMS) is a chronic benign condition seen in some adults in endemic areas, mainly tropical Africa, New Guinea and Vietnam. This results from an abnormal immunological response to malaria and is characterized by enormous splenomegaly, high titres of circulating anti-malaria antibody and absence of malaria parasites in peripheral blood smears. Hyperimmunoglobulinaemia (IgM, but not IgG), cryo-globulinaemia, reduced C3 and presence of rheumatoid factor without arthritis are other features (Paniker, 2017).

A normocytic normochromicanaemia is present, not responding to hematinics or anthelmintics. TSS differs from various other types of splenomegaly's seen in the tropics in its response to anti-malarial treatment, and histological changes in spleen (dilated sinusoids lined with reticulum cells showing erythro-phagocytosis, lymphocytic infiltration of pulp) and liver (marked sinusoidal infiltration with lymphocytes). The liver is also congested, enlarged and pigmented. Numerous pigment-laden Kupffer cells dot the liver. Changes are also seen in bone marrow, kidney and adrenals (Paniker, 2017).

2.7.7.5 Cerebral malaria

In cerebral malaria, lesions occur in the central nervous system. These consist of congestion of the meninges and brain, occlusion of capillaries in brain, numerous petechial perivascular hemorrhages, and necrotic lesions in mid zonal brain tissue, with peripheral glial reaction (malarial granuloma) around occluded blood vessels (Paniker, 2017).

2.7.7.6Dissminated intravascular coagulation (DIC)

DIC is a life-threatening acute,subacute,or chronic coagulation disorder occurring as a secondarycomplication in variety of diseases.it is characterized by activation ofcoagulation cascade,which leads to the formation of microthrombin through the microcirculation of the body. Sometime the coagulopathy is localizedto specific organ,but it often presents an

uneven distribution. Two major mechanism trigger DIC :release of TF into the circulation or endothelial injury. (Francischetti*et al*., 2008)

2.7.8 Laboratory diagnosis

Diagnosis of malaria involves identification of malaria parasite or its antigens in the blood of the patient. Although this seems simple, the efficacy of the diagnosis is subject to many factors. The different forms of the four malaria species; the different stages of erythrocyticschizogony; the endemicity of different species; the population movements; the inter-relation between the levels of transmission, immunity, parasitemia, and the symptoms; the problems of recurrent malaria, drug resistance, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deeper tissues; and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis can all have a bearing on the identification and interpretation of malaria parasitemia on a diagnostic test (Srinivas, 2015).The microscopic tests involve staining and direct visualization of the parasite under the microscope.

For more than hundred years, the direct microscopic visualization of the parasite on the thick and/or thin blood smears hasbeen the accepted method for the diagnosis of malaria in most settings, from the clinical laboratory to the field surveys (Srinivas, 2015). The useful examination of a well-prepared and well-stained blood film currently remains the "gold standard" for malaria diagnosis.

The most commonly used microscopic tests include the peripheral smear study and the quantitative buffy coat (QBC) test. The simplest and surest test is the time-honoured peripheral smear study for malarial parasites. None of the other newer tests have surpassed the 'gold standard' peripheral smear study (Srinivas, 2015).

Light microscopy of thick and thin stained blood smears remains the standard method for diagnosing malaria. Thick smears are 20-40 times more sensitive than thin smears for screening of Plasmodium parasites, with a detection limit of 10–50 trophozoites/μl. Thin smears allow one to identify malaria species (including the diagnosis of mixed infections), quantify parasitemia, and assess for the presence of schizonts, gametocytes, and malarial pigment in neutrophils and monocytes (Srinivas, 2015). The peripheral blood smear provides comprehensive information on the species, the stages, and the density of parasitemia. The efficiency of the test depends on the quality of the equipment and

reagents, the type and quality of the smear, skill of the technician, the parasite density, and the time spent on reading the smear (Srinivas, 2015).

The test takes about 20 to 60 minutes depending on the proximity of the laboratory and other factors mentioned above. Before reporting a negative result, at least 200 oil immersion visual fields at a magnification of 1000× should be examined on both thick and thin smear, which has a sensitivity of 90%. The level of parasitemia may be expressed either as a percentage of parasitized erythrocytes or as the number of parasites per microliter of blood. In non-falciparum malaria, parasitemia rarely exceeds 2%, whereas it can be considerably higher (>50%) in *P. falciparum* malaria. In nonimmune individuals, hyperparasitemia (>5%parasitemia or > 250,000 parasites/μl) is generally associated with severe disease(Srinivas, 2015).

The smear can be prepared from blood collected by vein puncture, finger prick and ear lobe stab. In obstetric practice, cord blood and placental impression smears can be used. In fatal cases, post-mortem smears of cerebral grey matter obtained by needle necropsy through the foramen magnum, superior orbital fissure, ethmoid sinus via the nose or through fontanelle in young children can be used (Srinivas, 2015).

Many of the new technologies for malaria diagnosis incorporate immune-choromatographic procedure, where conjugated monoclonal antibodies are the key reagents. Currently many rapid diagnostic tests (RDTs) are widely used for the diagnosis of malaria.

These RDTs are simple lateral-flow immunochromatographic tests that detect parasite specific antigens released from red blood cells. Two of the tests, the ICT Malaria Pf/Pv and ParaSight-F detect histidine rich protein-2 (HRP-2), a protein produced by asexual stages and young gametocyte of *P.falciparum*.

The third test optimal detects Plasmodium lactate dehydrogenase (PLDH), a marker protein for the intra- erythrocytic form of the malaria parasite. HRP-2 is an abundant protein produced by all blood stages of *P. falciparum*. Also, there is insufficient data available to determine the ability of this test to detect the 2 less common species of malaria, *P.ovale* and *P.malariae*(Verma*et al.,* 2013). Therefore, all negative RDTs must be followed by microscopy to confirm the result (CDC, 2014).

Although the rapid diagnostic assays offer a number of attributes that make them attractive for use in the developing world (minimally trained personnel find them easy to use, no equipment is required, and samples can be read with the naked eye), they cannot quantify

the level of parasitemia or malarial species, they aren't reliable in the presence of low-level (and occasionally even very-high-level) parasitemia, they remain positive for 7 to 14 days after treatment (CDC, 2014). Alternative microscopic methods have been tried, including faster methods of preparation, dark-field microscopy, and stains like benzo-thiocarboxypurine,acridine orange and rhodamine-123.

Acridine orange has been tried as a direct staining technique, with concentration methods such as thick blood film or the centrifugal quantitative buffy coat system and with excitation filter in the Kawamoto technique. Inability to easily differentiate the Plasmodium species, requirements of expensive equipment, supplies and special training as well as the high cost limit the use of these methods (Srinivas, 2015).

2.7.9 Treatment

Malaria is an entirely preventable and treatable disease. The primary objective of treatment is to ensure a rapid and complete elimination of the Plasmodium parasite from the patient's blood in order to prevent progression of uncomplicated malaria to severe disease or death, and to chronic infection that leads to malaria-related anemia. From a public health perspective, treatment is meant to reduce transmission of the infection to others, by reducing the infectious reservoir and by preventing the emergence and spread of resistance to antimalarial medicines (WHO, 2016).

2.8Previous studies

In a studydone by Abdelrahman (2014), conducted in Sudan about evaluation of platelets count and platelet indices in patients with acute malaria in Shendilocality, the results, showed that 67% of patients with thrombocytopenia and mean platelet volume (MPV), and Platelet crit (PCT) values were exhibited significant decrease, but there was no change in platelet distribution width (PDW).

Ascend study In Nigeria done by Marsh*et al.,* 2015, the results showed that 77% of patients with thrombocytopenia, (74%) are significant decreased in (MPV and PCT), no significant difference in (PDW).

In third study in Ireland by Combes*et al.,* 2006, suggested there is a strong association between thrombocytopenia and outcome in malaria, suggesting a role for platelets in the pathogenesis of malaria. The thrombocytopenia is likely due to platelet activation possibly through an interaction between PfEMP1 on plasmodium and CD36 on platelets. They concluded that, platelet activation by plasmodium has two potential consequences. It can lead to the formation of micro-aggregates of infected red blood cells and platelets which can occlude blood vessels and it also leads to binding to and activation of the endothelium.

Forth done in Pakistan by Sheikh*et al.*, 2010, which had high percent (85.5%) of patients with thrombocytopenia, 90.2 % of patients showed low MPV and 81.3 % showed low PCT.

CHAPTER III

Materials and Methods

3.1 Study design

This is analytical case control study conducted in Khartoum state from January to December 2020

3.2 Study area

This study was conducted in Al Amal Hospital in Khartoum, Sudan.

3.3 Study population

Known adult Sudanese diagnosed with Malaria.

3.4 SampleSize

Hundred participates were enrolled in this study; 50 diagnosed with Malaria as patients' group. Other 50 were normal without Malaria used as control group in this study.

3.5 Sampling

Blood was collected using sterile disposable plastic needle EDTA vacutainer, using aseptic standard non-traumatic vein puncture technique and immediately complete blood count was done, platelets count, andPLT indices). Thick and thin film prepared immediately after blood collection and stain with Giemsa .

3.6 Sampling technique

Random sampling technique

3.7Supplies, Material and Equipment

Microscope ,Giemsa stained blood films to be examined ,immersion oil ,automated hematology analyzer.

3.8 Data collection

The data was collected using pre-prepared questionnaire; which include personal data, medical data and laboratory investigations to perform some hematologicalparameters.

3.9 Data analysis

The analysis of the data was performed using Statistical Package for Social Sciences (SPSS, version 23.0). The statistical analysis of data was done using mean, standard deviation, and independent t-test. The test was referenced for p values and p value of less than 0.05 considered significant. Correlation test used to compare significant value .

3.10 Ethical consideration

Ethical consideration was taken from College of Medical Laboratory Science Sudan University of Science and Technology. Informed consent was taken fromeach patient before data collection.

3.11 Methodology

3.11.1 Principle

The Sysmex KX-21N is a quantitative automated hematology analyzer for in vitro diagnostic use for determining 17 hematological parameters. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections. The Sysmex KX-21N analyzer directly measures the WBC, RBC, HGB, HCT, PLT, LYM#, MIXED# and NEUT#. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MIXED%, NEUT%.

The KX-21N counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is converted to methemoglobin, and read photometrically at 555 nm.

3.11.2Specimen

- Required specimen: Whole blood anticoagulated with EDTA preferred.
- Specimen volumes required: Optimal draw is a tube drawn to capacity. The collection tube should be filled to a minimum of one-half full for acceptable results. An EDTA micro-container filled above the 250 uL line is adequate for testing in the whole blood mode.
- Unacceptable specimens including those listed below must be redrawn:
  - Clotted samples or those containing clots, fibrin strands, or platelet clumps. All specimens will be checked visually for obvious clots prior to sampling by the analyzer.
  - Check capillary tubes manually with a toothpick for clots.
  - Grossly hemolyzed samples.
  - Samples drawn above an IV.

- Characteristics that may affect test results are: lipemia, icterus, and cold agglutinins.
- Stored Specimen Stability
  - If stored at 4^{o}C within 6 hours of collection, EDTA blood samples with normal results may be analyzed up to 48 hours without significant loss of differential stability.
  - Sample stability at room temperature is 8 hours. Samples stored at room temperature may exhibit an increase in MCV, and HCT, and a decrease in MCHC after 16 hours. These changes may be minimized by refrigeration.
  - Capillary tubes are stable 4 hours when stored at 2-8^{o}C (warm and remix before analyzing).
  - Allow refrigerated samples to come to room temperature for 30 minutes then mix by hand inversion before analysis.
- Do not place samples on a mechanical rocker. Constant rocking may cause PLT clumping and alter white cell membranes resulting in inappropriate flagging.

3.11.3Reagents / Materials

3.11.3.1 Supplies

- Deionized water.
- Lint-free, plastic lined lab wipes.
- "filler" pipette supplied with the unit or a 5 cc Syringe.
- Clorox Ultra bleach (Use when Cellclean is indicated).
- Sysmex reagents.
- Tri-level commercial controls, Eightcheck-3WP X-TRA.
- Sysmex SCS-1000 whole blood calibrator.
- To ensure that the new reagent is completely cycled into the system, cycle the KX-21N analyzer 10 times before running controls and/or patients.
- Run 2 levels of commercial QC to monitor reagent performance. Document QC “OK” on reagent log.

3.11.3.2 Sysmex Reagents

- A diluent and bleach are used on the Sysmex KX-21N analyzer. Reagents and bleach are stored at room temperature and are to be used within the manufacturer’s expiration date on each container.

- Record date received, date opened and date expired on container. Record the lot, date and expiration date on the Reagent Replenishment Record.
- The reagents are azide free, and intended for in vitro diagnostic use only.

3.11.4Calibration

Initial calibration is performed during installation and verified bi-annually during preventive maintenance (PM) by the Sysmex Diagnostics Field Service Representative. Calibration compensates for any bias inherent to the pneumatic, hydraulic and electrical system that may affect the accuracy of results. Calibrators traceable to reference methods are used in the calibration of the instrument. WBC differential parameters are calibrated in the factory prior to shipment, and verified by the field service representative upon installation. The laboratory must verify calibration every 6 months or on an "as needed" basis to ensure accuracy of system. Calibration is also required if one or more of the following occur:

- Critical parts are replaced such as manometers, apertures or detector circuit boards.
- Controls show an unusual trend or are outside of acceptable limits and cannot be corrected by maintenance or troubleshooting.
- When advised by Sysmex Diagnostics Field Service Representative.

Calibration verification may be performed by review and documentation of all three levels of commercial control, proficiency testing results and patient control testing results. The operator may calibrate HGB and HCT with normal fresh whole blood, or use SCS-1000 calibrator to calibrate WBC, RBC, HGB, HCT, and PLT. Before calibration, ensure that the KX21N is both clean and precise.

3.11.5Quality control

EIGHTCHECK-3WP X-TRA Instructions for Use

- Remove EIGHTCHECK-3WP X-TRA vials from refrigerator and allow them to come to room temperature (18-25oC), for approximately 15 minutes.
- Mix vials by gentle end to end inversion until the cell button in the bottom of the vial is completely suspended. Do not use a mechanical rocker.
- Wipe residual blood off the cap and screw threads of the vial before replacing the cap.
- Return vials to the refrigerator promptly after use.

3.11.6 Complete blood count procedure

Complete blood count measured using Sysmex KX2IN (automated hematology analyzer).

Blood sample were aspirated, measured to a predetermined volume, diluted at thespecified ratio, and then fed into each transducer. The transducer chamber has aminute hole called the aperture. On both sides of the aperture, there are theelectrodes between which flows direct current. Blood cellswhich aresuspended inthe diluted sample pass the aperture, causing direct resistance to change betweenthe electrodes. As direct current resistance changes, the blood cell size wasdetected as electrode pulses. Blood cell count was calculated by counting thepulses, and a histogram of blood cell sizes was blotted by determining the pulsesizes. Also, analyzing a histogram makes it possible to obtain various analysis data(Sysmex Corporation, 2014).

Hemoglobin measurement was directly done in the WBCs chamber by spectrophotometer by formation of chromogencyanomethmoglobin for lytic solution without cyanide measurement of the blank hemoglobin was done for each analytical cycle and during the start-up rising steps (Sysmex Corporation, 2014). Reagents needed were checked for expiry date before use, the samples were analyzed according to the protocol recommended.

CHAPTER IV

Results

4.1 General characteristics of the study population

This study was conducted on 100 subjects, 50 Patients diagnosed with malaria and 50 healthy volunteers as control group .55 (55%) were males and 45(45%) were females (table4.1). The age of the subjects ranged between 30-73 years old with mean age of 51 years old. The age groups were divided into 30-40, 41-51, 52-62, and 63-73 years old. The frequency ofeach group as follow:23%,21%,32% &24% respectively (table4.2).

Table 4.1: Frequency of gender

Gender	Frequency	Percentage (%)
Male	55	55%
Female	45	45 %
Total	100	100 %

Table 4.2: Frequency of age groups

Age groups (years)	Frequency	Percentage (%)
30-40 years	23	23.0
41-51 years	21	21.0
52-62 years	32	32.0
63-73years	24	24.0
Total	100	100 %

.

Table 4.3: Comparison of mean PLTs and PLT indices between cases and controls

The platelet profile investigate the result showed statistically significant difference in patient with malaria and all parameter when compared with control group.

Parameters	Mean ±S. D Cases	Mean ±S.D controls	*P*. value
PLTs Count	108.7 ± 22.2	286.6 ± 32.4	0.00
PDW	13.7 ± 1.9	11.2 ± 1.5	0.00
MPV	10.9 ± 0.90	9.4 ± 0.85	0.00
PLCR	29.1 ±8.6	21.2 ± 6.0	0.00
PCT	0.39 ± 0.16	0.27 ± 0.08	0.00

Table 4.4: Correlation between study parameters and Age

The correlation between study parameter and age showed no relation between parameter and age.

Parameters	Pearson correlation	*P*. value
PLTs Count	0.069	0.49
PDW	0.095	0.34
MPV	-0.075	0.45
PLCR	0.045	0.65
PCT	0.011	0.91

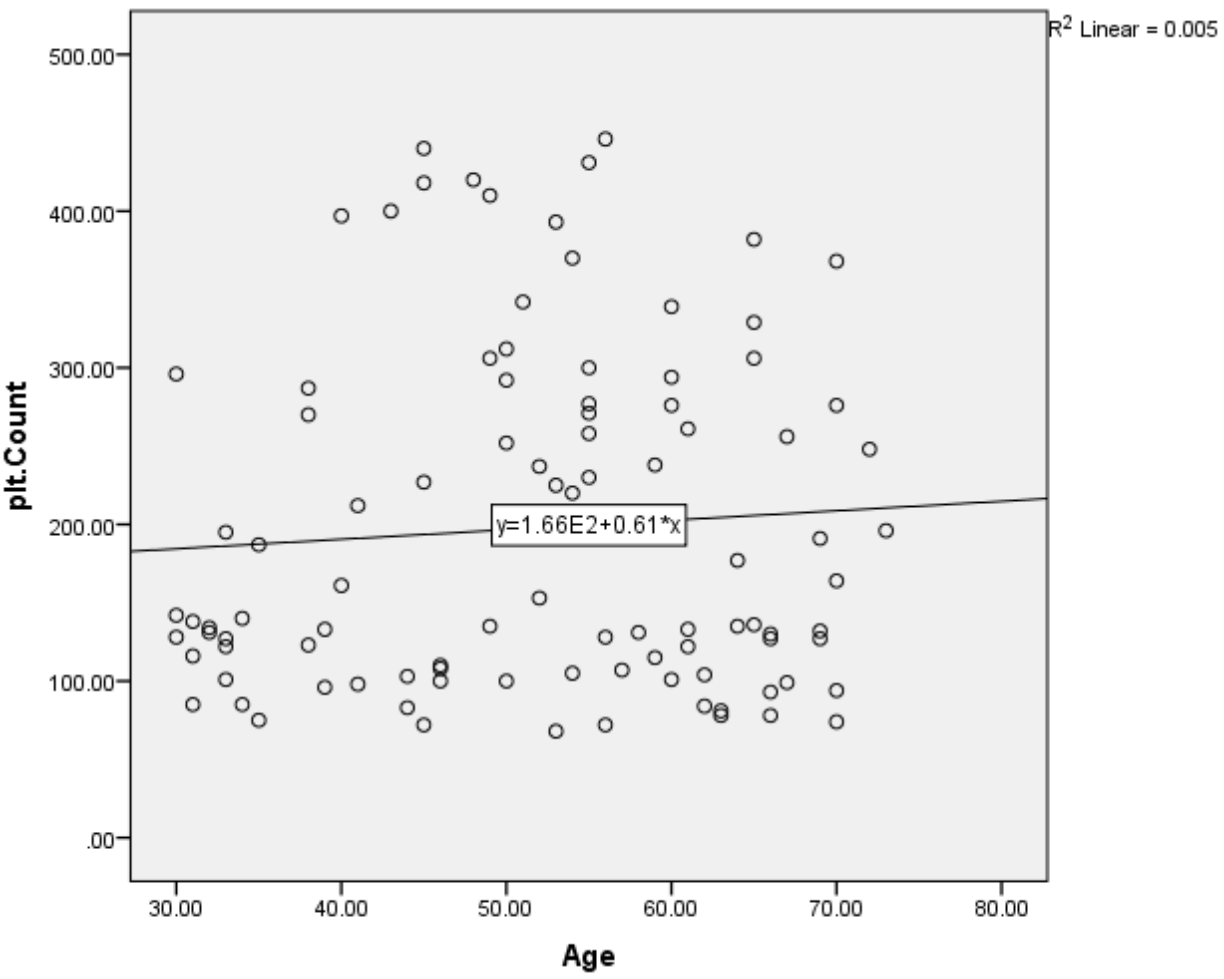

Figure 4.1: Correlation between PLT count and age

There was no correlation between PLT and age, *P* value (0.49).

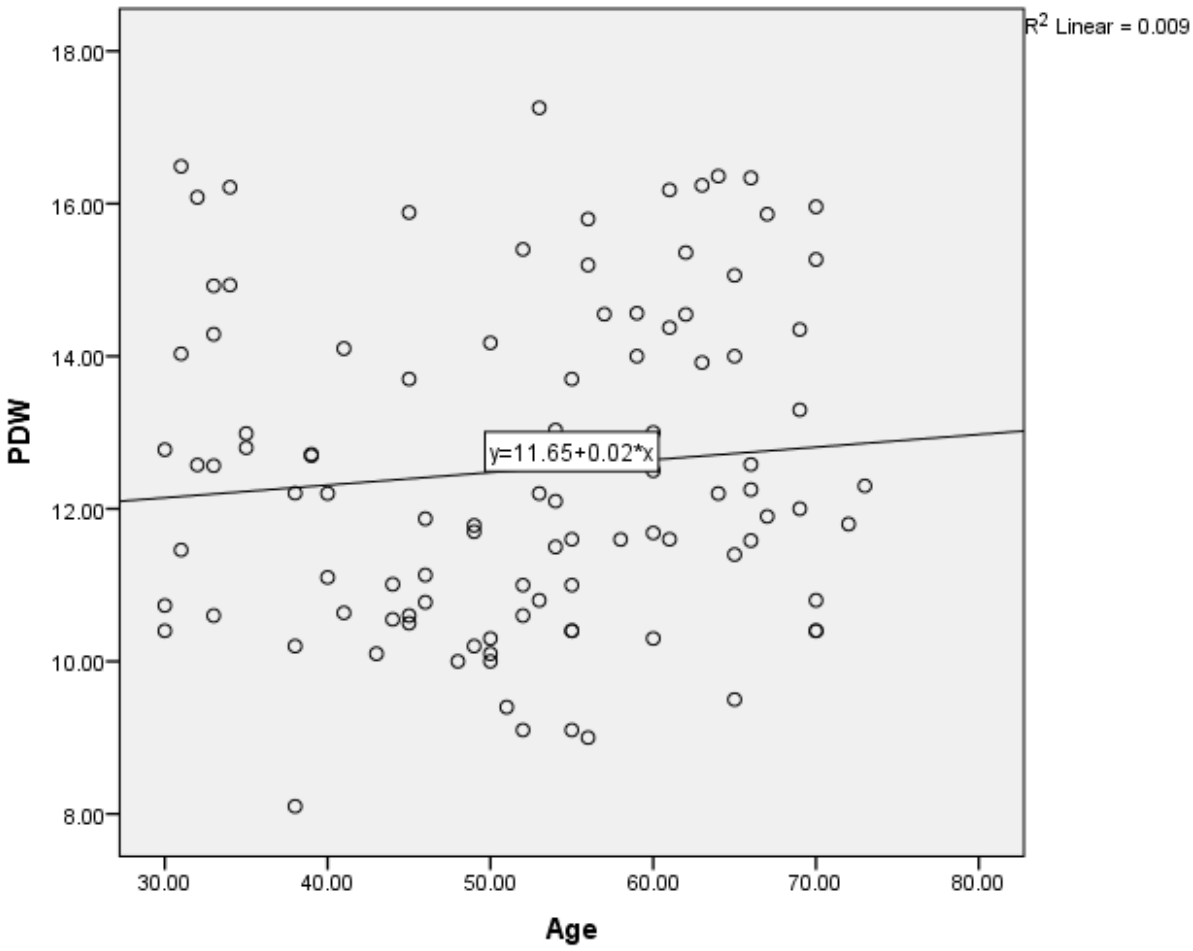

Figure 4.2: Correlation between PDW and age

There was no correlation between PDW and age, *p* value (0.34).

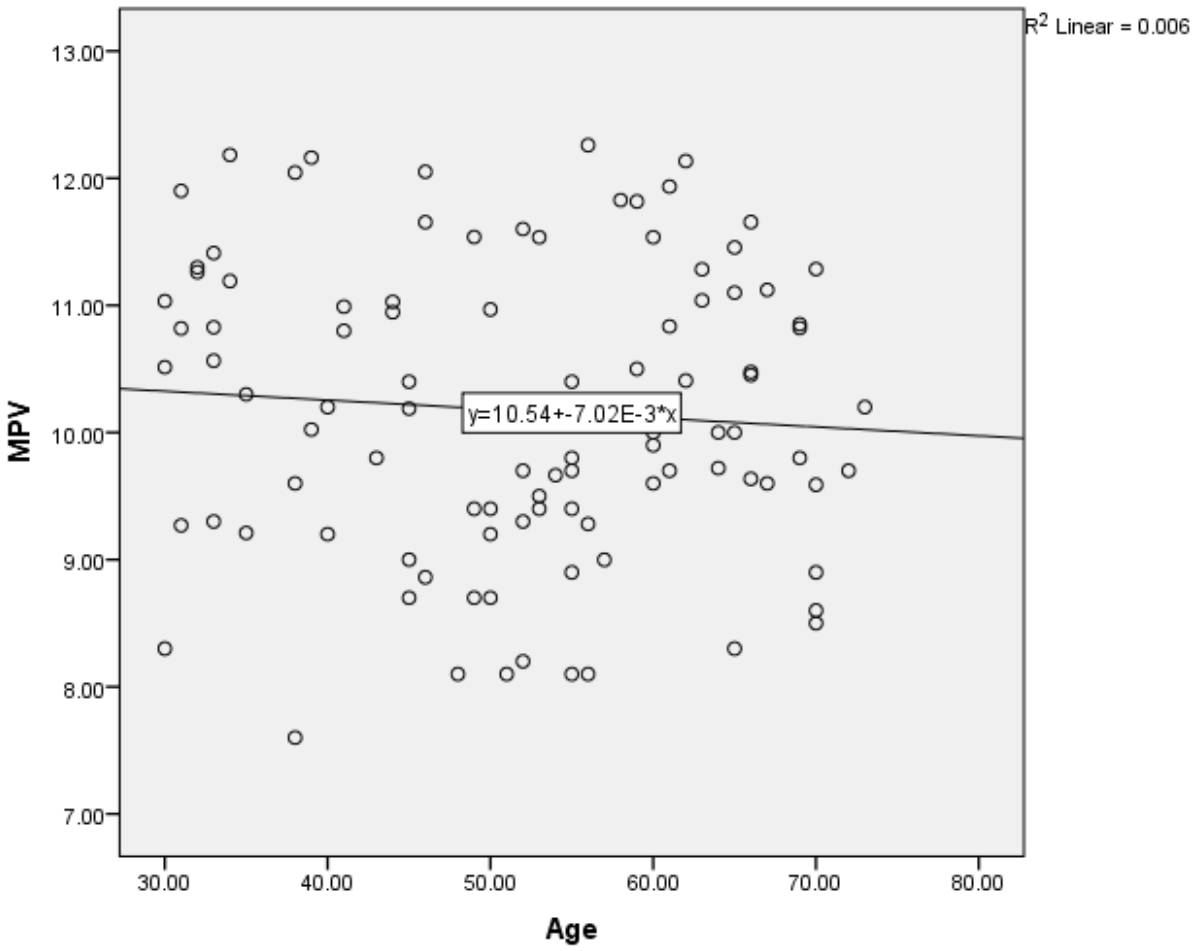

Figure 4.3: Correlation between MPV and age

There was no correlation between MPV and age, *P* value (0.45).

CHAPTER V

Discussion, Conclusion and Recommendations

5.1 Discussion

Thisis case control study conducted among 50 patients diagnosed with Malaria, (55%) were males and (45%) were females. The age of the subjects ranged between 30-73 years old with mean age of 51 years old. The age groups were divided into 30-40, 41-51, 52-62, and 63-73 years old. The frequency of each group as follow: 23%,21%,32% &24% respectively.

Blood samples were analyzed using sysmexKX-21N. Platelet count was decreased among case group when compared to control group *P*.value was (0.00) that means there was a significant difference. While comparing PDW, MPV, PLCR and PCT between case and control revealed that significant differences were obtained as p value for each was (0. 000).

This finding was similar to a previous studydone in Nigeria by Erhabor*et al.*,2014which showed that 53 % were males and47 % were females out of 100 cases.

In present study showed that most of cases had low PLTs counts. These results were similar from another one whichshowed that there was a relation between platelets count among Malaria infection as (p-value < 0.0001) (Kotepui*etal*., 2014).

Thrombocytopenia is the most common haematological disorder in patients with malaria and one of the major concerns when accompanied by multiple organ failures (Greinacher*et al.*,2010).

In our study, thrombocytopenia was the main manifestation among patients with malaria, though the reduction in platelet count was not associated with parasitaemia. Although some patients presented severe thrombocytopenia, none of them presented bleeding. Very low platelet counts during malaria are considered transient, for those who do not present bleeding, platelet transfusions are unnecessary (Ogah*et al.*,2013). Platelet function is compromised in thrombocytopenia caused by malaria, and this is generally evidenced by changes in its parameters (Bassat*et al.*,2008). The thrombocytopenia is usually not associated with bleeding even in patients with very severe form (platelets less 10 x 103/microliter), and there was a complete recovery after treatment, so thrombocytopenia is

a significant indicator for malaria, especially in correlation with typical fever and hypotension.

According to our present findings, PDW and MPV are the two most important haematological predictors of malaria infection. This is in line with a recent finding where Al-Salahy*et al.* reported that patients in Hajjah, Northwest Yemen with malaria parasitemia had significantly lower platelet counts compared to healthy subjects. Previous studies have shown that patients with complicated malaria had reduced haematological parameters such as platelet counts, which provided relatively good predictors for the diagnosis of malaria infection(Idro.2011).

On the other hand, the significant differences observed in the haematological parameters between parasitemic Ugandan patients and non-parasitemic Ugandans were only observed in the monocyte and the platelet count (Chiabi*et al.*,2009). No significant difference was found between the PLT counts or MPV (Manning*et al.*,2011).

In the current study, a PDW was 13.7 % and MPV was 10.9 fL were the main predictors for malaria. Previous studies have reported an increased MPV level in malaria (Gogia*et al.*,2012) Interestingly, Chandra *et al* reported that an MPV > 8 fL had a sensitivity and specificity of 70.8% and 50.4% for the diagnosis of malaria, respectively (Naha*et al.*,2012).The higher PDW and MPV values in malaria could be explained by bone marrow formation of megakaryocytes to compensate for the low absolute platelet count during acute malaria infection, A significantly higher level of the key platelet growth factor (thrombopoietin) has been reported in patients with malaria (Woyessa*et al.*,2012). Furthermore, the parasitized RBCs could increase in platelet sensitivity to adenosine diphosphate (ADP), prompting secretion of dense granules(Oduroet al.,2017). Haematological parameters formalaria-infested blood may vary depending on the level of malaria endemicity, presence of haemoglobinopathies and nutritional status (Kaushik*et al.*,2012).

5.2 Conclusion

The study concluded that *P. falciparum and P.*vivaxinfection cause significant changes in total platelets countandindicesin different levels of parasitemia. Also mixedinfection causes significant changes in total platelets count andindicesin different levels of parasitemia.

In conclusion, the study revealed that total platelets count, PDW and MPV were the main predictors among hematological parameter affected by malaria.

5.3 Recommendations

• Further studies should be done on mixed infections to detect their effect onthe complete blood count parameters.

• Further studies should be done on platelets functions test among malaria infected patients.

• Further studies should be done among malaria infected patients to detect their effect on coagulation system.

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Collage of Graduates Studies

Department of Hematology and Immunohematology

Appendix (A)

Questionnaire About: Estimation of Platelet Count and Platelet Indices in Malaria Patients in Khartoum state.

Serial number: …….

Gender: Male ☐ Female ☐

Age: ……years

Results:

Platelet count	
PLCR	
MPV	
PCT	
PDW	

Appendix (B)

SysmexKX-21N

SysmexKX-21N