بسم الله الرحمن الرحيم

**Republic of Sudan**

**Ministry of Higher Education and scientific Research**

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***Faculty of Graduate Studies and Scientific Research***

# Assessment of Platelet and D.dimer in Adult Malarial Patients in Shendi Locality River Nile State, Sudan

A thesis Submitted for partial fulfillment of the Msc .Degree in Haematology

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# الآية

**قال الله تعالى :**

بسم الله الرحمن الرحيم

﴿ اللَّهُ لا إِلَهَ إِلَا هُوَ الْحَيُّ الْقَيُّومُ لَا تَأْخُذُهُ سِنَةٌ وَلَا نَوْمٌ لَهُ مَا فِي السَمَاوَاتِ وَمَا فِي الأَرْضِ مَنْ ذَا الَذِي يَشْفَعُ عِنْدَهُ إِلَا بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلَا يُحِيطُونَ بِشَيْءٍ مِنْ عِلْمِهِ إِلَا بِمَا شَاءَ وَسِعَ كُرْسِيُّهُ السَمَاوَاتِ وَالْأَرْضَ وَلَا يَؤُدُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ ﴾

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# الإهـــداء

**إلهي لا يطيب الليل إلا بشكرك ولا يطيب النهار إلى بطاعتك .. ولا تطيب اللحظات إلا بذكرك .. ولا تطيب الآخرة إلا بعفوك .. ولا تطيب الجنة إلا برؤيتك**

**الله جل جلاله**

**.. إلى من بلغ الرسالة وأدى الأمانة .. ونصح الأمة .. إلى نبي الرحمة ونور العالمين**

**سيدنا محمد صل الله عليه وسلم**

**إلى من أحمل .. إلى من كلله الله بالهيبة والوقار .. إلى من علمني العطاء بدون انتظار أسمه بكل افتخار .. أرجو من الله أن يمد في عمرك لترى ثماراً قد حان قطافها بعد طول انتظار وستبقى كلماتك نجوم أهتدي بها اليوم وفي الغد وإلى الأبد**

**أبي الغالي : ابراهيم عبد القادر ابراهيم**

**إلى من كان قلبها مدرسة لي .. إلى من تسربت دواخلها دون استئذان**

**إلى من استقبلتني بابتسامة حين أتيتها باكياً.. إلى من أثرت على نفسها من أجلنا**

**إلى من علمتنا العطف والحنان . الصبر – التسامح بل كل الأشياء الجميلة**

**إلى من لا أستطيع وصفها من تمنيت تقبيل تراب قدميها عند نجاحي**

**فأنا أعترف أنني مدينة بكل ما وصلت إليه وما أرجو أن أصل إليه من الرفعة إلى أمي الملاك عفواً أمي لا أستطيع مكافئتك أسأل الله أن يكافئك بالجنة مع محمد صل الله عليه وسلم فأنتِ لا أقول أعظم امرأة بل أقول أعظم شيء في تاريخ حياتي**

**إلى أمي الغالية وصال محمد حامد**

**إلى من قاسموني رحم أمي وشاركوني حنان أبي**

**إلى من صنعوا وكللوا لي نجاحي**

أخواتي وأخواني الأعزاء

أهديكم بحثي عبقا تفوح منه رائحة اجتهادكم معي يطيبه وجودكم بين جنبي وتجمله فرحتكم وسعادتكم التي هي مبتغاى وسر سعادتي.

سأشكركم قدر استطاعتي ولكن سامحوني لأنني لأستطيع أن أوفيكم وأجزيكم بقدركم السامي فبارك الله فيكم ولا حرمني دعواتكم

**إلى أخواني الذين لم تلدهم أمي.....إلى من تحلو بالإخاء وتميزوا بالوفاء .... إلى ينابيع الصدق الصافي ، إلى من سعدت وبرفقتهم في دروب الحياة الحلوة والحزينة سرت...إلى من كانوا معي على طريق النجاح والخير ....إلى من عرفت كيف أجدهم وعلموني أن لا أضيعهم....**

زميلاتي وصديقاتي العزيزات

# الشكر والعرفان

سيبقي في حنايا النفس لأهل الفضل عرفاناً جميلا  فاكتب أحرفاً بمداد النور وابقي

انشد الشكر الجزيل...

الشكر إلي الله أولاً ...
هناك أناس يقف اللسان عاجزاً عن شكرهم فلا عجب من ذلك فهم أهل الشكر والثناء ...
هكذا كنت شموعا أوقدت المعرفة والعلم في دربنا
أساتذتي  الأجلاء  ...
ومشرف البحث  الذي لم يبخل بجهده ووقته حتى خرج بحثي بهذا الشكل...

**الدكتور :حمزة احمد حسن**

## Abstract

**Background** : Malaria is a major health problem in the tropics with high morbidity and mortality rate .

**Methods**: This is a descriptive cross-sectional study conducted at Shendi Teaching Hospital in Shendi town to analyse the effect of malaria on platelet and D.dimer level in the period between (march 2018- July 2018). The study included (30) patients who were diagnosed as malaria cases and the study groups were compared with (20) healthy volunteers as a control group. The diagnosis of malaria carried out by thick blood film . platelet was performed by using an automated counter and D.dimer was performed by using sandwich immunodetection method,by ichroma™ Reader .

**Results**: The study revealed that the malaria patients were; (33%) male and (67%) female, the mean of age was (35 year) distributed as (60%) have (20-35) years old, and 40% have 36-50 year .The mean values of plt and D.dimer in case group was 200.03 $10^9$ /l and 1188 ng/dl respectivly .the mean value of plt and D .dimer in control group was 261.65 x $10^9$ /l and 172.10.ng/dl respectivly .The mean value of plt and D.dimer in cases with moderate degree of clinical manifestation compare with control group was 264.23x $10^9$ /l) and 479.46 ng/ dl respectively

The mean value of plt and D . dimer in cases with severe degree of clinical manifestation compared with control group was 150.94 x$10^9$ /l) and 1751.18 ng / dl respectivly

The mean value of plt and D .dimer incases with one time evidence of malaria per year compared with control group was 247.87 x$10^9$ /l and 480.20 ng/gl respectively .The mean value of plt and D . dimer in cases with more than one time evidence of malaria per year and control group was 150.40 x$10^9$ /l and 1920.53 ng / dl respectively .

# المستخلص

**مدخل :** .مرض الملا ريا هو المشكلة الاساسية في المناطق الموبؤة ويصاحبه مستوى عالي من الوفيات والامراضية0

**منهجية الدراسة** : أجريت هذه الدراسة المقطعية في مستشفي شندي التعليمي بمدينة شندي لتحديد مدى تاثير مرض الملاريا علي الصفائح الدموية وجزيئات الفبرين المتكسرة في الفترة ما بين (مارس2018 – –يوليو2018م). وكانت عينة الدراسة عبارة عن 30 مريض تم اختيارهم بصورة عشوائية. وقورنت نتائج الدراسة مع 20 متطوع سليم كمجموعة ضابطة.

**النتائج:** أظهرت الدراسة أن المرضي 33% منهم ذكور و 67% منهم إناث وكان متوسط أعمارهم 35 عاما، 60 % منهم أعمارهم من 20 – 35 سنة و40 **%** منهم أعمارهم من 36 – 50 سنة0

كما أظهرت الدراسة أن متوسط تعداد الصفائح الدموية وجزيئات الفبرين 200×$10^9$/ ل و 1188 نانوجم/ دسل على التوالي في مجموعة الدراسة 0

وجدت الدراسة ان متوسط تعداد الصفائح الدموية وجزئيات الفبرين المتكسرة في المجموعة القابضة كانت 261×$10^9$/ل و 172 نانوجم/ دسل على التوالي 0

وجدت الدراسة ان متوسط تعداد الصفائح الدموية وجزئيات الفبرين المتكسرة في الاشخاص الذين لديهم أعراض خفيفة للملاريا كانت 2640×$10^9$/ل و 479 نانوجم / دسل على التوالي 0

كما وجدت الدراسة ان متوسط تعداد الصفائح الدموية وجزئيات الفبرين المتكسرة في الاشخاص الذين لديهم أعراض شديدة للملاريا كانت 150×$10^9$/ل و 1751 نانوجم /دسل على التوالي 0.

وجدت الدراسة ان متوسط تعداد الصفائح الدموية وجزئيات الفبرين المتكسرة في الاشخاص الذين يتعرضون للملاريا مرة واحدة في السنة كانت 247×$10^9$/ل و 480 نانوجم / دسل على التوالي.

ووجدت الدراسة ايضا ان متوسط تعداد الصفائح الدموية وجزئيات الفبرين المتكسرة في الأشخاص الذين يتعرضون للملاريا أكثر من مرة في السنة كانت150×$10^9$/ل و 1920 نانوجم / دسل على التوالي 0

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## List of abbreviations

| | |
|---|---|
| BSA | Bovine serum albumin |
| CM | Cerebral malaria |
| DC | Direct current |
| EDTA | Ethyl di Amin tetra acidic acid |
| FDP | Fibrin degradation product |
| FEU | Fibrinogen equivalent units |
| FP | Fibrino peptide |
| G | Glycoprotein |
| HLA | Human leucocyte antigens |
| HPA | Human platelet antigens |
| P | Plasmodium |
| PBS | Phosphate buffer saline |
| PRBCS | Plasmodium falciparum parasitized red blood cells |
| PT | Pro thrombin time |
| RF | Radio frequencies |
| SLS | Sodium lauryl sulfate |
| SP | Species |
| SPSS | Statistical package of social science |
| VTED | Venous thrombo embolic disease |
| VWF | Von wile brand factor |

# Chapter One

## Introduction

## Rationale

## Objectives

# 1. Introduction, Rationale and objectives

## 1.1. Introduction

Malaria is caused by the protozoan parasite Plasmodium. Human malaria is caused by four different species of Plasmodium: P. falciparum, P. malariae, P. ovale and P. vivax. Malaria is an acute febrile illness with an incubation period of 7 days or longer. Thus, a febrile illness developing less than 1 week after the first possible exposure is not malaria.Malaria remains a major cause of morbidity and mortality in more than 90 countries and accounts for at least 1 million deaths every year and 450 million disease episodes annually [1–5]. Malaria is caused by Plasmodium sp. transmitted to the host by bite of the blood-feeding Anopheles sp. mosquitoes, which inject sporozoite-infected saliva [6–10].

The most severe form is caused by P. falciparum; variable clinical features include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhoea and abdominal pain. Other symptoms related to organ failure may supervene, such as acute renal failure, pulmonary oedema, generalized convulsions, circulatory collapse, followed by coma and death.The initial symptoms, which may be mild, may not be easy to recognize as being due to malaria.The World Health Organization estimates that in 2016 malaria caused 216 million clinical episodes, and 445,000 deaths .In Sudan it is public health problem, it lead to an estimated 7,5-10 million cases and 35,000 death every year .[7] Thrombocytopenia in vivax malaria appears to be immune mediated [23] and occurs in the absence of blood coagulation activation [24]. In contrast, thrombocytopenia in falciparum malaria is most often accompanied by activation of the coagulation cascade [25] among other mechanisms (*e.g.* immune-mediated lyses, peripheral destruction) [26].

Cases of malaria are known to be associated with variable degrees of coagulopathy as evident from abnormalities of screening coagulation assays in a large number of patients during the course of the illness [1]. A direct interaction

between the parasites and the endothelium of the microcirculation causes endothelial cell injury and sets up a series of reactions characterized by release of a large variety of cytokines and inflammatory mediators by the endothelium, the leucocytes and the other cells in the body. These in turn activate the coagulation pathway leading to widespread thrombin deposition in small arteries and arterioles (disseminated intravascular coagulation) and fibrinolysis [2]. This explains the abnormalities of a number ofcoagulation parameters, including the markers of thrombosis and fibrinolysis that have been reported in malaria [1–3]. Such the platelet count , D-Dimer and fibrin degradation products.

## 1.2. Rationale

Malaria is a mosquito-born infectious disease affecting humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the *Plasmodium* type.[2] Malaria causes symptoms that typically include fever and tiredness.

Malaria has several serious complications  severe headache, low blood sugar, and hemoglobin in the urine with renal failure may occur.[2] Complications may include spontaneous bleeding, coagulopathy and shock [7] There was not studies in the effect of malaria on D. dimer  in Shendi so we will achieve  to do this study .

## 1.3. Objectives

### 1.3.1. General objective

-To determine the platelet count and D.dimer in malaria patients.

### 1.3.2. Specific objectives

1-To evaluate concentration of D .dimer in malaria patients.

2-To estimate the platelet count in malaria patients.

3-To determine the effect of recurrent malaria infections on platelet count and D.dimer.

# Chapter Two

## Literature review

# 2. Literature Review

## 2.1. Malaria

Malaria is a mosquito-born infectious disease affecting humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the *Plasmodium* type.[2] Malaria causes symptoms that typically include fever, tiredness, vomiting, and headaches.[1] In severe cases it can cause yellow skin, seizures, coma, or death.[1] Symptoms usually begin ten to fifteen days after being bitten.[2] If not properly treated, people may have recurrences of the disease months later.[2] In those who have recently survived an infection, reinfection usually causes milder symptoms.[1] This partial resistance disappears over months to years if the person has no continuing exposure to malaria.[1]

The disease is most commonly transmitted by an infected female *Anopheles* mosquito.[2] The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood.[2] The parasites travel to the liver where they mature and reproduce.[1] Five species of *Plasmodium* can infect and be spread by humans.[1] Most deaths are caused by *P. falciparum* because *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria [1-2] The species *P. knowlesi* rarely causes disease in humans.[2] Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests.[1] Methods that use the polymerase chain reaction to detect the parasite's DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity.[5]

Malaria is a serious and sometimes fatal disease caused by a parasite that commonly infects a certain type of mosquito which feeds on humans. People who get malaria are typically very sick with high fevers, shaking chills, and flu-like illness. Although malaria can be a deadly disease, illness and death from malaria can usually be prevented. About 1,700 cases of malaria are diagnosed in the United States each year. The vast majority of cases in the United States are

in travelers and immigrants returning from countries where malaria transmission occurs, many from sub-Saharan Africa and South Asia.The most severe form is caused by P. falciparum; variable clinical features include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhoea and abdominal pain. Other symptoms related to organ failure may supervene, such as acute renal failure, pulmonary oedema, generalized convulsions, circulatory collapse, followed by coma and death.The initial symptoms, which may be mild, may not be easy to recognize as being due to malaria.It is important that the possibility of falciparum malaria is considered in all cases of unexplained fever starting at any time between 7 days after the first possible exposure to malaria and 3 months (or, rarely, later) after the last possible exposure. Any individual who experiences a fever in this interval should immediately seek diagnosis and effective treatment and inform medical personnel of the possible exposure to malaria infection. Falciparum malaria may be fatal if treatment is delayed beyond 24 h after the onset of clinical symptoms

The forms of human malaria caused by other Plasmodium species cause significant morbidity but are rarely life-threatening. Cases of severe P. vivax malaria have recently been reported among populations living in (sub)tropical countries or areas at risk. P. vivax and P. ovale can remain dormant in the liver. Relapses caused by these persistent liver forms ("hypnozoites") may appear months and rarely several years, after exposure. Relapses are not prevented by current chemoprophylactic regimens with the exception of primaquine. Latent blood infection with P. malariae may be present for many years, but it is very rarely life-threatening.

In recent years, sporadic cases of travellers' malaria due to P. knowlesi have been reported. Humans can be infected with this "monkey malaria" parasite while staying in rainforests and/or their fringe areas in south-east Asia, within the range of the natural monkey hosts and mosquito vector of this infection. These areas include parts of Cambodia, China, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand and Viet Nam. The parasite has

a life-cycle of 24 h and can give rise to daily fever spikes occurring 9–12 days after infection. Symptoms may be atypical. Severe P. knowlesi malaria with organ failure may occur, and sporadic fatal outcomes have been described. P. knowlesi has no persistent liver forms and relapses do not occur. Travellers to forested areas of south-east Asia where human P. knowlesi infections have been reported should protect themselves against mosquito bites between dusk and dawn to prevent infection and take the usual chemoprophylaxis where indicated

### 2.1.1. Life cycle

A mosquito causes an infection by a bite. First, sporozoites enter the bloodstream, and migrate to the liver. They infect liver cells, where they multiply into merozoites, rupture the liver cells, and return to the bloodstream. The merozoites infect red blood cells, where they develop into ring forms, trophozoites and schizonts that in turn produce further merozoites. Sexualforms are also produced, which, if taken up by a mosquito, will infect the insect and continue the life cycle.

In the life cycle of *Plasmodium*, a female *Anopheles* mosquito (the definitive host) transmits a motile infective form (called the sporozoite) to a vertebrate host such as a human (the secondary host), thus acting as a transmission vector. A sporozoite travels through the blood vessels to liver cells (hepatocytes), where it reproduces asexually (tissue schizogony), producing thousands of merozoites. These infect new red blood cells and initiate a series of asexual multiplication cycles (blood schizogony) that produce 8 to 24 new infective merozoites, at which point the cells burst and the infective cycle begins anew.[30]

Other merozoites develop into immature gametocytes, which are the precursors of male and female gametes. When a fertilized mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito gut. The male and female gametocytes fuse and form an a fertilized, motile zygote. Ookinetes develop into new sporozoites that migrate to the insect's salivary glands, ready to infect a new vertebrate host. The sporozoites are injected into the skin, in the saliva, when the mosquito takes a subsequent blood meal.[12]

### 2.1.2.pathohysiology

Malaria infection develops via two phases: one that involves the liver (exoerythrocytic phase), and one that involves red blood cells, or erythrocytes (erythrocytic phase). When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver where they infect hepatocytes, multiplying asexually and asymptomatically for a period of 8–30 days.[11]

After a potential dormant period in the liver, these organisms differentiate to yield thousands of merozoites, which, following rupture of their host cells, escape into the blood and infect red blood cells to begin the erythrocytic stage of the life cycle.[11] The parasite escapes from the liver undetected by wrapping itself in the cell membrane of the infected host liver cell.[12]

Within the red blood cells, the parasites multiply further, again asexually, periodically breaking out of their host cells to invade fresh red blood cells. Several such amplification cycles occur. Thus, classical descriptions of waves of fever arise from simultaneous waves of merozoites escaping and infecting red blood cells.[11]

### 2.1.3. Complications

Malaria has several serious complications. Among these is the development of respiratory distress, which occurs in up to 25% of adults and 40% of children with severe *P. falciparum* malaria. Possible causes include respiratory compensation of metabolic acidosis, noncardiogenic pulmonary oedema, concomitant pneumonia, and severe anaemia. Although rare in young children with severe malaria, acute respiratory distress syndrome occurs in 5–25% of adults and up to 29% of pregnant women.[10] Coinfection of HIV with malaria increases mortality.[6] Renal failure is a feature of blackwater fever, where hemoglobin from lysed red blood cells leaks into the urine.[2]Infection with *P. falciparum* may result in cerebral malaria, a form of severe malaria that involves encephalopathy. It is associated with retinal whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever.[6]

Enlarged spleen, enlarged liver or both of these, severe headache, low blood sugar and hemoglobin in the urine with renalfailure may occur.[2] Complications may include spontaneous bleeding, coagulopathy and shock [7]

**2.2. Platelet**

Platelets are tiny blood cells that help your body form clots to stop bleeding. If one of your blood vessels gets damaged, it sends out signals that are picked up by platelets. The platelets then rush to the site of damage and form a plug, or clot, to repair the damage.[13]

**2.2.1 Thrompopoiesis**

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte-the megakaryoblast-arises by process of differentiation from the haemopoietic stem cell [14]. The megakaryocyte matures by endomitotic synchronous replication (i.e. DNAreplication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. Apichlre of mature polyploid megakaryocytes is shown in. Very early oninvaginations of plasma membrane are seen, called the demarcation, which evolves through the development of the megakaryocyte into a highly branched network At a variable stage in development, most commonly at the eight nucleus stage, the cytoplasm becomes granular. Mahlre megakaryocytes are eXh'emely large, with an eccentric placed 2single lobu lated nucleus and a low nuclear to cytoplasmic ratio [13]. Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to 1000-5000 platelets. The time interval from differentiation of the human stem cell to the production. thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys. Tlu'ombopoietin increases the via c- Mpl receptor [15]. Platelet levels start to rise 6 days start of therapy and remain high for 7-10 days. Unfortlmately, tlu'ombopoietin is not available for routine clinical practice. Platelets also have c-Mpl receptors for thrombopoietin and remove it from the

circulation. Therefore, levels are high in thrombocytopenia as a result of marrow aplasia and vice versa. The normal platelet count is approximately 250 x 109/L (range 150-400 x 109/L) and the normal platelet lifespan is 7-10 days. Up to one-third of the marrow output of platelets may be trapped at any one time in the normal spleen but this rises to 90% in cases of massive splenomegaly. [13-15].

**2.2.2. Platelet structure:**

Platelets are extremely small and discoid, 3.0 x0.5 11m in diameter, with a mean volume 7-11 fL. The ultrash'uchlre of platelets is represented in platelet production giving rise to 1000-5000 platelets. The time interval from differentiation of the human stem cell to the production of platelets averages approximately 10 days. Tlu'ombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys. Tlu'ombopoietin increases the number and rate of maturation of megakaryocytes via c-Mpl receptor. Platelet levels start to rise 6 days after the start of therapy and remain high for 7-10 days. Unfortlmately, thrombopoietin is not available for routine clinical practice. Platelets also have c-MP receptors for thrombopoietin and remove it from the circulation [16]. Therefore, levels are high in thrombocytopenia as a result of marrow aplasia and The normal platelet count is approximately rise 6 days after the start of therapy and remain high for 7-10 days. rise 6 days after the start of therapy and remain high for 7-10 days. Unfortlmately, tlu'ombopoietin is not available for routine clinical practice. Platelets also have c-Mpl receptors for thrombopoietin and remove it from the circulation. Therefore, levels are high in thrombocytopenia as a result of marrow aplasia and The normal platelet count is approximately events leading to platelet plug formation during haemostasis. Adhesion to coHagen is facilitated by glycoprotein la (Grla). Glycoproteins Ib (defective. in Bernard-Soulier syndrome) and IIb/IIIa (defective in tlu'ombasthenia) are important in the attachment of platelets to von Willebrand factor (VWF) and hence to vascular subendothelium. Also the receptor for binding site for lib /IIIa is also the receptor for binding site for lib /IIIa is also the receptor foThe plasma membrane

invaginates into the platelet interior to form an open membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids(previously known as platelet factor 3) are of particular importance in the conversion of coagulation factor X to Xa and prothrombin (factor II) to thrombin (factor IIa). The platelet contains three types of storage [14].

### 2.2.3 Platelet antigens:

Several platelet surface proteins have been fould to be important antigens in platelet-specific autoimmunity and they have been termed human platelet antigens (HPA). In most cases, two different alleles exist, termed a or b alleles (e.g. HPA-la). Platelets also express ABO and human leucocyte antigen( HLA) class I. [16]

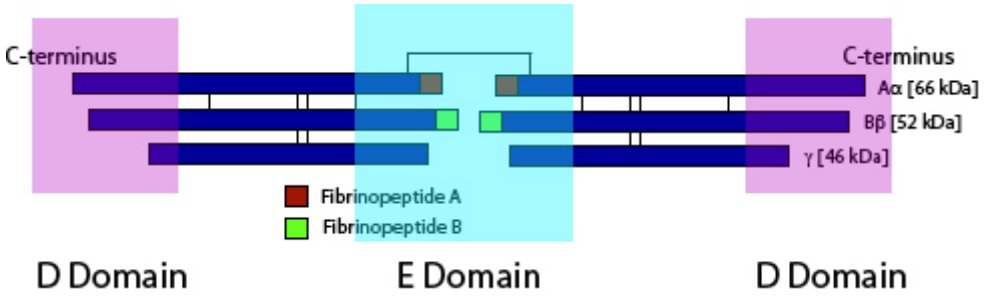
## 2.3. D. dimer

### 2.3.1. Definition:

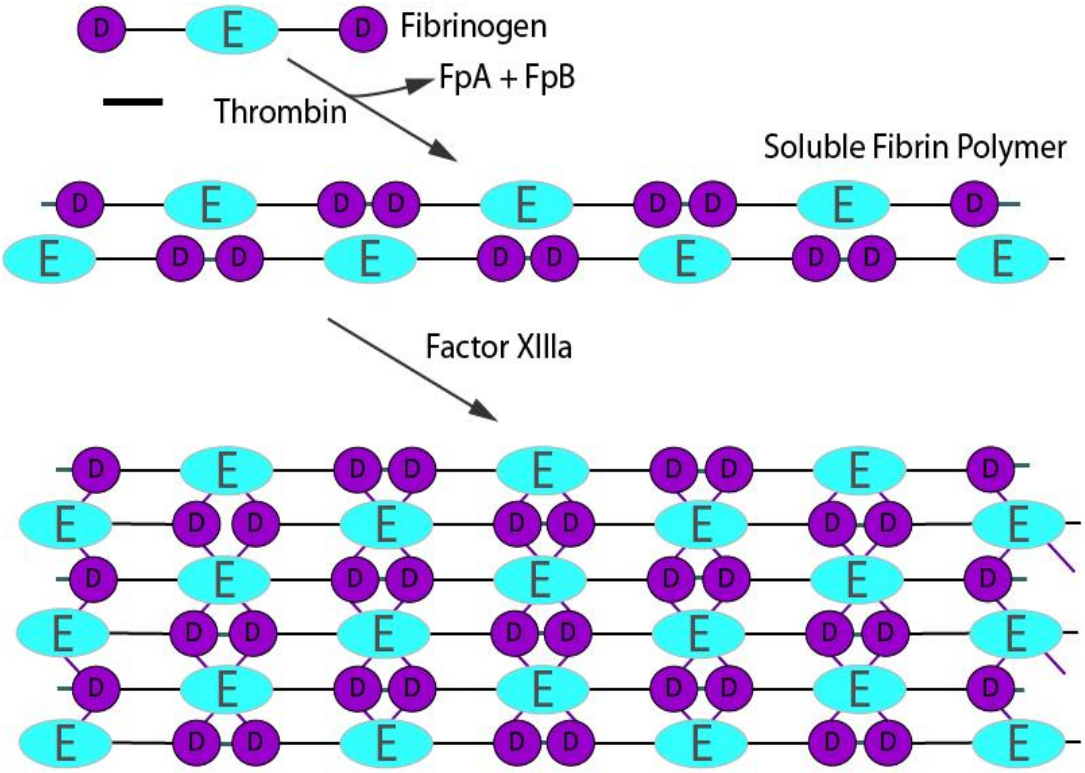
D-dimer (or D dimer) is a fibrin degradation product (or FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two D fragments of the fibrin protein joined by a cross-link.[17]

D-dimer concentration may be determined by a blood test to help diagnose thrombosis. Since its introduction in the 1990s, it has become an important test performed in patients with suspected thrombotic disorders. While a negative result practically rules out thrombosis, a positive result can indicate thrombosis but does not rule out other potential causes. Its main use, therefore, is to exclude thromboembolic disease where the probability is low. In addition, it is used in the diagnosis of the blood disorder disseminated intravascular coagulation.[17]Fibrinogen consists of three pairs of polypeptide chains: two Aα, Bβ and γ. These are linked together by 29 disulphide bonds in such a way that N-terminal regions of the 6-polypeptide chains meet to form a central E-domain. The C-terminal regions [Aα, Bβ and γ] for the D-domain and these are joined by α-helical ropes to the central E-domain to give the characteristic [18]

Fibrinogen structure.




Activation of fibrinogen by thrombin [IIa] cleaves the two short peptides from the N-terminal regions of the Aα and Bβ chains - these peptides are known as Fibrinopeptide A [FpA - 16 amino acids] and Fibrinopeptide B [FpB - 14 amino acids] respectively. Removal of the N-terminal sequences from the Aα and Bβ chains chains reveals new N-terminal sequences in the Aα and Bβ chains located within the E domain, known as 'knobs.' These knobs can interact spontaneously with the D-domains to form fibrin polymers. Under the influence of factor XIIIa, cross-linking of these fibrin polymers [between glutamine and lysine amino acids] occurs to form cross-linked fibrin polymers. [19]



To recap the sequence is:

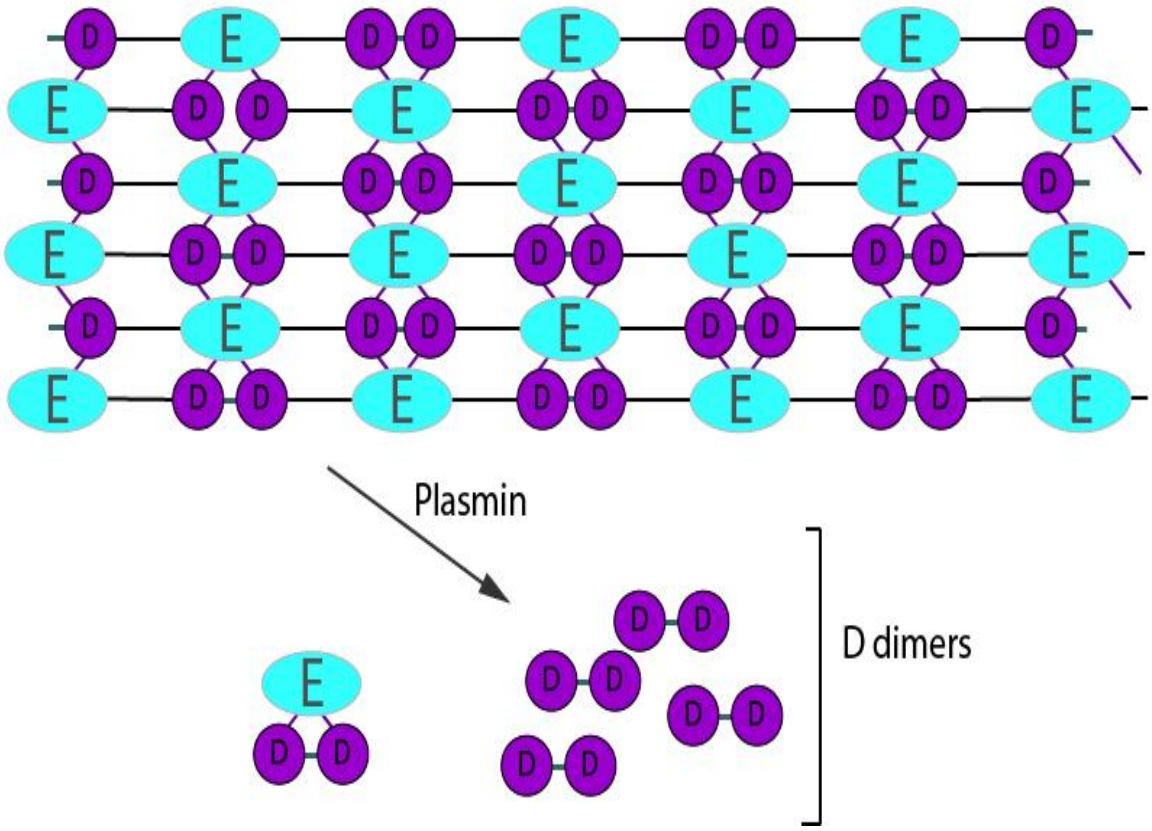
Fibrinogen → Fibrin monomer → Soluble fibrin polymer [non cross-linked] → Cross-linked fibrin polymer Thrombin activation of factor XIII is accelerated by the presence of non–cross-linked fibrin but is inhibited by fully cross-linked fibrin. This autoregulation serves to help limit factor XIIIa activity to sites of fibrin clot formation. The principal sites of cross-linkage induced by FXIIIa are between the γ-domains in the D domains of adjacent fibrin monomers and between the carboxyl-terminal ends of the α chains of fibrin monomers. [18]

### 2-3.2 Degradation of Fibrinogen and Non-cross Linked Fibrin

Fibrinogen is degraded by plasmin generating a series of fragments [see above. Partial degradation of the γ chain gives rise to Fragment X and then further digestion of this fragment gives rise to Fragment Y. Digestion of Fragment Y gives rise to Fragment E and two D Fragments.[18-20].

### 2.3.3 Degradation of Cross-Linked Fibrin

Digestion of cross-linked fibrin by plasmin gives rise to a variety of fragments including D dimers. These define the breakdown of cross-linked fibrin [i.e. fibrin that has been cross-linked by FXIIIa] as the cross-linking links D-fragments. Digestion by plasmin cleaves cross-linked fibrin in a random order to generate a series of soluble fragments [so called 'X-oligomers'] of varying molecular weights and including D dimers. In each of these breakdown steps, the *γ–γ* cross-links are retained and so D-dimers are present. Although the diagram below shows D-dimers in isolation, in practise they exists as a variety of D-Dimer containing oligomers e.g. EDD. The process is dynamic and more fragments are generated as the process proceeds. D-dimers create a neo-epitope and it is this that is detected by specific antibodies used in the various detection systems [21]




**2.3.4 raised in:**

• Pregnancy

• Malignancy

• Infection

• DIC

• Vaso-occlusive sickle cell crisis

• Surgery

• Burns

• Liver disease

• Snake bites

• Atrial fibrillation

• Renal failure

• Cardiac failure

• Venous thromboembolic disease [VTED]

### *2.3.5 Comments*

1. No standard reference preparation [i.e. international standard] currently exists for D-dimers.

2. Units for reporting D dimers values include: Fibrinogen Equivalent Units/mL [FEU/mL] and µg/mL.

3. Fibrinogen equivalent units express the concentration of fibrin degradation products in terms of the gravimetrically determined mass of fibrinogen from which they were derived.

Two µg FEU/mL have an immunoreactivity similar to 1 µg/mL of purified D-Dimer.

4. False positive D dimers assays have been see in association with the presence of Rheumatoid Factor

### *2.4. previous studies*

1. A retrospective chart review of all non-immune travelers hospitalized with malaria during 01/2000–12/2014 at the Sheba Medical Center, Israel. Admission and peak D-dimer levels were compared among malaria patients, according to *Plasmodium* species and severity.[3]

Results

Complete laboratory data was available for 94/168 travelers hospitalized with malaria, with 68.1% caused by *P. falciparum*. Admission D-dimer levels were significantly higher in *P. falciparum* malaria compared to non-*falciparum* malaria cases (3585±7045 and 802±1248ng/dL respectively, p=0.04). Admission D-dimer levels were higher in patients with severe compared to non-severe *P. falciparum* malaria (4058±3544 & 3490±7549ng/dL), however the difference was short of statistical significance (P=0.06). Peak D-dimer levels were also significantly higher in severe and non-severe *P. falciparum* than in non-*falciparum* cases.

2. study done by Narendra kumar ,Shyam Babu , Uttam Chand and Kiran Sahere in india to find out the frequency and the degree of thrombocytopenia in

patients with malaria. In 230 patients with malaria positive were investigated with platelet count.[28]

Results:

In the study group of 230 patients: 130 (56.51%) were positive for *Plasmodium vivax*, 90 (39.13%) were positive for *P. falciparum* and 10 (4.34%) had mixed infection with both *P. vivax* and *P. falciparum*. Out of 130 cases detected with *vivax* malaria, 100 cases had thrombocytopenia. Out of 90 cases detected with *falciparum* malaria, 70 cases had thrombocytopenia. Among 10 cases of mixed infection, 9 cases had thrombocytopenia.

3. Study in India done by Anirban Dasgupta, Sandeep Rai and Amar Das Gupta.[9]

Results;

Screening coagulation tests and assays for thrombosis and fibrinolysis were performed in 80 cases of malaria at presentation and during the course of the disease. Close correlation between the degree of thrombocytopenia (observed in >97% cases) and the presence hemorrhagic manifestations at presentation, and improvement in the platelet count in parallel with clinical recovery emphasised the role of platelets in the pathogenesis of coagulopathy in malaria. A potential selection bias resulting from inclusion of only patients admitted at a tertiary care hospital could explain the higher incidence (27.5%) of clinical bleeding observed in this study compared to that reported in the literature. Although a significant correlation between overt bleeding and abnormal PT/INR and APTT (observed in 20–37% cases) could not be demonstrated, a good correlation existed between normal screening coagulation tests and the absence of bleeding complications. Elevated D-Dimer and FDP levels in almost all cases (90%) of both types of malaria confirmed the high prevalence of disseminated intravascular coagulation and fibrinolysis. A correlation between rising D-Dimer levels and the incidence of bleeding was observed. Follow up studies in six cases with complications showed normalization of platelet counts and of screening coagulation assays with clinical recovery. D-Dimer and FDP levels

however, remained elevated in most of these cases indicating the continuation of a smouldering coagulopathy even after full clinical recovery possibly due to the persistence of residual damage to the cells caused by the parasitic infection. Knowledge of this fact is important for avoiding unnecessary investigations and longer hospital stay in patients admitted with malaria.

4. Samuel Crocodile, Wassmer Terrie Taylor,. Calman Alexander MacLennan, Maxwell Kanjala Mavuto Mukaka and Malcolm Edward Molyneux do study in Platelet-Induced Clumping of *Plasmodium falciparum*-Infected Erythrocytes from Malawian Patients with Cerebral Malaria—Possible Modulation in Vivo by Thrombocytopenia [27]

**Results:**

Platelets may play a role in the pathogenesis of human cerebral malaria (CM), and they have been shown to induce clumping of *Plasmodium falciparum*-parasitized red blood cells (PRBCs) in vitro. Both thrombocytopenia and platelet-induced PRBCclumping are associated with severe malaria and, especially, with CM. In the present study, we investigated the occurrence of the clumping phenomenon in patients with CM by isolating and coincubating their plasma and PRBCs ex vivo. Malawian children with CM all had low platelet counts, with the degree of thrombocytopenia directly proportional to the density of parasitemia. Plasma samples obtained from these patients subsequently induced weak PRBC clumping. When the assays were repeated, with the plasma platelet concentrations adjusted to within the physiological range considered to be normal, massive clumping occurred. The results of this study suggest that thrombocytopenia may, through reduction of platelet-mediated clumping of PRBCs, provide a protective mechanism for the host during CM.

# Chapter Three

## Materials and Method

# 3. Materials and methods

## 3.1. Study design:

This is a cross sectional descriptive study design based on venous blood sample of 30 adults in Shendi to determine the platelet count and D. dimer in malaria patients.

## 3.2. Study area:

The study was conducted at Shendi teaching hospital which located in Shendi town in Sudan, during the period between march 2018. Shendi is a town in northern Sudan, situated on the east bank of the Nile 150 km northeast of Khartoum. Shendi is also about 45 km southwest of the ancient city of Meroe. Located in the River Nile state, Shendi is the center of the Ja'aliin tribe and an important historic trading center. Its principal suburb on the west bank is Al-Matamma. A major traditional trade route across the Bayuda desert connects Al-Matamma to Marawi and Napata, 250 km to the northwest.

## 3.3. Study Population:

A total of (30) samples collected of Study group of malaria disease patients and (20) samples collected of healthy individuals as control group.

### 3.3.1. Inclusion criteria:

1. Only smear-proven patients with malaria positive test.
2. Only patients admitted in the hospital were enrolled in the study.
3. Both male and female patients from the age of 20 to 50 years were included

### 3.3.2. Exclusion criteria:

1. Cases with mixed malarial infection were excluded from the study
2. Pregnant women were excluded.
3. Patients with acute hepatitis, leptospirosis, Salmonella and those with other bacterial and viral infections; immune-compromised individuals, e.g. AIDS .

### 3.4. Data collection tools:

Data was collected using self-administrated pre-coded questionnaire which specifically designed to obtain information that helped in study.

### 3.5. Blood Sampling:

Venous blood collected using sterile disposable plastic syringe after cleaning the venipuncture area with (70%) ethanol, the blood added to the anticoagulant and gently mix.The sample centrifuge at (1300 rpm) for (15min) to obtain plasma.

### 3.6. Methods:

**3.6.1. Platelet count** was done by using Mindray Haematology Analyzer (Mindray bc-3000):

**3.6.1.1. Principle**: blood cells can be broadly divided into three categories .red blood cells, White blood cells and platelets. The analyzer measures the number of cells and distinguishing between their types according to size using sheath flow DC detection. Electrical current is passed through a solution; this method measures the changes in electrical resistance that occurs when blood cells pass through detection aperture. This instrument performs haematology analyses according to the RF/DC detection method, Hydro Dynamic Focusing (DC Detection), and sodium lauryl sulphate (SLS) haemoglobin method. The radio frequencies and direct current (RF/DC detection method) detects the volume of blood cells by changes in direct- current resistance.

**3.6.1.2. Procedure:**

RBCs count, Hct, Hb concentration, haematimetric indices (MCV, MCH, and MCHC), RDW, WBCs and platelets counts were measured by using an automatic blood cell counter (Mindray -3000 analyzers). The assay was performed according to the instructions provided by the manufacturer. The analyzer was controlled by normal control, abnormal high and abnormal low.

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

### 3.6.2. D.dimer method:

#### 3.6.2.1. Principle:

The test uses the sandwich immunodetection method, such that the detection antibody in buffer binds to D-Dimer in the plasma sample and antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocelluose matrix. The more D-Dimer antigen in the plasma, the more antigen-antibody complexes are accumulated on test strip. Signal intensity of fluorescence on detection antibody reflects amount of antigen captured and is processed by ichroma™ Reader to show D-Dimer concentration in the specimen. The working range of ichroma™ D-Dimer test is (50 – 10,000 ng/ml). [21] [22]

**3.6.2.2. Reference Value**: 500 ng/ml.

#### 3.6.2.3. Components and reagents:

Ichroma™ D-Dimer consists of Cartridge, an ID Chip, and Detection Buffers.

- The test cartridge contains a test strip; on the membrane of which, antibodies against D-Dimer and streptavidin have been immobilized at the test line and the control line respectively.
- Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. [25] sealed test cartridges are packed in a box which also contains an ID chip.
- The detection buffer pre-dispensed in a tube contains fluorochrome-labeled anti-D-Dimer antibodies, fluorescent-labeled biotin-BSA, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is dispensed in each detection buffer tube. [25] detection buffer tubes are packed in a separate pouch which is further packed in a Styrofoam box provided with ice packs for the purpose of shipment. [23].

#### 3.6.2.4. Test procedure:

1. (10µL) of serum/plasma/control sample was transfered using a transfer pipette to a tube containing the detection buffer.

2. The lid of the detection buffer tube closed and mixed the sample thoroughly by shaking it about (10 times). (The sample mixture must be used immediately.

3. Pipette out (75 µL) of a sample mixture and dispensed it into the sample well on the test cartridge.

4. The sample-loaded test cartridge was left at room temperature for (12 min).

5. For scanning, inserted into the test cartridge holder of the ichroma™ Reader. Ensure proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose.

6. Press 'Select' button on the ichroma™ Reader to start the scanning process.

7. Ichroma™ Reader will start scanning the sample-loaded test cartridge immediately.

8. The test result was read on the display screen of the ichroma™ reader.[23]

**3.7. Ethical consideration:**

The consent of the selected individuals to the study was taken after being informed with all detailed objectives of the study and it is health emphasis in the future.

**3.8. Data analysis:**

The collected data code in master sheet and proceed for analysis using SPSS version 11.5 . (mean, standard deviation, standard error mean, P.value by using independent T.test).

# Chapter Four

## Results

# 4. Results

## 4.1clinical data:

A total of (30) blood samples collected from malaria patients and (20) samples collected as control from healthy individuals including the frequency of sex was 10 males (33%) and 20 females (67%), frequency of age groups 20-35 years 18 (60%), 36-50 year 12 [4o%].

**Table (4.1): Distribution of study population according to sex and age:**

| Characteristic | | Frequency | Percent % |
|---|---|---|---|
| Study groups | Case | 30 | 60% |
| | Control | 20 | 40% |
| Sex | Male | 10 | 33% |
| | Female | 20 | 67% |
| Age/years | 20-35 year | 18 | 60% |
| | 36-50 year | 12 | 40% |

Participation to treatment used in malaria patients reflected that; 15 (50%) were not indicate any treatment of malaria or, while 15 (50%) were indicate malaria treatment. On the other hand, 13 (43%) were indicate other medications, while the remaining 17 (57%) were not indicated. Table (4.2).

**Table (4.2): Distribution of Study Population According to Risk Factors:**

| Characteristic | | Frequency | Percent % |
|---|---|---|---|
| Malaria treatment | Yes | 15 | 50% |
| | No | 15 | 50% |
| Other medications | Yes | 13 | 43% |
| | No | 17 | 57% |

## 4.2 Laboratory Data:

The mean values of plt and D.dimer in case group was 200.03 x $10^9$ /l and 1188 ng/dl respectivly .the mean value of plt and D .dimer in control group was 261.65 x $10^9$ /l and 172.10.ng/dl respectivly . Table [4.3].

**Table (4.3): Comparison between case and control in platelet count and D. dimer:**

| Groups | | Number | Mean | SD | P.value |
|---|---|---|---|---|---|
| platelet count | Case | 30 | 200.0 | 97.05 | 0.003 |
| | Control | 20 | 261.6 | 71.24 | |
| D.dimer ng/dl | Case | 30 | 1188 | 2228.48 | 0.001 |
| | Control | 20 | 172.1 | 42.60 | |

The mean value of plt and D.dimer in cases with moderate degree of clinical manifestation and control group was 264.23x $10^9$ /l) and 479.46 ng/ dl respectively table (4. 4 )

**Table (4.4) comparison between cases with moderate degree of clinical manifestation and control group in plt and D. dimer:**

| Groups | | Number | Mean | SD | P.value |
|---|---|---|---|---|---|
| platelet count | Case | 13 | 264.23 | 83.23 | 0.004 |
| | Control | 20 | 261.6 | 71.24 | |
| D.dimer ng/dl | Case | 13 | 497.46 | 742.70 | 0.005 |
| | Control | 20 | 172.1 | 42.60 | |

The mean value of plt and D .dimer in cases with severe degree of clinical manifestation and control group was 150.94 $x10^9$ /l) and 1751.18 ng / dl respectively table (4.5)

**Table [4.5] comparison between cases with severe degree of clinical manifestation and control group in platelet count and D. dimer**

| Groups | | Number | Mean | SD | P.value |
|---|---|---|---|---|---|
| platelet count | Case | 17 | 150.94 | 77.26 | 0.000 |
| | Control | 20 | 261.6 | 71.24 | |
| D.dimer ng/dl | Case | 17 | 1751.18 | 2794.76 | 0.000 |
| | Control | 20 | 172.1 | 42.60 | |

The mean value of plt and D .dimer in cases with one time evidence of malaria per year and control group was 247.87 $x10^9$ /l and 480.20 ng/dl respectively table [4.6]

**Table (4.6 ) comparison between cases with one time evidence of malaria per year and control group in platelet count and D. dimer;**

| Groups | | Number | Mean | SD | P.value |
|---|---|---|---|---|---|
| platelet count | Case | 15 | 247.87 | 86.61 | 0.004 |
| | Control | 20 | 261.6 | 71.24 | |
| D.dimer ng/dl | Case | 15 | 480.20 | 690.10 | 0.002 |
| | Control | 20 | 172.1 | 42.60 | |

**The mean value of platelet and D .dimer in cases with more than one time evidence of malaria per year and control group was 150.4 x$10^9$ /l and 1920.5 ng/dl respectively table (4.7)**

| Groups | | Number | Mean | SD | P.value |
|---|---|---|---|---|---|
| platelet count | Case | 15 | 150.40 | 81.51 | 0.002 |
| | Control | 20 | 261.6 | 71.24 | |
| D.dimer ng/dl | Case | 15 | 1920.53 | 2943.08 | 0.003 |
| | Control | 20 | 172.1 | 42.60 | |

# Chapter Five

## Discussion

## Conclusion

## Recommendations

# 5. Discussion, conclusion and Recommendations

## 5.1. Discussion

The results of this study demonstrated that the mean value of platelet was slightly decrease and the mean value of plasma D. dimer was moderately increase when compare with healthy individual in control group , p value [ 0.004] , it similar to study done by Narendra kumar ,Shyam Babu , Uttam Chand and Kiran Sahere in india to find out the frequency and the degree of thrombocytopenia in patients with malaria.in 100 cases had thrombocytopenia. Out of 90 cases detected with *falciparum* malaria, 70 cases had thrombocytopenia. Among 10 cases of mixed infection, 9 cases had thrombocytopenia .

In this study we found that , the mean value of platelet was normal and the mean value of plasma D. dimer was moderately increase in cases with moderate degree of clinical manifestation, and in cases with one time evidence to malaria per year when compare with healthy individual in control group , p value ( 0.005) , it is similar to study done by Samuel Crocodile ,Wassmer Terrie Taylor,. Calman Alexander MacLennan , Maxwell Kanjala Mavuto Mukaka and Malcolm Edward Molyneux do study in Platelet-Induced Clumping of Plasmodium falciparum-Infected Erythrocytes from Malawian Patients with Cerebral Malaria—Possible Modulation in Vivo by Thrombocytopenia ,it is show that Both thrombocytopenia and platelet-induced PRBCclumping are associated with severe malaria and, especially, with CM.

The results showed the mean value of platelet was decrease and the mean value of plasma D. dimer was highly increase in cases with severe degree of clinical manifestation when compare with healthy individual in control group , p value [ 0.000] .it similar to study e by in India done by Anirban Dasgupta , Sandeep Rai and Amar Das Gupta .Screening coagulation tests and assays for thrombosis and fibrinolysis were performed in 80 cases of malaria at presentation and during the course of the disease. Close correlation between the degree of thrombocytopenia (observed in >97% cases) and the presence

hemorrhagic manifestations at presentation. Elevated D-Dimer and FDP levels in almost all cases (90%) of both types of malaria .

The result of this study denoted that the mean value of platelet was slightly decrease and the mean value of plasma D. dimer was significantly increase in cases with more than one time evidence to malaria per year , when compare with healthy individual in control group , p value [ 0.000] . ). It is similar to a retrospective chart review of all non-immune travelers hospitalized with malaria during 01/2000–12/2014 at the Sheba Medical Center, Israel. Admission and peak D-dimer levels were compared among malaria patients, according to *Plasmodium* species and severity, which demonisterated that complete laboratory data was available for 94/168 travelers hospitalized with malaria, with 68.1% caused by *P. falciparum*. Admission D-dimer levels were significantly higher in *P. falciparum* malaria compared to non-*falciparum* malaria cases ($3585 \pm 7045$ and $802 \pm 1248$ ng/dL respectively, $p = 0.04$). Admission D-dimer levels were higher in patients with severe compared to non-severe *P. falciparum* malaria ($4058 \pm 3544$ & $3490 \pm 7549$ ng/dL), however the difference was short of statistical significance ($P = 0.06$). Peak D-dimer levels were also significantly higher in severe and non-severe *P. falciparum* than in non-*falciparum* cases.

A direct interaction between the parasites and the endothelium of the microcirculation causes endothelial cell injury and sets up a series of reactions characterized by release of a large variety of cytokineThese in turn activate the coagulation pathway leading to widespread thrombin deposition in small arteries and arterioles (disseminated intravascular coagulation) and fibrinolysis [2]. This explains the abnormalities of a number ofcoagulation parameters, including the markers of thrombosis and fibrinolysis that have been reported in malaria [1–3]. Such the platelet count , D-Dimer and fibrin degradation products.

## 5.2. Conclusion

- The mean value of platelet was normal in cases with moderate degree of clinical manifestation, cases with one time evidence to malaria per year and decrease in cases with severe degree of clinical manifestation and in cases with more than one evidence to malaria per year, when compared with control group.
- The mean value of D.dimer was normal in cases with moderate degree of clinical manifestation, cases with one time evidence to malaria per year and increase in cases with severe degree of clinical manifestation and in cases with more than one evidence to malaria per year, when compared with healthy individual in control group.

## 5.3. Recommendations

1-Health education, is important factor in lowering the percent of malaria.

2-we need to improve general population awareness of malaria complications.

3-More investigations should be done for malaria patients, to determine which risk factors of malaria on other systems in the body.

4- Further study in this topic should be done with increase sample size and study area to obtain accurate result with quality control in hematology lab.

# Chapter Six

## References

## Appendices

## 6.1 References

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# Appendix I

# Questionnaire

## Assessment of Platelet and D.dimer in Adult Malarial Patients in Shendi Locality River Nile State, Sudan

Demographic and Clinical Features of Included Patients

- *No. of Case* ☐
- *Age* ☐
- *Occupation* ☐
- *Address* ☐

*Gender*

*Female* ☐

*Male* ☐

*Age*

*20-35 [ ]*

*36-50 [ ]*

- *Degree of clinical manifestation*
- *Moderate [ ]*
- *Severe [ ]*
- *Evidence of malaria per year*
- *One time [ ]*
- *More than one time [ ]*
- *Indication of malaria treatment recently*
- *Yes [ ]*
- *No [ ]*
- *Indicate of other medications*
- *Yeas [ ]*
- *NO [ ]*

## Appendix II

# إقـــرار بالموافقـــة

الاسم:----------

العمر:----------  العنوان:----------

أوافق بمحض ارادتي بالمشاركة في البحث العلمي المتعلق بدراسة التغييرات في الصفائح الدموية وجزيئات الفبرين المتكسرة لدي مرضي الملاريا في مستشفى شندي التعليمي.

**الطالبة/ صفاء إبراهيم عبد القادر إبراهيم**

بعد أن شرح لي بأنه لا يترتب عليه أي أذى جسدي أو نفسي واعلم أن المشاركة في هذا البحث لن تؤثر بأي حال من الأحوال في الرعاية الطبية التي أتلقاها كما أنه يحق لي بدون إبداء أسباب الانسحاب من هذا البحث في أي مرحلة من مراحله.

**البحث بإشراف:**

**د.حمزة احمد حسن**

التوقيع : ----------  التاريخ :----------