Sudan University of Science and Technology

College of Graduate Studies

Evaluation of Platelets Indices in Malaria Patients in Khartoum State

تقييم عدد الصفائح الدموية ومؤشرات الصفائح الدموية عند مرضى الملاريا بولاية الخرطوم

A Research Submitted for Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science

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الآيه

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَالضُّحَى (1) وَاللَّيْلِ إِذَا سَجَى (2) مَا وَدَّعَكَ رَبُّكَ وَمَا قَلَى (3) وَلَلْآخِرَةُ خَيْرٌ لَكَ مِنَ الْأُولَى (4) وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَى (5) أَلَمْ يَجِدْكَ يَتِيمًا فَآوَى (6) وَوَجَدَكَ ضَالًّا فَهَدَى (7) وَوَجَدَكَ عَائِلًا فَأَغْنَى (8) فَأَمَّا الْيَتِيمَ فَلَا تَقْهَرْ (9) وَأَمَّا السَّائِلَ فَلَا تَنْهَرْ (10) وَأَمَّا بِنِعْمَةِ رَبِّكَ فَحَدِّثْ(11

صدق الله العظيم

سورة الضحى

Dedication

This work is dedicated TO

MY BELOVED MOTHER

MY BELOVED FATHER

MY BELOVED BROTHER AND SISTERS

TO MY FAITHFUL FRIENDS AND COLLEAGUES .

AND ALL SUDANESE PEOPLE

Acknowledgment

In the name of Allah , the most merciful, the most compassionate all p raise is to Allah , the lord of the worlds , and prayers and peace upon Mohammed His servant and messenger .

First and foremost , I must acknowledge my limitless thank to Allah , t he ever magnificent ; the ever thankful , for His help and bless . I am t otally sure that this work would have never become truth , without H is guidance .I am a deep debt of gratitude to my university for giving my an opportunity to complete this work .

I am grateful to all people , who worked hard with me from the begin ning till the completion of the present research specially my supervis or D . Abdallah Musa Abdalla Mohamed , who has been always gener ous during all phases of the research , and I highly appreciate the effo rts expended by him .

Last but not least , deepest thanks go to my friends Manahil , Amany , Zohida , Momena , who took part in making this thesis real

Abstract

Malaria is a life threatening disease caused by parasites that are trans mitted to people through the bites of infected female anopheles mosq uitoes . In Sudan there is a high prevalence of malaria. A number of st udies have been conducted to determine the magnitude of the effect of malaria and the mortality rate among members of the community and these one of them , these study to determined the effect of malari a in Khartoum patient .

This is a case control study conducted at Saad Rashwan hospital in K hartoum state at the period of February to April 2020. The study aim ed to measure platelet count and indices in malaria patient .

Two hundred blood sample were collected in ethylene deaminatetraa citic acied (EDTA) containers , 100 samples from malaria patient and 100 sampls from apparently healthy volunteers as control. Platelet c ount and platelet indices were measured using automated hematolog ical analyzer (Sysmex BC-3000 PLUS).The study showed a thromboc ytopenia among malaria patients when compared to the control. Mea n of cases $233X10^9$/L, while the mean in control group is $290X10^9$ (P. value= 0.00). MPV was increased in patients when compared to cont rol group (Mean of patient is 9.3, Mean of control is 8.4, P. value= 0.0 0). PCT showed no significant difference when compared to control g roup mean in patients 0.2197 , Mean in Control group is 0.2427, P. v alue=0.150). PDW was decreased in patients comparing to control gr oup (Mean of patients is 11.372, means of control group is 14.031 *P. v alue* = 00.0).

In this study the age and gender had no effect on platelet count ,MPV, PDW and PCT.

The study concluded that malaria altered the blood count causing thr ombocytopenia and increased MPW and decreased in PDW but no sig nificance difference in PCT.

مستخلص البحث

الملاريا من الامراض التي تهدد الحياة تسببه الطفيليات التي تنتقل عن طريق لدغات بعوض الاذ وفليس المصابة وهو مرض يمكن الوقاية منه والشفاء .في السودان يوجد انتشار عالي للملاريا و معدل الاصابه بها بين المجتمع, وقد اجريت العديد من البحوث للتعرف على حجم تاثير الملاريا ومعدل الوفيات بين افراد المجتمع وهذه الدراسه واحدة منهم للتعرف على تاثير الملاريا على م رضى الخرطوم .

صممت هده الدراسة كدراسة حالات وشواهد ,اجريت في ولايه الخرطوم في مستشفى سعد رشد وان في الفترة من فبراير حتى ابريل وحتى مايو 2020.هدفت هدة الدراسة الى قياس عدد الصـ فائح الدموية ومعاملات الصفائح الدموية في المرضى المصابين بالملاريا .

مئتي عينة دم في حاويات تحوي مادة مانعة للتجلط , EDTA 100 عينة شخصت اصابتهم بالم لاريا و100 عينة اصحاء كضوابط عدد الصفائح الدموية ومؤشرات الصفائح الدموية تم تحديده ا باستخدام محلل الدم الالي ()Sysmex BC-3000

اوضحت النتائج ان بنقص الصفائح الدموية في المصابين بالملاريا , متوسط عدد الصفائح الـ دموية في المرضى اظهر .زيادة دا دلالة احصائية مقارنة بالظوابط 233 المرضى 290 , م عامل الضبط 0.0 PV ,) ومتوسط حجم الصفائح في المرضى لم يظهر تاثير دا دلالة احصائي ة(9.3 المرضى 8.4معامل الضبط 0.00 PV,) ومعيار الصفائح الدموية اظهر نقصانا دا د لالة احصائية في المرضى الظوابط (11.372المرضى 14.031 معامل الضبط .00 PV 0) .

اوضحت الدراسة بان العمر والجنس لا تؤثر على معاملات الصفائح الدموية .خلصت الدراسة الـ ى ان الملاريا تسبب خلل في عدد الصفائح الدموية مسببة زيادة في عدد الصفائح الدموية ونقصا ن في توزيع الصفائح .ولا يوجد تغيير في معيار لصفائح .

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Plt	Platelets
MPV	Mean platelets volume
PDW	Platelet distribution risk
PCT	Platelet crit
MK	Magakaryocte
TPO	Thrompopitein
GP	Glycoprotein
TXA2	Thromboxan A2
GPCR	Glycoprotein cell receptor
ADP	Adenosine Di phosphate
PAR	Protease activated receptors
ITAM	Immune receptor tyrosine based activation motif
VWF	Vonwelibrand factor
MPC	Main platelet component
PCDW	Platelet component distribution width
CM	Cerebral malaria
PF4	Platelet factor 4
Ie	Infected erythrocyte

CHPTER I

Introduction

Platelet are disc shaped , a nucleate cellular fragments derived from megakaryosytes. (Heemskerk *et al,*2002) .

Platelets are extremely small and discoid , 3.0 X 0.5mm in diameter w ith mean volume 11 fl . produced in bone marrow by fragmentation o f the cytoplasm of megakaryocyte ,one of the largest cell in the body . The normal platelet count is approximately 250 X 10^9 cell/l (range 1 50- 400X10^9 cell/l)and the normal life span is 7-10 days. (Hoffbrand, 2006)

In addition to reduction in number of platelets, functions of platelet is also compromised which is generally evident in platelet .Platelet is re sponsible for initiation of the haemostatic mechanisms repair injury t o vascular endothelium .The four major platelet function include plat elet adherence, platelet activation and secretion , platelet aggregate on interaction with coagulation factor.(Deutsch,2006)

Platelet also plays important role in inflammation and depending upo n severity of bacterial infection changes in platelet count and indices also has been reported,further platelet activation also alters mean pl atelet volume (MPV) and platelet distribution width (PDW). Platelet c rit is also another marker for measurement biomass which combines mean platelet volume with absolute platelet count. MPV, PDW and pc t are considered as markers of platelet activation and altered in differ ent clinical condition and they were also altered in malaria, however the relation between platelet indices and clinical outcome were contr oversial especially between altered PDW or PCT and severity of mala ria. (Leal *et al.,2013)*

Malaria is a major health problem with increased morbidity and mort ality .It is a vector-borne infection caused by unicellular parasite of th e genus Plasmodium. Plasmodia are obligate intracellular parasites th at are able to infect and replicate within the erythrocytes. There are a hematological abnormalities that are observed in patient with malari a includes anemia and thrombocytopenia . (Spinello et al.,2012)

The major Plasmodium spices is plasmodium falciparum, plasmodiu m vivax plasmodium oval and plasmodium malaria. Malaria is a paras itic disease that involves igh fever , shivering chills ,flu like symptoms and anemia . this infection is caused by parasite known as plasmodiu m ,transmitted by anopheles mosquitoes .After infection the parasite enter the bloodstream and infect the red blood cells. The parasites m ultiply inside the red cell which then break open to infect more red ce ll. The first symptoms typically occur 10 days to 14 days after the infe ction .

Platelets are the second most abundant cell of the circulation after re d blood cells (RBCs) and the principle regulators of hemostasis. Platel ets can also integrate host immune responses through production of i mmunomodulatory molecules and via cell-to-cell interactions with w hite blood cells (WBCs), and may have host-protective roles in infecti ous disease. Platelets are an abundant source of antimicrobial molecu les ,it has broadspectrum pathogen-killing activities ,and required for host-mediated pathogen control and host survival in some infectious disease models. Clinically, low latelet counts are often associated wit h a poor prognosis and increased risk for infection. (Steven *et al.,*201 8)

Hematological changes associated with malaria are well recognized, but the specific changes may vary with demographic factors, nutritional status, hemoglobinopathy, background, malaria endemicity levels, and malaria immunity. Changes in platelet counts during acute malaria were reported in the several medical literatures, such as *P. falciparum* infections; these changes are the major cause of serious and complicated disease. Many studies have also report the association of thrombocytopenia with *P.vivax* infection. Peripheral destruction, excessive sequestration of platelets in spleen and excessive use of platelets associated with the disseminated intravascular coagulation phenomenon are underlying mechanisms of thrombocytopenia in malaria infection . Addition with reduction in platelet (PLT) count and platelet function, changes in the volume and other features of platelet cells are the generally evidenced in these patients .Platelet activation alters the morphology change of platelets, included mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT), which is a reliable measurement of platelet biomass . All of these indices are considered as markers of platelet activation and alteration in different clinical conditions. (Orathai et al, 2016)

1.2 Rationale

Malaria is a serious and fatal disease caused by plasmodium species . Plasmodium falciparum is a most dangerous form of malaria and call ed malignant malaria,this diseases affected many part of the world pa rticularly in africa while reliable data are scarce 100 of millions of pe ople were likely infected with malaria and 10 of millions died (WHO 2020),the incidence in sudan was estimated to be about 9 milion epis odes in 2002 and the number of the death about 44.000 DAYLYdue to mortality ,episodes and anemia.children under 5 years of age had the highest Barden males had the highest incidence and mortality (Safa I Abdalla *et.al,* 2007) .Platelet are shown to be more active to kill the parasite infected red blood cell .Platelet count and indices could be us eful in diagnosis of malaria .

Malaria is associated with different degree of reduction platelet count and alteration of platelet indices and may be used as probable indica tor for malaria in endemic regions and therefore encourage the labor atory physicians for more depth search of the parasite microbiology. This study is aimed to measure platelet count and indices to prove thi s theory .

1.3 Objectives

1.3.1 General objective

To measure platelets indices in malaria patient .

1.3.2 Specific objectives

To measure platelets count and indices (MPV,PDW ,PCT) in cases and control.

To compare between gender and platelet indices .

To compare between gender and type of malaria.

To compare between age and type of malaria ,gender and case and co ntrol.

CHAPTER II

Literature Review

2.1 Platelets

Platelets are blood cells that are released from bone marrow megaka ryocytes and circulate for approximately 10 days. They possess granu lar cytoplasm with no nucleus and their diameter when seen in a Wri ght stained peripheral blood film averages 2.5 nm with a subpopulati on of larger cells, 4-5 um. Mean platelet volume (MPV), as measured i n a buffered isotonic suspension flowing through the impedance-base d detector cell of a nature of platelet remained afield of interest only f or biologists . A nucleate ,discoid platelet are the smallest blood parti cles which unveil their dynamicity through their morphology . clinical profiling instrument, is 8-10fL. (Laroche et.al., 2020). Platelet were d iscovered by Giulio Bizzozero in 1882,but for many decades the dyna mic and multifunctional . (Kakali,2014) .

Megacaryocyte are undergo fragmentation of their cytoplasm to prod uce platelet under the control of humoral agent like thrombopoietin . (Kamath et.*al*., 2001).

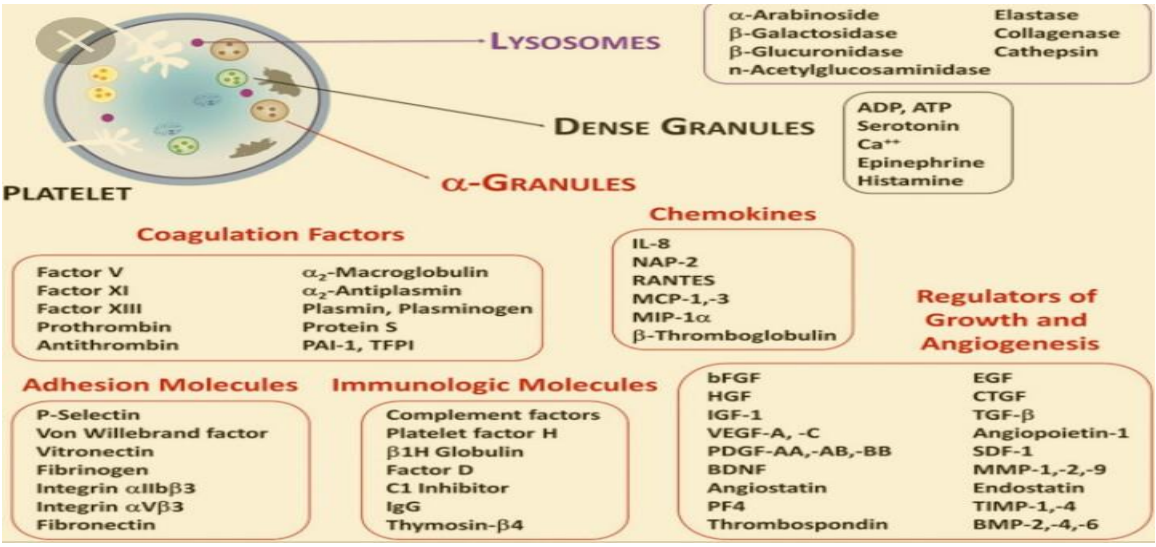
They have a pivotal role in haemostasis by forming the initial haemos tatic plug that provides a surface for the assembly of activated coagul ation factors leading to formation of fibrin stabilized platelet aggrega tes and subsequent clot retraction .

2.2 Platelets granules

Platelets have two type of granules Alpha granules which contain P.se lectin, fibrinogen ,fibrionctin, factor v ,factot VIII , factor IV , platelet d

rived growth factor and tumor groth factor-a (TGT-a) and Gamma gra nules or dense granules which contain adenosine triphosphate (ATP) , adenosine diphosphate (ADP)+ calcium (ca) serotonin , histamine andepinephrine. (Heemskerk *et.al*., 2002)

In general, more gaint platelets are younger ,more reactive and aggre gable than older platelets ,consequently the younger platelets contain more granules ,secrete more serotonin and B- thromboglobulin ,and produce thromboxan A2 than smaller platelets these can produce pro -coagulant effect which lead to thrombotic vascular complications ,in brief changes in(MPV)reflect the state of thrombogenesis. (Kodiiatte *et.al*., 2012). large platelets more active than small platelets. (Mangal pally et.al.,2010) .Platelet also have a critical role in normal haemost asis ,and thrombotic disorders. The development of megakaryocytes i s controlled by thrombopoietin which bind to c-mpl on the surface of platelets and megakaryoctes. Platelet membrane glycoproteins media te binding to subendothelial tissue and aggregation into haemostic pl ugs .Thrombocytopenia and disorder of platelet function cause petec hiae and mucocutaneous bleeding (James, 2000). Platelets are the sec ond most abundant cell of the circulation after red blood cells (RBCs) and the principle regulators of homeostasis. Platelets can also integra te host immune responses through production of immunomodulator y molecules and via cell-to-cell interactions with white blood cells (W BCs), and may have host-protective roles in infectious disease. Platele ts are an abundant source of antimicrobial molecules,have broad-spe ctrum pathogen-killing activities, and are required for host-mediated pathogen control and host survival in some infectious disease models . (Steven Kh and Bridget,2018).



Figer 1 : platelets granules and it's function .

Table 1 : show the platelet granules and there biological effect

Substance	Biological effect
Alpha granules	
Platelet derived growth factor	**Matrix deposition**
Transforming growth factor beta	**Matrix deposition**
Transforming growth factor alpha	**Epitheliazation**
Insulin like growth Factor binding protein 3	**Matrix deposition**
Platelet factor 4	
Alpha thromboglobulin	**Activation of groth factors**
	Activation of groth factors
Dense granules	
Adenosine diphosphate	**Platelet aggregation**
Calcium	**Platelet aggregation**
Serotonin	**Vasoconstriction**
Cytosol	
Von willebrand factor VIII	**Mediator of platelet adhesion**
Fibronectin	**Ligand of platelet aggregation**
Fibrinogen	**Ligand of platelet aggregation**
Thrombospondin	**Ligand of platelet aggregation**
Factor V	**Hemostasis**
Platelet activation factor	**Platelet activation**
Thromoxan A2	**Vasoconstriction**
12Hydroxyeicosateranoic acid (12 HETE)	**Vasoconstriction**

2.3Platelet production

Platelet formed and released into the bloodstream by precursor cells called megakaryocytes which reside within the bone marrow .The pr oduction of platelets from megakaryocytes is a systematic and regula ted process that is thought to occur either in the bone marrow or has been shown more recently in the lung, the platelet is exposed to in th e vessel as well as the limitations imposed on the platelet due to the a bsence of a nucleus ; the lifespan of the platelet is limited to between 5 to 7 days following formation and separation from the megakaryoc yte. During its normal life cycle, platelets decrease in size such that yo ung platelets are measurably larger than older platelets. At the end of their life in the vessel or following full activation of the platelet and i ncorporation into a forming clot in the vessel, they are removed from the vessel by neutrophils and macrophages and transported to the sp leen for removal from the body. (Patel *et al*.,2015)

Thrombopoietin is the primary humoral regulator of megakaryocyte differentiation and platelet number under steady state conditions. It i s synthesized in the liver and kidney and mediates its effects through its receptor c-Mpl which is present on megakaryocyte and platelet m embranes. Levels of thrombopoietin are controlled via binding to and internalization into cells expressing the receptor. When platelets and megakaryocytes are decreased in number, less thrombopoietin is re moved from plasma, and the thrombopoietin level rises, while when platelet numbers increase, more thrombopoietin is cleared from the plasma and the thrombopoietin level falls again.(Daly 2011)

Thrombocytopenia Defined as a platelet count less than $150X10^9$/L, t hrombocytopenia is usually an acquired disorder. Causes include incr

eased platelet consumption, splenomegaly, drugs or infection-mediated bone marrow suppression, and bone marrow failure. Increasingly, however, inherited forms of thrombocytopenia, caused by mutations in genes encoding proteins involved in the differentiation of megakaryocytes and platelet production, which can result in autosomal dominant, autosomal recessive, and X linked recessive forms of inherited thrombocytopenia, are being recognized.

Thrombocytosis Defined as a platelet count exceeding the upper limit of the normal range (>400X10^9/L),is associated with an increased risk of thrombosis .Primary thrombocytosis can be either inherited or acquired and is caused by alterations targeting hematopoietic cells while secondary thrombocytosis is due to external factors such as chronic inflammation or cancer. (Daly 2011)

2.4 Megakaryocytopoiesis

Megakaryocytes (MKs) are named for their large nucleus, i.e., mega (large) karyo(nucleus) cyte (cell), and are large polyploid blood cells with diameters ranging from 20 to 100 nm. (Ming and Alan 2012) .

Megakaryopoiesis and thrombopoiesis are controlled by multiple cytokines and growth factors, although thrombopoietin is the key regulator. (Seong *et al*,2016)

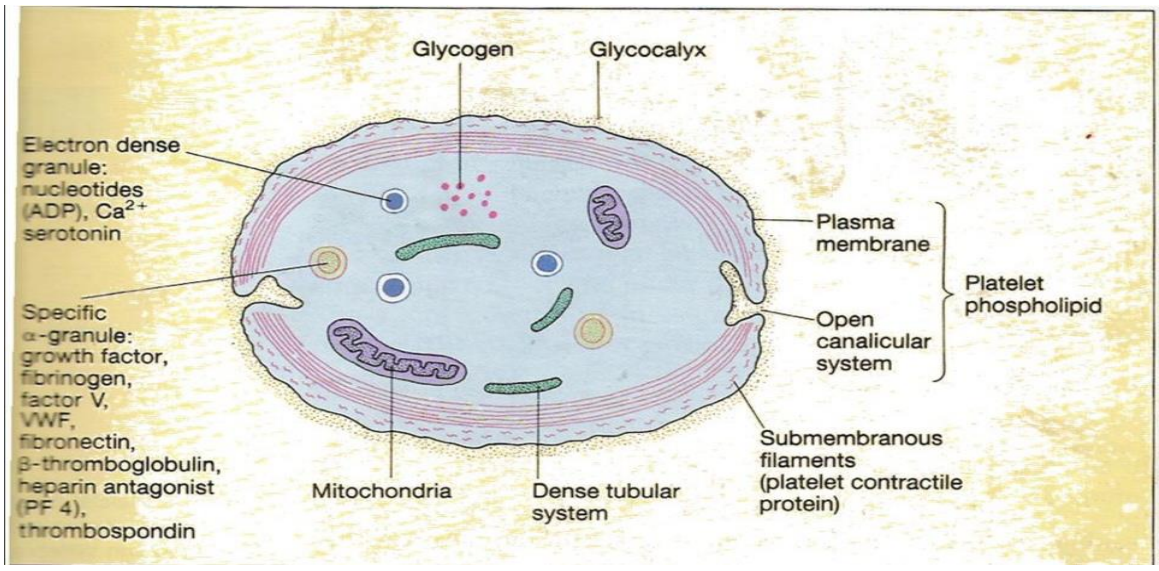
Megakaryocytopoiesis involves the commitment of haematopoietic stem cells, and the proliferation, maturation and terminal differentiation of the megakaryocytic progenitors. Circulating levels of thrombopoietin (TPO), the primary growth-factor for the megakaryocyte (MK) lineage, induce concentration-dependent proliferation and maturation

of MK progenitors by binding to the c-Mpl receptor and signalling ind uction..(Varda *et al*., 2006**)**

2.5 Platelet structure

Platelet plasma membrane is a standard bilayer composed of protein s and lipids . The predominant lipids are phospholipids, which form t he basic structure, and cholesterol, which distributes asymmetrically throughout the phospholipids. The phospholipids form a bilayer with their polar heads oriented toward aqueous environment toward the plasma externally and the cytoplasm internally. Their fatty acid chain s, esterified to carbons 1 and 2 of the phospholipid triglyceride backb one, orient toward each other, perpendicular to the plane of the mem brane, to form a hydrophobic barrier sandwiched within the hydroph ilic layers .The neutral phospholipids in platelets support platelet acti vation by supplying arachidonic acid, an unsaturated fatty acid that b ecomes converted to the eicosanoids prostaglandin and thromboxane A2 during platelet activation. Phosphatidylserine flips to the outer su rface upon activation and is the charged phospholipid surface on whi ch the coagulation enzymes, especially coagulation factor complex VII I and IX and coagulation factor complex X and V, assemble. Cholester ol stabilizes the membrane, maintains fluidity, and helps control the t ransmembranous passage of materials. Anchored within the membra ne are glycoproteins and proteoglycans; these support surface glycos aminoglycans, oligosaccharides, and glycolipids. The plasma membra ne is selectively permeable, and the membrane bilayer provides phos pholipids that support platelet activation internally and plasma coag ulation externally.. Platelet Granules: α-Granules, Dense Granules, an d Lysosomes There are 50 to 80 α-granules in each platelet. Unlike th

e nearly opaque dense granules, α-granules stain medium gray in os mium-dye transmission electron microscopy preparations. The α-gra nules are filled with proteins, some endocytosed, some synthesized w ithin the megakaryocyte . As the platelet becomes activated, α-granul e membranes fuse with the SCCS. Their contents flow to the nearby m icroenvironment, where they participate in platelet adhesion and agg regation and support plasma coagulation. There are 2–7 dense granul es per platelet. Also called dense bodies, these appear later than α-gr anules in megakaryocyte differentiation and stain black (opaque) wh en treated with osmium in transmission electron microscopy. Small molecules are endocytosed and are stored in the dense granules .(La roche 2020)



Figer 2 : show platelet structure .

2.6 The platelet shape change

Blood platelets attracted the attention of early microscopists by the o utstanding capacity to change physical properties in response to vess el wall injury or foreign substances . To ensure vascular integrity, the se corpuscles developed a variety of reactions, including shape chang e, adhesion, aggregation, granule release and formation of procoagula nt surface. The microtubules play important role in the formation of platelets. In mature cells, they form the peripheral ring, which suppor ts the flattened platelet shape. It gives the cell essential rigidity to ma rginate in blood flow and to slide along vessel walls. Morphological "d isk-tosphere" transformation is the one of early events and occurs fol lowing even the weakest stimulation . It can be considered as the uni versal hallmark of platelet activation, along with the intracellular calc ium concentration. The dramatic morphological change is provided b y the unique inner architecture of platelets, which incorporates the m arginal band of microtubules, the submembrane cortex and the actin cytoplasmic network

2.6.1 Structure of resting platelets

The shape of the resting platelet is flattened, which is why it is called "platelet". The first attempts to characterize its morphology were ma de with phase contrast microscopy. This approximating model is ofte n used to model platelets in optical and hydrodynamic simulations . P latelet possess a submembrane cortex mainly consisting of spectrin, but also actin, myosin and intermediate filaments . The cortex provid es tension to the platelet surface. This results in formation of "wrinkl es" on thelipid bilayer, which serve as a membrane reservoir when ac tivation and spreading occur . In the absence of the internal cytoskele

ton, the cell would adopt the spherical shape, since it has the minimal surface area with the given volume. This is actually the case for bloo d granulocytes. However, the peripheral ring of microtubules in plate lets stretches the membrane and flattens the cell . (Alexander,*et.al*.,20 18)

2.7.2 Platelet adhesion

Platelets perform a central role in haemostasis and thrombosis. They adhere to subendothelial collagens exposed at sites of blood vessel in jury via the glycoprotein (GP) Ib-V-IX receptor complex, GPVI and int egrin . These receptors perform distinct functions in the regulation of cell signaling involving non-receptor tyrosine kinases adaptor protei ns, phospholipase C and lipid kinases such as phosphoinositide 3-kin ase. They are also coupled to an increase in cytosolic calcium levels a nd protein kinase C activation, leading to the secretion of paracrine/a utocrine platelet factors and an increase in integrin receptor affinities . Platelets possess several cell-surface receptors that allow them to ad here to sites of tissue damage and spread to form a monolayer of cells that covers the exposed tissue. Spreading is accompanied by the secr etion or synthesis of several prothrombotic factors, such as ADP, sero tonin and thromboxane A2, which act in an autocrine/paracrine fashi on and activate or prime approaching platelets . During platelet activ ation, inside-out signalling upregulates the affinity of several platelet integrins .This binds to the bivalent ligand fibrinogen, which is prese nt in the plasma and is released by activated platelets. The resulting p latelet aggregation leads to the assembly of a platelet thrombus. **(**Jon atha**,** 2004**)**

2.7.3 Platelet Activation

Platelet activation is stimulated by bound platelet secretion products and local prothrombotic factors such as tissue factor. Multiple pathways can lead to platelet activation. There are two principle activating pathways in platelets . GP Ib-IX-V, GP VI, or C-type lectin like receptor 2 are all membrane glycoproteins exclusively expressed in platelets and megakaryocytes and have closely related signal transduction pathways. GP VI is thought to be the major signaling receptor involved in platelet activation on exposed collagen. Following GP VI interactions with collagen, platelets initiate strong activation and release the content of alfa and dense granules. Platelet activation through receptors containing the immune receptor tyrosine based activation motif (ITAM) sequence. Most soluble agonists released by activated cells such as ADP, thromboxane A2, and thrombin trigger platelet activation through GPCR. This increases the cytosolic calcium concentration and activates specific signaling pathways. ADP released from damaged endothelial cells and activated platelets acts on platelet P2Y1 and P2Y12 GPCR, which causes further platelet activation and release of ADP. P2Y12 receptor sustains platelet activation in response to ADP and therefore has a central role in this process. TxA2 produced and released by stimulated platelets also activates further platelets via GPCR, that promoting plug formation. Trombin is the most strong platelet agonist and also responsible for converting fbrinogen into fbrin to stabilize the platelet plugs. Trombin activates platelets through protease-activated receptors (PAR) on the platelet surface via GPCR . Other agonists like epinephrine, prostaglandin E2, and serotonin can also utilize GPCR to potentiate platelet responses . All these platelet signaling events converge upon the final common pathway of platelet activation, the f

unctional upregulation of integrin adhesion receptors . The most imp ortant is the activation of the GP IIb/IIIa receptor which results in the cross-linking of fbrinogen or vWF between receptors, leading to plat elet aggregation. This promotes further the recruitment of additional platelets to the site of vascular injury, allowing the subsequnt thromb us formation. (Jonathan ,2004)

2.8 Platelet hemostasis

Hemostasis is a process to prevent hemorrhage by keeping the blood within the damaged vessel walls. Blood vessels must maintain their i ntegrity to effectively deliver blood to the body's vital organs and tis sues . when damage occurs to the wall of a blood vessel ,the physiolog ical ,reparative mechanism of hemostasis is prevents further bleedin g . Hemostasis occurs in 2 phases : platelet activation to form a static plug and stabilization of this plug through extrinsic and intrinsic coa gulation pathways, known as primary and secondary hemostasis resp ectively . (saad and schoenberger,2019) .This complex process contin gent on the complex interaction of platelets, plasma coagulation casc ades, fibrinolytic proteins, blood vasculatures and cytokine mediator s. Upon tissue injury, the hemostatic mechanism employs a plethora of vascular and extravascular receptors, in accordance with the blood components, to seal off the impairments to the vasculature and closi ng it off from the encircling tissues. Normal hemostatic responses can be organized into six different important phases, which fall under thr ee major categories of hemostasis Primary hemostasis: Blood vessel c ontraction /vasoconstriction Platelet plug formation upon platelet ad hesion and aggregation. Secondary hemostasis: Activation of the coag ulation cascade Deposition and stabilization of fibrin. Tertiary hemos

tasis: Dissolution of fibrin clot Dependent on plasminogen activation . (Mercy *et.al*, 2016)

2.9 Formation of primary haemostatic plug

The primary role of the platelet in circulation is to help maintain pri mary hemostasis and blood flow within the vessel. In order to accom plish this goal, the platelet flows through the vessel in close proximity to the vessel wall due to the biophysical nature of the blood constitu ents and shear forces within the vessel. This close proximity to the ve ssel wall allows for a quick response when a vascular insult or injury occurs. This response is typically thought to occur in several stages st arting with adhesion to the subendothelial extracellular matrix throu gh initial interaction of the matrix with specific receptors on the plate let including the GP1b/V/IX complex binding to Von Willebrand facto r as well as GPVI and αIIβ1 receptors on the platelet surface binding t o the collagen component of the extracellular matrix. Following this i nitial tethering of the platelet to the vessel wall, subsequent firm adh esion results in signal transduction within the platelet and flattening of the initially round or "plate" looking platelets. Secondary to firm ad hesion, which results in the initial clot or thrombus formation, the act ivated platelets bound within the thrombus will begin to incorporate new platelets from circulation through platelet interactions mediate d by the integrin receptor αIIbβ3.(Gale,2011)

2.10 Platelet indices

Platelet indices are biomarkers of platelet activation. (Budak et.al.,2 016). Platelet indices (PI) ,platelet crit ,mean platelet volum (MPV) an d platelet distribution width (PDW) are a group of derived platelet pa ramerters obtained as a part of the automatic complete blood count .

Emerging evidence suggests that platelet may have a diagnostic and prognostic value in certain disease . (Budak *et.al*, 2016)

Mean platelet volume (MPV), is considered as a marker of platelet act ivation. Red cell distribution width (RDW) refers the size variations o f erythrocytes. (Dogan 2016)

Platelet indices are potentially useful markers for the early diagnosis of thromboembolic diseases. An increase in both mean platelet volum e (MPV) and platelet distribution width (PDW) due to platelet activati on, resulting from platelet swelling and pseudopodia formation was h ypothesized. Several investigators have used a series of platelet indic es measured by hematology analyzers, given the fact that platelet acti vation causes morphologic changes of platelets. The mean platelet vol ume (MPV) is probably the most extensively studied platelet activatio n marker. Recently, novel platelet indices such as mean platelet comp onent (MPC) and platelet component distribution width (PCDW) hav e been investigated as prospective platelet activation markers. Howe ver, not all hematology analyzers examine these indices. The present effort for finding simple and widely used platelet activation indices fo cused on the fact that platelet activation causes morphologic changes of platelets, including both the spherical shape and pseudopodia form ation. Platelets with increased number and size of pseudopodia differ in size, possibly affecting platelet distribution width (PDW). The poss ibility whether platelet activation increases MPV and PDW as expecte d was examined. Moreover, the issue whether pseudopodia formatio n could cause specifc changes, supporting the differential diagnosis b etween platelet activation and other causes of platelet swelling was a ssessed .(Vagdatli *et al*., 2010).

2.10.1 Mean Platelet volume

Platelet size has been demonstrated to reflect platelet activity and se ems to be a predictive and prognostic biomarker of cardiovascular ev ents. It is associated with a variety of prothrombotic and proinflamm atory diseases. The aim is a review of literature reports concerning c hanges I n the mean platelet volume (MPV) and its possible role as a biomarker in inflammatory processes and neoplastic diseases Literat ure data indicate that mean platelet volume (MPV) can provide impor tant information on the course and prognosis in many pathological co nditions, such as cardiovascular diseases, respiratory diseases, Croh n's disease, rheumatoid arthritis, juvenile systemic lupus erythemato us, diabetes mellitus, and the majority of neoplastic diseases . (Aleksa ndra *et.al*.,2019).Mean Platelet volume measure the average size and activity of platelet found in blood ((Guclu *et al*., 2013)

MPV : 8.9 -11.8 fl (Maluf *et al*., 2015) ninety five percent of individual s had an MPV between 7.2 and 11.7 fl (Demirin *et al*., 2011)

2.10.2 Platelet distribution widith

PDW is more specific marker of platelet activation , which cause mor phologic change of platelete, among both the spherical shape and pse udopodia formation since is dose not increase during simple platelet swelling .platelet with an increase number and size of pseudopodia di ffer in size ,possibly affecting platelet distribution widith. (Vagdatli *et .al.,* 2010) . Is an indicator of platelet size variation . (Guclu *et.al*, 201 3) .It also increase in platelet anisocytosis . (Osselaer *et.al*.,1997) .

The normal range of PDW is : 9.6 - 15.3 fl . (Maluf *et.al*., 2015)

2.10.3 Platelet crit

Context-Plateletcrit is a measure of total platelet mass. Values vary de pending on mean platelet volume resulting in overlap between norm al platelets, thrombocytopenia and thrombocytosis. The normal rang e of plateletcrit which could screen thrombocytopenia, thrombocytos is and normal platelet count. (Vani 2013)

2.11 Normal values

platelet count in humans ranges from $150X10^9$/L to $400X10^9$/L. Give n that platelets have a circulating lifespan of around 10 days, and that about one third of platelets are sequestered in the spleen at any time , approximately $100X10^9$ of these small anucleate cells must be releas ed from mature megakaryocytes into the circulation each day in orde r to maintain a normal platelet count. (Daly ,2010)

The normal ranges for MPV, PDW and PCT for this analyzer are as foll ow: Platelet count: 150.00 – 450.00 × 10^3 /µl; MPV: 7 -11 fL; PDW: 15 .0 – 17.0 %; PCT: 0.108 – 0.282 %, respectively . (Orathai *et.al*.,2016)

2.12 Malaria

Malaria is a life-threatening disease caused by parasites of genus plas modium that are transmitted by Malaria vectors to man through the bites of infected female Anopheline mosquitoes. There are four plasm odium species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodiu m malariae*, *plasmodium ovale*) causes human malaria, among which the *P. falciparum* and *P. vivax* are the most common and *P. falciparum* is the most deadly. (Niamatullah *et.al*.,2014)

According to world health organization (WHO) report in 2013 it is es timated about 207 million cases of malaria in 2012 and accounts for a n estimated 627000 deaths. A vast majority, about 85% malarial case s were in African Region, followed by the South-East Asian Region (1 0%) and 4% from East Mediterranean region. 89% of the death cases reported from African Region followed by East Mediterranean (6%) and 5 % from South-East Asia Region. (Gupta *et.al*., 2013)

Malaria is one of the most common infectious diseases and a great pu blic health problem worldwide, particularly in Africa and south Asia. The organism that causes the most dangerous from of malaria is a mi croscopic parasite called plasmodium falciparum (Talaro,2012)

Malaria kills more than 400 000 people each year. Although most dea ths are caused by Plasmodium falciparum, all Plasmodium species ca n cause severe and fatal infection. Malaria pathogenesis is driven pri marily by parasite biomass and modulated by host innate and adapti ve immune responses. Thrombocytopenia is common in all malarias and is a risk factor for mortality in African children with falciparum malaria, Southeast Asian adults and children with falciparum and viv ax malaria, and adults with knowlesi malaria. (Steven Kho and Bridg et,2018)

2.13 Plasmodium species

There are four type of plasmodium species that cause Malaria in hum an :Plasmodium falciparum ,Plasmodium vivax ,Plasmodium ovale ,Pl asmodium malarei .

2.13.1 *Plasmodium* falciparum

Plasmodium (Laverania) falciparum is the highly pathogenic and mos t deadly parasite causing malaria in humans. It was discovered in 188 0 by Charles Alphonse Laveran, a French Army Surgeon, deployed in Constantine (Algeria) and originally named by himself*Oscillaria mala riae*. Examining under a microscope a drop of blood from a young sol dier with fever, Laveran observed some spherical and crescent-shape d bodies with actively moving filaments ; he was looking at exflagellat ion of a male gametocytes of *P.falciparum* a phenomenon that was su bsequently explained by Maccallum. Exflagellation of the microgamet ocyte in the life cycle of malarial parasites occurs in the stomach of m osquitoes after ingestion of an infected blood meal but in rare cases it can be observed also in the peripheral blood smear of infected huma ns, generally as the consequence of extended delay in slide preparati on or following warming. (Spinello *et al*.,2012)

plasmodium falciparum is a protozoan parasite, on of the species of p lasmodium that cause malaria in humans. It is transmitted by the fem ale Anopheles mosquito .malaria cause by this species (also called m alignant of falciparum malaria) is the most dangerous form of malari a , with the highest rates of complication and mortality . As of the late st World Health organization report in 2014 , there were 198 million case of malaria worldwide in 2013 , with an estimated death of 584,0 00 . (WHO, 2014)

2.13.2 plasmodium vivax

Plasmodium vivax of the world. It accounts for more than half of all m alaria is prevalent in many regions malaria cases in Asia and Latin A merica. Despite the high prevalence of disease caused by this parasite

, research into its effects has lagged disproportionately (Dhanpat *et al* .,2004)

Plasmodium vivax is the most geographically widespread species am ong human malaria parasites .Immunopathological studies have sho wn that platelets are an important component of the host innate imm une response against malaria infections.(Cho Naing and Maxin ,2018)

Plasmodium vivax is a protozoal parasite and a human pathogen. The most frequent and widely distributed cause of recurring (benign tert ian) malaria , p.vivax is one of the four species of malaria parasites th at commonly infect humans it is less virulent than plasmodium falcip arum ,the deadliest of the four . but vivax malaria can lead to severe d isease and death due to splenomegaly (a pathologically enlarged sple en) .(Davidson, 2004) .

2.13.3 plasmodium oval

Plasmodium ovale was discovered in 1922 by Stephens who observe d it in the blood of an East african patient with malaria erythrocytes with oval shape and fimbriated edges and named the parasite *P.ovale*. Using the sequences of the small subunit ribosomal RNA (SSUrRNA) gene it has been established that *P.ovale* belong to 2 genetic haplotyp es named classic and variant. Both the classical and variant types are morphologically indistinguishable and occurred in sympatry worldwi de ; based on the observation that no evidence of inter-or intragenic r ecombination could be observed among samples coming from differe nt part of the world .(Spinello *et al.*, ,2012)

plasmodium oval is a species of parasitic protozoa that cause tertian malaria in humans .It is one of several species of plasmodium parasit

es that infect primarily they are associated with haemostasis , which i s to initiate blood coagulation ..(Kakali and Maitree ,2014)

2.13.4 Plasmodium malariae

Plasmodium malariae responsible of the "quartan malaria", is present worldwide in all major malaria-endemic regions but with a scattered distribution. Infections caused by *P.malariae* are most common in su bSaharan Africa and southwest Pacific and less frequently encounter ed in Asia, Middle East, Central and South America. The life cycle of d evelopment (respectively 48- and 72-hours) and the periodicity of th e fever paroxysm were elegantly explained by Camillo Golgi in 1886. The parasite is characterized by a slow development either in the *Ano pheles* mosquito (15 days) and in human (15 days in the liver, 72 hou rs in the blood). *P.malariae* is considered to be the precursor of *P.bras ilianum* a parasite that infects New World monkeys and has naturally adapted to it; both *Plasmodia* are able to infect either humans and mo nkeys. *P.malariae* is responsible of low grade parasitaemia , rarely ex ceeding 30,000 parasites permicroliter, probably as a consequence of the low number of merozoites produced per erythrocytic cycle toget her with the 72-hour developmental cycle and the preference to infec t older erythrocytes. The pre-patent period for *P.malariae* is extremel y variable with a range of 16 to 59 days. No quiescent liver stage form s have been identified for *P.malariae* but this parasite is able to persis t in the blood with low level parasitaemia for extremely long periods and perhaps for the entire life of the human host causing recrudescen ce even after more than 30-40 years or longer. (Spinello *et.al*., 2012)

2.14 Life cycle

Plasmodium life cycle have tow hosts : mosquito and humans. Sexual reproduction takes place in the mosquito and the parasite is transmit ted to the humans when mosquito takes a blood meal . (Kayser *et.al*, 2005)

Plasmodium sporozoites are the product of a complex developmental process in the mosquito vector and are destined to infect the mamm alian liver. Attention has been drawn to the mosquito stages and pree rythrocytic stages owing to recognition that these are bottlenecks in t he parasite life cycle and that intervention at these stages can block tr ansmission and prevent infection. Parasite progression in the Anophe les mosquito, sporozoite transmission to the mammalian host by mos quito bite, and subsequent infection of the liver are characterized by extensive migration of invasive stages, cell invasion, and developmen tal changes. Preparation for the liver phase in the mammalian host be gins in the mosquito with an extensive reprogramming of the sporoz oite to support efficient infection and survival . The malaria parasite l ife cycle constitutes one of the most complicated and fascinating life c ycles of any organism and thus poses intriguing areas of study for cell biology, molecular biology, and immunology alike. A major part of th e complexity associated with the malaria parasite life cycle is due to t he parasite's ability to change its cellular and molecular makeup, whi ch is controlled by a genome with more than 5000 recognized genes and develop in intracellular and extracellular niches in the mammalia n host and the mosquito vector. Malaria parasite effectively compens ates for losses by growth and replication in cellluar niches hidden aw ay from the host's immune responses . Parasite stages that suffer suc

h severe losses are the ookinete and the sporozoite, both of which for m and migrate within the insect vector . The ookinete develops from a zygote in the bloodfed mosquito midgut lumen, a product of fertiliz ation of a female macrogamete by a male microgamete.The ookinete i s the only invasive stage that is not preceded by a replication step, an d thus ookinete numbers are a direct product of the number of fertiliz ation events. The ookinete starts its short journey by traversing the midgut epithelial cell layer from the apical side and then egresses fro m the basal end to reach the basal lamina. This invasion step is accom panied by a severe reduction in ookinete numbers due to the interve ntion of host protective mechanisms . The surviving ookinetes becom e sessile and transform into oocysts. The oocyst is the only parasite d evelopmental stage that grows extracellularly and results in the form ation of sporozoites. Sporozoites are released into the mosquito body cavity and invade the salivary glands, and they suffer severe losses o n this journey . The ookinete and the sporozoite are thus bottleneck s tages in the malaria parasite life cycle. Sporozoites are transmitted d uring the next mosquito blood meal and initiate liver infection in the mammalian host. Liver infection does not result in overt pathology b ut leads to a 10,000- fold amplification of parasite numbers,culminati ng in the release of exoerythrocytic merozoites into the bloodstream, which in turn infect erythrocytes to initiate the pathogenic erythrocyt ic cycle .(Ahmed S.I. Aly1 *et.al*.2009,)

2.15 Life cycle of plasmodium

Plasmodium is a parasite that grows intracellularly. The life cycle of p lasmodium develops in two phases: an asexual phase in the human h ost and a sexual phase in the carrier, the *Anopheles* mosquito. The spo

rozoites transmitted during a blood meal rapidly penetrate from the blood stream into the liver parenchymal cells in which they replicate asexually. Depending on the plasmodium species, this so-called schiz ogony phase lasts between 5-7 days in *P. falciparum* and between 6-18 days in the other species. Schizogony, formerly referred to as mer ogony, is the asexual replication of the protozoae. After schizogony is completed, the swollen liver cell ruptures and releases the mobile me rozoites into the blood stream. These adhere to the red blood cells via specific surface receptors Then, they enter the red blood cells and tu rn into trophozoites. At the end of the 48- to 72-hour erythrocytic ph ase, the schizonts will have formed in the red blood cells. During this phase, so-called seal-ring shapes (vacuoles with parietal nuclei) may f orm . From decayed red blood cells, new merozoites may be released which can infect further red blood cells. A part of the merozoites diffe rentiates within erythrocytes into sexual stages, forming macro- and microgametocytes. In the intra-erythrocytic vacuoles, haemozoin is f ormed as an insoluble metabolite of haemoglobin, called malaria pig ment. After ingestion of male and female gametocytes during a blood meal, a motile flagellated zygote is formed in the midgut of the *Anoph eles* mosquito. This zygote moves into the salivary gland. An oocyst is formed releasing sporozoites which can infect a new human host via the saliva of the mosquito.(Blut and Untergruppe ,2019)

2.15.1Life cycle in human

Malaria remains a major cause of death and morbidity worldwide acc ounting for the majority of malaria mortality, with infections by Plas modium falciparum though the less virulent P. vivax, and probably P. ovale, also contribute significantly to morbidity. Plasmodium sporozo

ites injected by an infected mosquito migrate to the liver and initiate the hepatic stage of the parasite life cycle by invading hepatocytes wi thin which they multiply and differentiate into schizonts containing t housands of hepatic merozoites. These merozoites are subsequently r eleased into the blood where they initiate the erythrocytic stage by in vading and replicating within red blood cells (RBCs). Some of these as exual blood parasites differentiate into gametocytes that will ensure parasite transmission to the mosquito vector. P. vivax and P. ovale sh ow a slightly different life cycle within the mammalian host, as some sporozoites once in the liver do not develop immediately into schizon ts, but remain at an uninucleate stage, in a quiescent form named hyp nozoite, before resuming hepatic development on the impulse of still unknown factors, causing relapses weeks, months or even years after the primary infection. (Vale´rie *et.al*.,2015)

2.15.2 Life cycle in mosquitoes

Continuous host habitat changes are always associated with severe lo sses for the malaria parasite, leading to great fluctuations in populati on densities. This is due mostly to the action of host defense mechani sms initiated upon infection . However, the malaria parasite effectivel y compensates for losses by growth and replication in cellular niches hidden away from the host's immune responses . Parasite stages that suffer such severe losses are the ookinete and the sporozoite, both of which form and migrate within the insect vector. The ookinete develo ps from a zygote in the bloodfed mosquito midgut lumen, a product o f fertilization of a female macrogamete by a male microgamete. Notab ly, the ookinete is the only invasive stage that is not preceded by a re plication step, and thus ookinete numbers are a direct product of the

number of fertilization events. The ookinete starts its short journey b y traversing the midgut epithelial cell layer from the apical side and t hen egresses from the basal end to reach the basal lamina. This invasi on step is accompanied by a severe reduction in ookinete numbers d ue to the intervention of host protective mechanisms . The surviving ookinetes become sessile and transform into oocysts. The oocyst is th e only parasite developmental stage that grows extracellularly and re sults in the formation of sporozoites. Sporozoites are released into th e mosquito body cavity and invade the salivary glands, and they suffe r severe losses on this journey . The ookinete and the sporozoite are t hus bottleneck stages in the malaria parasite life cycle. Sporozoites ar e transmitted during the next mosquito blood meal and initiate liver i nfection in the mammalian host. Liver infection does not result in ove rt pathology but leads to a 10,000- fold amplification of parasite num bers, culminating in the release of exoerythrocytic merozoites into th e bloodstream, which in turn infect erythrocytes to initiate the patho genic erythrocytic cycle. (Ahmed *et.al*., 2009**)**

2.17 Symptoms of malaria

Plasmodium falciparum is the most prevalent malaria parasites on th e African continent, responsible for most malaria deaths globally. It c ontributes to most childhood parasitic infection and presents with sig ns and symptoms such as recurrent fever, fatigue, and body joint pain s. Other symptoms may include headache, nausea, chills, sweating, pa llor and body weakness. Children with severe malaria frequently dev elop one or more of the following symptoms: severe anemia, respirat ory distress, cerebral malaria or thrombocytopenia. (Renate *et.al*.,20 20**)**

2.18 Diagnosis of malaria

Malaria parasite affects multiple human organs such as brain spleen, l iver gastrointestinal tract (GIT), Gall bladder and blood vessels. Ther efore the clinical picture may be of wide range that is from simple ma laise to life threatening central nervous expression like coma. Blood a bnormalities have been observed in patients with malaria; Anemia an d thrombocytopenia is the most common (Wickramasinghe, 2000; Kh an, 2008).The frequency and severity of thrombocytopenia appears t o vary with the severity of infection and the type of malarial parasites (Leowattana *et.al*., 2010; Kochar *et*. *al*., 2010)

Also we diagnosis malaria by using :

Thick and thin blood smear, Rabid diagnostic test (antigen testing), Molecular test (polymerase chin reactions PCR) and Antibody test (s erology). WHO 2006

2.19 Haematological Parameters as an Indicator of Malarial Infe ction

Malaria may be associated with complications which may be avoided by early diagnosis and treatment. Microscopic diagnosis showing pre sence of malarial parasites is needed for confirmation which at times may be unreliable and requires technical expertise. The present stud y was conducted to statistically analyze the haematological paramete rs including platelet indices which can give initial hint for malarial inf ection and therefore prompt the laboratory physician for active searc h of the parasite microscopically. Routine haematological parameters along with platelet indices (MPV and PDW) which are easily availabl e on automated cell counter were statistically analyzed to assess thei

r role as indicators for malaria. Leukocyte count and platelet count w ere significantly lower in cases of acute malaria . (Chandra, 2013**)**

Changes in blood cell counts are a well-known feature of malarial infe ctions. These changes involve major cell lines including red blood cell s (RBC), leukocytes and thrombocytes. Hematological changes in the course of a malaria infection, such as anemia, thrombocytopenia and l eukocytosis or leucopoenia are well recognized. These alterations var y with the level of malarial endemicity, background hemoglobinopath y, nutritional status, demographic factors, and also malaria immunity . Hyperparasitemia has been listed as one of the criterion of severe fal ciparum malaria by the World Health Organization (WHO) for more t han two decades . Previous studies have shown that there is a correla tion between parasite density and severity of malarial infections . Mo rtality is also correlated with the degree of parasitemia. Patients with the highest parasite densities also have the highest fatality rates . Ad ditionally, high parasitemia due to Plasmodium falciparum infection t akes a serious turn in anemia . Thrombocytopenia was also seen in th e majority of patients with malaria. It was also observed that at high parasitemias, the platelets were found to be significantly lower. It has been noted by previous studies that increasing levels of P. falciparu m parasite loads results in a decreased platelet count . The hematolog ical parameters (RBC, leukocyte, platelets, hemoglobin level (Hb), me an corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell dis tribution width (RDW) of patients infected with malaria were investi gated. (Manas *et.al*., 2015)

Thrombocytopenia is an early and consistent feature of malaria, but i ts pathogenesis remains incompletely understood. In falciparum mal aria there is increased platelet consumption as evidenced by shorten ed survival of radiolabelled platelets and the finding of plentiful meg akaryocytes in patients' bone marrow and appropriately elevated pl asma thrombopoietin levels . Both systemic microvascular sequestrat ion and endothelial activation may play a pathophysiological role, a hypothesis supported by the observation that the radiolabelled platel ets of patients with falciparum malaria are diffusely sequestered, rath er than pooling in the liver or spleen.Yet while thrombocytopenia is a ubiquitous laboratory finding, it had been thought to have limited cli nical significance, as major bleeding is relatively uncommon in the di sease. (Josh Hanson *et.al* 2015)

2.20 Effect of malaria on immune system

Anty malarial effector mechanisms as many of the surface antigens of malaria parasites and the parasite proteins inserted into the plasma membrane of the infected red blood cell are polymorphic or exhibit cl onal antigenic variation, It has been proposed that one may need to d evelop a diverse repertoire of antibodies capable of blocking parasite invasion and tissue adhesion in order to attain effective antiparasite i mmunity . Infection with a parasite variant that is not recognized by t he existing antibody repertoire may lead to uncontrolled parasite rep lication and therefore pathology. The gradual acquisition of clinical i mmunity (following repeated infection) parallels the development of a diverse antibody repertoire; these two observations may be causall y linked.Malaria-specific antibodies mediate a number of antiparasiti c effector functions including inhibition of cytoadherence , inhibition

of erythrocyte invasion and antibody dependent cytotoxicity and cell ular inhibition . Cell-mediated immune effector mechanisms include macrophage activation by NK cell-,T cell- or Th1-derived interferon g (IFN-g) for enhanced phagocytosis and killing of parasitized erythroc ytes , and inhibition of parasite growth and development inside hepat ocytes by CD8+ cytotoxic and IFN-g-producing T cells . (Artavan tsak onas et.al,2003)

Effect of malaria on platelets

Platelets play a critical role in the pathogenesis of malarial infections by encouraging the sequestration of infected red blood cells within th e cerebral vasculature. But platelets also have well-established roles i n innate protection against microbial infections. We found that purifi ed human platelets killed *Plasmodium falciparum* parasites cultured i n red blood cells. Inhibition of platelet function by aspirin and other p latelet inhibitors abrogated the lethal effect human platelets exert on *P. falciparum* parasites. Adaptive immunity to malarial infection accu mulates slowly over the lifetime of an individual living in an endemic region. Cross- immunity between isolates is low, and every new infec tion requires the development of a virtually novel immune response. Therefore, innate mechanisms that limit parasite growth within the r ed blood cell (RBC) are extremely important in determining survival, especially during the first few years of life, because clinical severity c orrelates closely with parasite mass. Several known mutations affect the RBC and decrease the severity of infection through either impedi ment of parasite entry or development within the cell . Thrombocyto penia is a common clinical accompaniment of *Plasmodium falciparum* ,*Plasmodium vivax*, malarial infections. Low platelet concentrations c

orrelate with increased parasite density and poor outcome . Platelets bind preferentially to infected RBCs and have been postulated to play a role in the pathogenesis of malarial infection,either positively or n egatively . Given the role platelets play in other infectious diseases . (McMorran *et.al* ,2008)

2.22 The protective role of platelet in malaria infection

The protective role played by platelets in human malarial infections h as yet to be directly answered, but there are a number of lines of evid ence that support the affirmative. Depletion of platelets (or inhibition of platelet function) in murine models of malaria where outcome is d etermined by bloodstream parasitemia (as opposed to the inflammat ory response-based cerebral malaria syndrome) results in reduced s urvival. Although a similar platelet depletion study is diffcult to perfo rm in humans, we do know that thrombocytopenia, which is a commo n clinical accompaniment of all malarial infections, has been correlate d with a poor outcome in falciparum malaria.A loss in the protective f unction afforded by platelets could explain this. Platelets have also be en implicated as susceptibility factors in the development of cerebral malaria (CM), a complex collection of syndromes specifc to *P. falcipar um* infections and a major cause of death. Central to the pathophysiol ogy of CM is the accumulation or sequestration of IE in the cerebral m icrovasculature, causing the obstruction of blood flow, leukocyte accu mulation, localized intravascular inflammation, activation and damag e of the endothelium and disruption of the bloodbrain barrier. Platele ts are often found at sites of IE sequestration in both human CM and mouse CM models where they are believed to mediate IE binding in t he microvasculature, and release molecules that affect endothelial cel

l viability and promote leukocyte adhesion. The parasite-killing activi ty of platelets may also contribute to the pathophysiology of CM. Plat elet-directed killing may moderate the local inflammatory responses to live parasites, or dead parasite toxins and exudates could contribut e to the inflammatory and cell-damaging milieu. we previously report ed that platelets protect during malaria infection by binding Plasmod ium infected erythrocytes (Ie) and killing the parasite within. More re cent studies have now revealed the platelet plasmocidal factor, platel et factor 4 (PF4) and the red cell-expressed Duffyantigen molecule as the central players in the parasite killingearly in an infection in a non antigen-specifc manner. It also allows time for the subsequent develo pment of an adaptive response, which is capable of clearing the infect ion and protecting against clinically symptomatic malaria. The latter response is antibody-mediated and provides memory-based protecti on. It is also antigen-specifc, and several years and many exposures a re needed to build an effective immunity against the multitudinous ar ray of parasite antigens in any given endemic region. Innate immune mechanisms are therefore crucial in all malarial infections to buffer a gainst the early growth of blood-stage parasites. We recently reporte d that platelets are an important component of the host innate immu ne response against malaria infection. activity of platelets . (McMorra n *et.al*,2013)

There are recent reports indicating that platelets may also have a pro tective role in uncomplicated malaria by killing Plasmodium in iRBCs . (Aggrey*et.al*., 2013)

CHAPTER III

Materials and methods

3.1 Study design

This study is case control study, conducted between February and A pril 2020 to evaluate platelets count and platelet indices of malaria p atients in Khartoum state .

3.2 Study population and sample size

Hundred patients with malaria and hundred healthy volunteer as a c ontrol group were enrolled in this study.

3.3 Inclusion criteria

Patient with malaria disease from saad Rashwan hospital in February to April 2020 .

3.4 Exclusion criteria

Malaria patient under treatment.

Malaria patient with other disease .

3.5 Ethical consideration

Ethical clearance was obtained for this study from saad rashwan hos pital , and the sample was collected after consent of participant were informed about the proseder of blood collection and the aim and ben efits of this study and take a permeation by their signature .

3.6 Tools of data collection

Data were collected using a personal interview questionnaire to the p atient including age ,gender , history of disease .

3.7 Sampling

Two hundred sample was collected from patient and healthy volunte er , 2.5 ml of venous blood was collected from each one using disposa ble syringes and sprit (70% alcohol) is used to sterilizing the area of collection , the blood is drawn in EDTA containers , measurement of platelet count and indices was determined within tow hours after coll ection of blood sample using BC -3000 plus auto hematology analyze r .

3.8 Determination of platelet count and indices by auto hematol ogy analyzer

BC -3000 plus auto hematology analyzer is a three part auto hematol ogy analyzer able to run 19 parameters per sample including : hemo globin level , packed cell volume , red cell concentration , mean corpu scular hemoglobin , mean cell volume , , mean corpuscular hemoglob in concentration ,white blood cell .Platelet count , mean platelet volu me , plateletcrit , platelet distribution width .

3.9 Platelet count measurement principle

Platelet is counted and sized by impedance method , this method is b ased on the measurement of change in electrical resistance produced by a particle , which in this case is blood cell , suspended in a conduc tive diluent as it passes through an aperture of known dimensions . a n electrode is submerged in the liquid on both side of aperture to cre

ate an electrical pathway as each particle passes though the aperture , a transitory change in the resistance between the electrode is produ ced . this change produce a measurable electrical pulse. the number o f pulses generated indicates the number of particles that passes throu gh the aperture . the amplitude of each pulse is proportional to the v olume of each particle . each pulse is amplified and compared to the e ternal reference voltage channels , which only accept the pulses of a c retin amplified . if the pulse generated is above the platelet lower thr eshold , it is counted as an platelet .

3.10 Data analysis

Data obtained was analyzed by statistical package of social since (SP SS version - 11) . soft were program to compare means and p. values at 0.05 by independent sample test .

CHAPTER IV

Results

The study was conducted to study platelet count and platelet indices i n malaria patient in Khartoum state . The study included two hundre d subject, 100 were diagnosed with malaria 57 (58.76%)of them wer e male and 43 (41.75) of them were female ; 100 healthy volunteers were selected as control 40 (41.24%) of them are male and 60(58.25) of them are female (Table4.1**)**

Table 4.1 Frequency of gender among malaria patient

Gender	Frequency	Percent
Male	57	59%
Female	43	41%
Total	100	100%

Mean platelet count of patients significantly decreased compared to controls (P.value 0.00), mean platelet volume (MPV) in patients were showed significant increased in patients compared to control group (P.value 0.00) ,platelet distribution width (PDW) was showed significant decreased in patients compared to control group (P.value 0.00) platelet crit (PCT) showed significant decreased in patients compared to control group (P.value 0.150) .(Table4.2 **)**

Table 4 .2 The blood platelets count , MPV ,PDW , PCT of study Population

Parameter	Groups	Means / SD	P . value
Platelets count (*10^9 cell/L)	Patients	233 /123	0.00
	Control	290 / 96	0.00
MPV (fl)	Patients	9.3 /1.4	0.00
	Control	8.4 /1.1	0.00
PDW (fl)	Patients	11.3 / 2.02	0.00
	Control	14.03 / 2.02	0.00
PCT %	Patients	0.21 /0.13	0.150
	Control	0.24/0.07	0.150

Frequency of thrombocytopenia among malaria patients (24%) of pa tients have thrombocytopenia(63%)were normal platelet count and (13%) with high platelet count .(Table 4.3)

Table 4.3 Frequency of thrombocytopenia among malaria patien ts

Platelet count	Frequency	Percent
Normal	63	63%
Low	24	24%
High	13	13%
Total	100	100%

Means and SD of age in patients decreased significantly compared to control (P.value 0.017),and the mean of age increased in type of plas modium falsipram compared to plasmodium vivax (Pvalue 0.026)and showed the mean and SD of age in male and female (P.value 0.335) . (Table 4.4)

Table 4.4 Means and SD of age in study population , type of plas modium and gender

Parameter		Means / SD	P . value
Age	Case	24.61 / 18 .128	0.017
	Control	31.06 / 19 .754	
Type of plasmodium	P.f	25.40 / 18.898	0.026
	P.V	18 .18/ 7 .705	
Gender	Males	29.19 /18.354	0.335
	Females	26.56 /19.1942	

Age of 40% of subject range (0-20) years and 37% (21-40) years and 15% (41-60) and 7% (61-80) and 1% (81-100) .**(**Table 4.5)

Table 4.5 distribution of age among study population

Age	**Frequency**	**Percent %**
0 – 20	80	40%
21 – 40	74	37 %
41 – 60	30	15 %
61 – 80	14	7 %
81 – 100	2	1 %
Total	200	100%

Man platelet count and SD of gender in study population (P.value 0. 788), mean platelet volume (MPV) in patients were showed (P.value 0.276) ,platelet distribution width (PDW) was showed (P.value 0.914) platelet crit (PCT) showed significant decreased in patients compar ed to control group (P.value 0.897) .Shows (Table 4.7)

Table 4.7The blood platelets count , MPV ,PDW , PCT of gender

Parameter	Gender	Means / SD	P . value
Platelets count (*10^9 cell/L)	Males	264.09 /114.634	0.788
	Females	259.75 /113.498	0.788
MPV (fl)	Males	9.043 /1.6159	0.276
	Females	8.833 /1.0668	0.276
PDW (fl)	Males	12.682 /2.1192	0.914
	Females	12.719 / 2.6876	0.914
PCT %	Males	0.2323 /0.12580	0.897
	Females	0.2302 /0.0993	0.897

CHAPTER V

Discussion

5.1 Discussions

In the present study ; the analysis of platelet count and indices in pati ent with malaria revealed a high frequency of thrombocytopenia . an d change in MPV and PDW but no change in PCT. All subject in this st udy were free from other disease can effect on platelet count and pla telet indices and did not take any medication .

In the result of current study mean of platelet count in patient is decr eased compared to mean of platelet count in control group which co nsistent with previous study that mentioned platelets count is decrea sed in malaria patient.

In this study mean of MPV is increased in patient group compare to t he control group that means the malaria is affect on this parameters .

PDW in this study showed decreased in mean of patient compared to the control group that means the malaria is affect on this parameter.

PCT in this study showed no significant difference in mean of patient compared to control group so it is clearly not effected by malaria .

In the result of this study showed that the age was not affected on pl atelet count ,MPV,PDW and PCT.

In the result of this study showed that the age was not affected with both male and female of the patient .

In the result of this study showed that the age in the type of malaria was significant affected that p.f are more than p.v.

in this study showed decreased in mean of patient age compared to t he control group that means the age is affect on this parameter.

In the result of this study showed that both male and female was not affected on platelet count ,MPV,PDW and PCT

In this study thrombocytopenia occurred in(24%) of malaria case thi s is lower comparison to study done in Hyderabad which had high pe rcent (69.18%) of thrombocytopenia ,while this result is agree with o ther study done in western Kenya which report thrombocytopenia i n (49%) of patient and significant inecreased in MPV and decreased o n PDW and no change in PCT.

5.2 Conclusions

The study concluded that malaria altered the blood count cousing thr ombocytopenia and increased MPW and decreased in PDW but no sig nificance deference in PCT platelet crit .and showed that the type of malaria plasmodium falciparum , plasmodium vivax , age and gender were not affected on platelet count and indices .

In this study showed that the age affected in the type of malaria that plasmodium falciparum are more than plasmodium vivax .

5.3 Recommendation

1. Routine investigation of platelet count and platelet indices with inv estigation of malaria to confirmation.

2. Further studies recommended with using a large sample size and d uration in different the 4 species .

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Appendix (A)

Sudan university of since and technology
Master research
Department hematology
Study of platelet count and indices in malaria patient on Khartoum stat
NO( )

Age .....
Gender : male ( ) female ( )
History of disease;
Bleeding disease ( )
Renal disease ( )
Liver disease ( )
Cancers :.....
....
Others :
.....
Investigation:
Platelet count ( )
MPV ( )
PDW ( )
PCT