Sudan University of Science and Technology

College of Graduate Studies

Measurement of Prothrombin Time and Activated Partial Thromboplastin Time in Sudanese Sickle Cell Anemia Patients in Khartoum State

حساب زمن البروثرومبين وزمن الثرمبوبلاستين الجزئي النشط لدى مرضى الأنيميا المنجلية السودانيين في ولاية الخرطوم

A dissertation Submitted in Partial Fulfillment of the Requirements for MS.c Degree in Medical Laboratory Science (Haematology and Immunohaematology)

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الآيـــــة

بسم الله الرحمن الرحيم

قال تعالى:

{يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ}

صدق الله العظيم

سورة المجادلة

الآية 11

Dedication

TO

My mother

A strong and gentle soul who taught me to trust

In Allah, believe in hard work and that so much

Could be done with little

My father

For being my first teacher

My brothers

For supporting and encouraging me to believe in my self

My friends

For being my guardian during my educational career

Acknowledgement

First of all, thanks to **ALMIGHTY ALLAH** for giving me patience and strength to complete this study.

It gives me great pleasure to conduct this study, and I would like to thank everyone who has made this possible.

It is most appropriate that I begin by expressing my undying gratitude to my supervisor **Dr: Mansor Mohammed Mansor,** for his invaluable guidance with his super talent, professional expertise and immense patience; showing great care and attention to details and without his guidance this study would have been impossible.

My thanks extend to laboratory staff at Laboratory Administration who performed investigations and measurements.

Special thanks to my close friends for their support.

My thanks also go to everyone, who helped me in this work to see the light.

Abstract

This is a case control study aimed to measure levels of, Prothombin time (PT), activated partial Thromboplastin time (APTT), platelet count in patients with homozygous sickle cell disease (HbSS), sickle cell trait (HbAS) and normal controls (HbAA).

The study period from August to October 2019, in Khartoum State in the Sickle Cell Clinic of Gaffer Ibn Auf Children Specialized Hospital and Al-ribat University hospital. The study population comprises two groups of children in different age-groups and gender. The first group of 50 individuals included are the children who are known to have sickle cell anemia (HbSS 41) and (HbAS 9). The second group of 50 individuals included are normal-healthy (HbAA) as a control group.

Venous blood sample was collected into tri sodium citrate (9:1) and centrifuged at 4000 rpm for 10 min to obtain plts poor plasma for coagulation study. PT and APTT were measured using (DIA Timer 2) and platelets count obtained from records.

The data collected using questionnaire, and analyzed using Statistical Package Social Science programme (Version 20). Students T test and One way ANOVA test were used to compare between means. *P. value* significant when ≤0.05.

PT (seconds) mean and SD were 15.4±1.7, 14.6±0.8 in cases and healthy subjects respectively, *P. values* were 0.000 and APTT (seconds) mean and SD were 40.8±4.9, 37.1±3.3 in cases and healthy subjects respectively, *P. values* were 0.000. Both PT and APTT were high in SCD patient compare to control group.

Mean level of platelets count (cumm) were 389±204, 281±71 in the cases and controls respectively, *P. value* was 0.01. Statistically there was significant deferens between cases group and control group in platelets count (cumm).increased platelet count in cases.

مستخلص البحث

تهدف هذه الدراسة الى قياس زمن التخثر وزمن التخثر الجزئي المنشط وعدد الصفائح الدموية في مرضى الأنيميا المنجلية (النوع المتماثل والنوع الحامل للمرض) ومقارنة هذه النتائج مع اخرين أصحاء.

فترة هذه الدراسة من أغسطس الى أكتوبر 2019 في ولاية الخرطوم في عيادة الأنيميا المنجلية بمستشفى جعفر بن عوف التخصصي للأطفال ومستشفى الرباط الجامعي.

ويتكون مجتمع الدراسة من مجموعتين من الأطفال بمختلف الجنس والأعمار، ضمت المجموعة الأولى 50 طفل من الأطفال المصابين بمرض الأنيميا المنجلية، (41 من النوع المتماثل) و(9 من النوع الحامل للمرض)، والمجموعة الثانية 50 طفل من الأطفال الأصحاء (مجموعة مقارنة). سحبت عينة وريدية من كل مشارك في انبوبة تحتوي على سترات ثلاثي الصوديوم (مضاد التجلط) بنسبة (1:9) وتم فصل البلازما ف جهاز الطرد المركزي بسرعة 4000 دورة في الدقيقة لمدة 10 دقائق للحصول على بلازما خالية من الصفائح الدموية لإجراء اختبارات التجلط.

قيس زمن التخثر وزمن التخثر الجزئي المنشط عن طريق جهاز الـ (DIA Timer 2). وجمعت نتائج الصفائح الدموية من السجلات.

تم جمع البيانات بواسطة استبيان وحللت باستخدام الحزمة الإحصائية للمجتمع (نسخة 20) أستخدم أنوفا (ANOVA) واختبار T لمقارنة الأوساط وكانت القيمة المطلقة متوافقة عند اقل من 0.05.

أظهرت هذه الدراسة المتوسط لزمن التخثر وزمن التخثر الجزئي المنشط (15.4 و40.8) ف المرضى مقارنة مع الأصحاء (مجموعة المقارنة) (14.6 و37.1) وكانت نتائج الاختبارين (زمن التخثر وزمن التخثر الجزئي النشط) مرتفعة لدى مرضى الأنيميا المنجلية مقارنة مع مجموعة الأصحاء. (القيمة المطلقة اقل من 0.05).

كما أظهرت هذه الدراسة ان متوسط عدد الصفائح الدموية هو (389) في المرضى و (281) في الأصحاء، من النتائج السابقة نستنتج أن هناك زيادة في عدد الصفائح الدموية ف المرضى مقارنة مع مجموعة الأصحاء. (القيمة المطلقة اقل من 0.05).

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List of Abbreviations

ACD: anemia of chronic disease

ADP: adenosine diphosphate

APC: Activated Protein C

APTT: activated partial thromboplastin time

AT: Antithrombin

ATP: adenosine triphosphate

BFU-E: burst-forming unit- erythroid

ECM: Extracellular Matrix

ESRD: End Stage Renal Disease

Hb: Haemoglobin

HbA1: Haemoglobin A1

HbA2: Haemoglobin A2

HbC: Haemoglobin C

HbF: Haemoglobin F

HbS: Haemoglobin S

HPLC: High-Pressure Liquid Chromatography

HSCs: haematopoietic stem cell

HSPCs: haematopoietic stem and progenitor cells

INR: International normalized ratio

MMPs: Matrix MetalloProteinases

NO: nitric oxide

P. value: Probability value

PA: Plasminogen Activators

PAR1: Protease-Activated Receptor-1

PCR: Polymerase Chain Reaction

PDGF: Platelet-Derived Growth Factor

PF: Platelet Factor

Plt: platelet

PS: phosphatidylserine

PT: prothrombin time

RBC: Red Blood Cell

ROS: Reactive Oxygen Species

SCD: Sickle Cell Disease

SPSS: Statistical Package for Social Science

TF: Tissue Factor

t-PA: tissue-type PA

u-PA: urokinase type PA

VWF: Von Willebrand Factor

Chapter One

Introduction, Rationale, Objectives

Chapter One

Introduction, Rationale, Objectives

1.1. Introduction:

Sickle cell disease (SCD) was first described in 1910, in a dental student who presented with pulmonary symptoms (Herrick, 1910). Herrick coined the term "sickle-shaped" to describe the appearance of the RBC of this patient. Given the patient's symptoms, he was not sure at the time whether the blood condition was a disease or a manifestation of another disease (Herrick, .1924). Sickle cell disease (SCD) is clinically one of the most important haemoglobinopathies. It is characterized by haemolytic anaemia, an increased susceptibility to infections and vaso-occlusion that occurs in almost all vascular beds leading to ischaemic tissue injury with organ dysfunction and early death (Schnog *et al*, .2004). It is a set of autosomal recessive hereditary hemoglobinopathies caused by a point mutation in the gene coding the Beta hemoglobin subunit (Carroll, .2019). The disease is caused when the hemoglobin S allele is inherited with another abnormal beta globin allele – most commonly a second S allele, causing the classic version, sickle cell anemia (SCA)(Lanzkron *et al*, .2013). Mutation of the β-globin gene's sixth codon from GAG to GTG. Consequently, the sixth amino acid in the β-globin chain becomes valine instead of glutamic acid, leading to sickle cell hemoglobin (Du et al, .2019). Upon deoxygenation, sickle cell hemoglobin gathers into polymers. The polymers form long strands that distort the membrane, resulting in the sickle shaped red blood cells. These sickle cells are inflexible, have poorly deformability, and break easily, producing hemolysis and hematological complications, vaso-occlusion, infection, and organ dysfunction (Moerdler and Manwani, .2018). Repeated episodes of sickling damage the cell membrane and decreases the cell's elasticity. As a

consequence, these rigid blood cells are unable to deform as they pass through narrow capillaries, leading to vessel occlusion and ischemia (Obeagu *et al*, .2015). The terms "sickle-cell crisis" or "sickling crisis" may be used to describe several independent acute conditions occurring in patients with SCD (Vasaikar *et al,* .2015). The disease has no established cure to date except in a few patients who had successful bone marrow or stem cell transplantation. Although gene therapy for sickle cell anemia, the ultimate goal of cure, is not feasible at the present, significant strides have been made at the basic level to achieve the genetic correction of hemoglobinopathies (Ballas *et al*, .2012). Coagulation is a dynamic process and the understanding of the blood coagulation system has evolved over the recent years in anaesthetic practice. Although the traditional classification of the coagulation system into extrinsic and intrinsic pathway is still valid, Normal coagulation pathway represents balance between the pro coagulant pathway that is responsible for clot formation and the mechanisms that inhibit the same beyond the injury site. Imbalance of the coagulation system may occur in the perioperative period or during critical illness, which may be secondary to numerous factors leading to a tendency of either thrombosis or bleeding (Palta *et al,* .2014). Hypercoagulation state is another prominent feature of sickle cell disease and is mediated by activation of both intrinsic and extrinsic coagulation pathways. Growing evidence demonstrates that coagulation may not only contribute to the thrombotic complications, but also to vascular inflammation associated with this disease (Sparkenbaugh and Pawlinski, 2013). Chronic hypercoagulable or prothrombotic state is generally known to be one of the factors that contribute to vaso-occlusion and progressive end-organ damage in sickle cell disease (Stuart and Yamaja, .2001). This study therefore aims at determining the actual value of some coagulation profiles (PT, APTT) among patients with SCA in steady state in this environment and compare with subjects with normal hemoglobin genotype.

1.2. Rationale:

Sickle cell diseases are wide spread haemoglobinopathy occurring throughout the world. In Sudan, sickle cell diseases are one of the major health problems, especially in western Sudan where the sickle gene is quite frequent. It is very common and its complications cause a lot of morbidity and mortality. The most serious complication of this condition is vaso-occlusion that results in severe pain and organ dysfunction. Sickle cell disorders are associated with a hypercoagulable state that may contribute to the vaso-occlusive episodes observed in the disorder. To what extend changes in coagulation process occur in Sudanese individuals with sickle cell disease has had limited study. To clarify the association between the clinical and haematological findings that may occur in these individuals, we examined a group of sickle cell disease patients to determine to what extend those changes in coagulation screening do occur. These provide evidence for clinician to manage their patients probably and thus reduce the risk of serious complications and improve the health care services for sickle cell disease patients in the future.

1.3. Objectives:

1.3.1 General objective:

Measurement of Prothrombin Time and Activated Partial Thromboplastin Time in Sudanese Sickle Cell Anemia Patients in Khartoum State

1.3.2. Specific objective:

- To measure PT, APTT, and platelet count in control group and patients.
- To compare results of PT, APTT, platelets count between control group and patients.
- To compare PT, APTT results between patients according to age and gender.
- To compare PT, APTT between HbSS and HbAS.

Chapter Two

Literature review

Chapter Two

Literature review

2. Literature review:

2.1. Blood:

Blood is a complex liquid tissue composed of plasma and formed elements or cellular components suspended in plasma. Contains many types of cells with very different functions, ranging from the transport of oxygen to the production of antibodies. Some of these cells function entirely within the vascular system, while others use the vascular system only as a means of transport and perform their function elsewhere (Alberts *et al*, .2002).

2.2. Erythropoiesis:

Red blood cells (RBCs) are generated from haematopoietic stem and progenitor cells (HSPCs) through the step-wise process of differentiation known as erythropoiesis (Nandakumar, .2016). Every second, the human body generates 2 million red blood cells, the first steps of erythroid differentiation involve an engagement phase, in which HSCs differentiate into more committed erythroid progenitors, from a common myeloid progenitor the megakaryocytic-erythroid progenitor and finally the burst-forming unit- erythroid (BFU-E). BFUEs are the first progenitor cells committed solely to the erythroid lineage (Zivot. 2018).

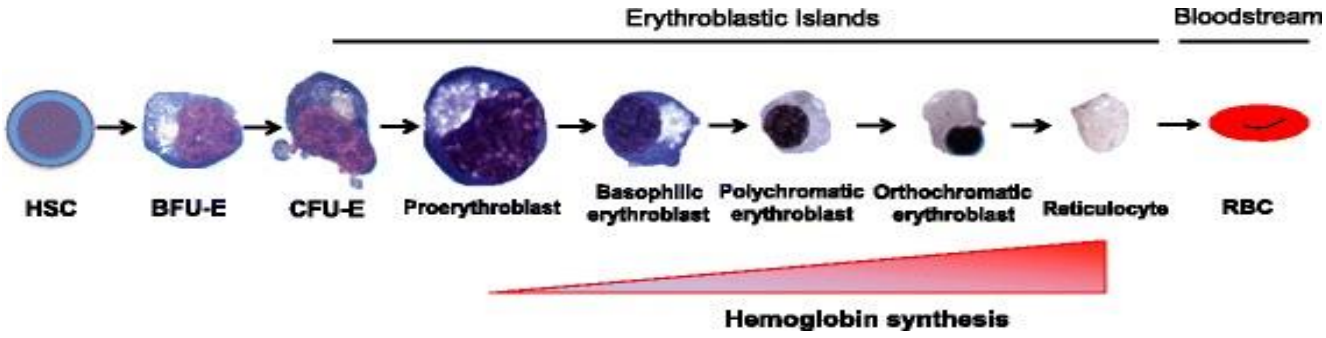

Figure (2-1): (Zivot. 2018)

2.3. Red blood cells (RBCs):

Mature erythrocyte is a round biconcave disc about 7 to 8 μm in diameter. Basic structural properties of various red cell components (haemoglobin, enzymes, and membrane) The mature circulating red blood cell is a membrane-encapsulated liquid compartment which contains a hemoglobin solution for oxygen delivery to--and carbon monoxide removal from the metabolizing tissues of an animal (Evans, 1989). RBCs may be directly involved in tissue protection and regulation of cardiovascular homeostasis by exerting further noncanonical functions, including nitric oxide (NO) metabolism and control of blood rheology, as well as erythrocrine function (i.e., by releasing bioactive molecules, including NO, NO metabolites, and ATP)(Kuhn *et al,* .2017). The circulatory lifetimes of red blood cells in humans of approximately 120 days (Yoshida, 2019). The interendothelial slit (IES) is the narrowest circulatory pathway in the human spleen where aged and diseased red blood cells (RBCs) are filtered (Li *et al,* .2018).

2.4. Hemoglobin (Hb):

Hemoglobin (Hb) is an essential component of the circulatory system of vertebrates (Lukin and Ho, 2004). Hemoglobin is a heterotetramer composed of α-like and β-like globin subunits, each bound to a heme prosthetic group. The major functions of Hb are to transport oxygen (O_2) from the lungs to peripheral tissues and carbon dioxide (CO_2) from the tissues to the lungs (Thom *et al*., 2013). Different types of human hemoglobins (Hbs) are exist, the adult type is most often studied; results on the embryonic and fetal types are usually interpreted within its framework (Manning, 2010). The expression of the eight different types of normal human Hbs during the embryonic, fetal, and adult stages of life represents a major model of developmental biology, which is currently explained by the ‘‘switching’’ on and off of the various globin genes, known as ontogeny (Manning *et al,* .2010). Table (2-1).

Hemoglobin disorders can be broadly **classified** into two general categories:

- Those in which there is a quantitative defect in the production of one of the globin subunits, either total absence or marked reduction. These are called the thalassemia syndromes.

- Those in which there is a structural defect in one of the globin subunits (Forget, 2013).

Table (2-1) Hemoglobin variants: (Horton-Szar *et al.,* 2012; Kawthalkar, 2013)

Developmental stage	Hemoglobin types	Chains
Embryonic	Hb Gower I Hb Gower II Hb Portland	ζ2ε2 a2ε2 ζ2γ2
Fetal	HbF (80%) HbA (20%)	α2γ2: higher affinity for O2
Adult	HbA (97%) HbA2 (2.5%) HbF (0.5%)	α2β2: Principal Hb of adult Hb α2δ2

2.5. Anemia:

2.5.1. Definition:

Anaemia is a condition in which the number of red blood cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiologic needs. Specific physiologic needs vary with a person's age, gender, residential elevation above sea level (altitude), smoking behavior, and different stages of pregnancy (World Health Organization, 2011). Anemia is not a disease but it is the expression

of underlying disease and from the treatment point of view, it is necessary to identify the cause of anemia (Nayak, et al., 2011).

2.5.2. Classification:

2.5.2.1. Anemia of decreased red blood cell production:

Caused by maturation defect and by proliferation defects, a third category, anemia of chronic disease (ACD) (His, 2012).

2.5.2.2. Increased destruction (hemolytic):

Gastrointestinal blood loss, menorrhagia, Membrane defects, Enzyme defects, Hemoglobinopathies. (Wahed, and Dasgupta, .2015).

2.5.2.3. Anemia due to deficiency of hematopoietic factor:

Iron deficiency, folate deficiency, vitamin B12 deficiency, erythropoietin deficiency (Wahed, and Dasgupta, .2015).

2.6. Sickle cell disease:

Sickle cell disease (SCD) refers to a group of genetic disorders defined by the presence of sickle hemoglobin (HbS), chronic hemolysis and multi-organ morbidity. More than 300 000 children were born with sickle cell anemia (SCA), the homozygous form of SCD, in 2010 and it is predicted that more than 400 000 children will be born annually by 2050 (Noubouossie *et al.*, 2016). Inherited autosomal recessively, either two copies of Hb S or one copy of Hb S plus another β-globin variant (such as Hb C) are required for disease expression (Ashley et al., 2000). HbS imparts sickle shape to red cells on low oxygen tension or deoxygenation. The term sickle cell disease includes all entities associated with sickling of the red blood cells (Nayak *et al*., 2011). Sickle cell anaemia is the most common and severe variant of sickle cell disease (SCD). It is a haematological disorder caused by a single nucleotide mutation that substitutes glutamic acid with

valine at the sixth position of HBB (the b-globin gene) (Sparkenbaugh and Pawlinski, .2013).

2.6.1. Normal Hb (Hb A) and Sickle Hb (Hb S):

Normal Hemoglobin is a heterotetramer composed of a-like and b-like globin subunits, each bound to a heme prosthetic group. The major functions of Hb are to transport oxygen (O2) from the lungs to peripheral tissues and carbon dioxide (CO2) from the tissues to the lungs (Thom *et al*., 2013). Sickle cell disease is caused only by the Hb S allelic variant of the P-globin gene. All individuals who are homozygous or compound heterozygous for Hb S exhibit some clinical manifestations of sickle cell disease (Ashley et al., 2000). In **sickle cell trait** (**heterozygous),** approximately 40% of the hemoglobin is HbS and 60% is HbA. Such individuals remain as asymptomatic carriers and their red cells sickle only with severe hypoxia. In contrast, **homozygous HbS** individuals with no HbA have full-blown sickle cell anemia. (Nayak *et al*., 2011).

2.6.2. Genetics and classification:

The Hb S mutation is the result of the substitution of valine for glutamic acid at position 6 in the β chain, Molecules of deoxyhemoglobin S have a strong tendency to aggregate and form polymers (Lichtman *et al*, 2017). This abnormal polymerization produces rigid, deformed RBCs, resulting in chronic hemolysis and vasoocclusive phenomena. SCD most commonly results from homozygous Hb S mutations (SS disease), but also is a consequence of compound heterozygosity for Hb S and Hb C (SC disease), β-thalassemia (S/β-thalassemia), or rarely other β-chain variants (His, 2012).

2.6.3. Epidemiology:

The global distribution of HbS is indicative of two factors: selection for carriers through their survival advantage in malaria-endemic regions and subsequent migration (Rees *et al,* 2010). The HbS allele was originally distributed throughout sub-Saharan Africa, the Middle East, the Mediterranean area and India. Carrier rates range from 5% to > 40% in these areas (Houwing et al, .2019). A geographic association with areas of high malaria prevalence has been determined to represent a lessened risk of developing falciparum malaria in heterozygotes (Lichtman *et al*, 2017). This clustering of hemoglobinopathies is assumed to reflect a selective survival advantage for the abnormal RBC, which presumably provide a less hospitable environment during the obligate RBC stages of the parasitic life cycle. Between 2% and 3% of American blacks carry a hemoglobin C allele. (Longo, .2010). Patients with Hb SS disease may have increased Hb F. The distribution of Hb F among the haplotypes of Hb SS are Hb F 5-7% in Bantu, Benin, or Cameroon; Hb F 7-10% in Senegal; and Hb F 10-25% in ArabIndian (Wahed and Dasgupta, 2015).

2.6.4. Pathophysiology:

HbS is caused by a mutation in the β-globin gene in which the 17th nucleotide is changed from thymine to adenine and the sixth amino acid in the β-globin chain becomes valine instead of glutamic acid. This mutation produces a hydrophobic motif in the deoxygenated HbS tetramer that results in binding between β1 and β2 chains of two haemoglobin molecules (Rees *et al,* .2010) This crystallization produces a polymer nucleus, which grows and fills the erythrocyte, disrupting its architecture and flexibility and promoting cellular dehydration, with physical and oxidative cellular stress ((Rees *et al,* .2010). the polymers can have a direct impact on the rbc plasma membrane, leading to the extracellular exposure of protein epitopes and glycolipids that are normally found inside

the cell. These changes and the aberrant expression of adhesion molecules on stress reticulocytes likely explain the increased adherence of sickle rbc to vascular endothelium (Frenette and Atweh, 2007). The vascular endothelium is also activated in SCD, and its role in the pathology of this disease has been extensively reviewed (Sparkenbaugh and Pawlinski, 2013). abnormal phosphatidylserine (PS) exposure of the sickle cell RBC membrane alters the adhesive properties of sickle RBCs leading to an increase in capillary transit time and stasis enhancing the potential for the activation of coagulation factors and cellular elements in the microvasculature and post-capillary venules (Pakbaz and Wun, 2014). Sickled erythrocytes are rigid, lysis-prone and interact with leucocytes and the vascular endothelium. This results in haemolytic anaemia and recurrent occlusion of the small vessels. Vaso-occlusion leads to ischaemic damage of tissues resulting in severe pain and cumulative organ damage. Subsequent reperfusion of ischaemic tissues promotes chronic inflammation by increased reactive oxygen species (ROS) production (Houwing et al, .2019). High granulocyte counts are a risk factor for death in sickle cell anemia. Granulocytes interact with sickle cells and endothelial cells and are stimulated to release injurious cytokines (Steinberg, 1999). The coagulation system is hyperactivated in SCD. While any process that increases adhesion of SRBCs or leukocytes to endothelial cells can cause microvascular occlusion and promote stasis-induced microthrombi due to coagulation activation, SRBC intrinsic and extrinsic abnormalities themselves can activate the coagulation system and platelets (Telen *et al,* .2019). Abnormal adenosine signaling via the adenosine A2B receptor results in increased sickling, which may be key to clinical manifestations, such as priapism (Lichtman *et al*, 2017).

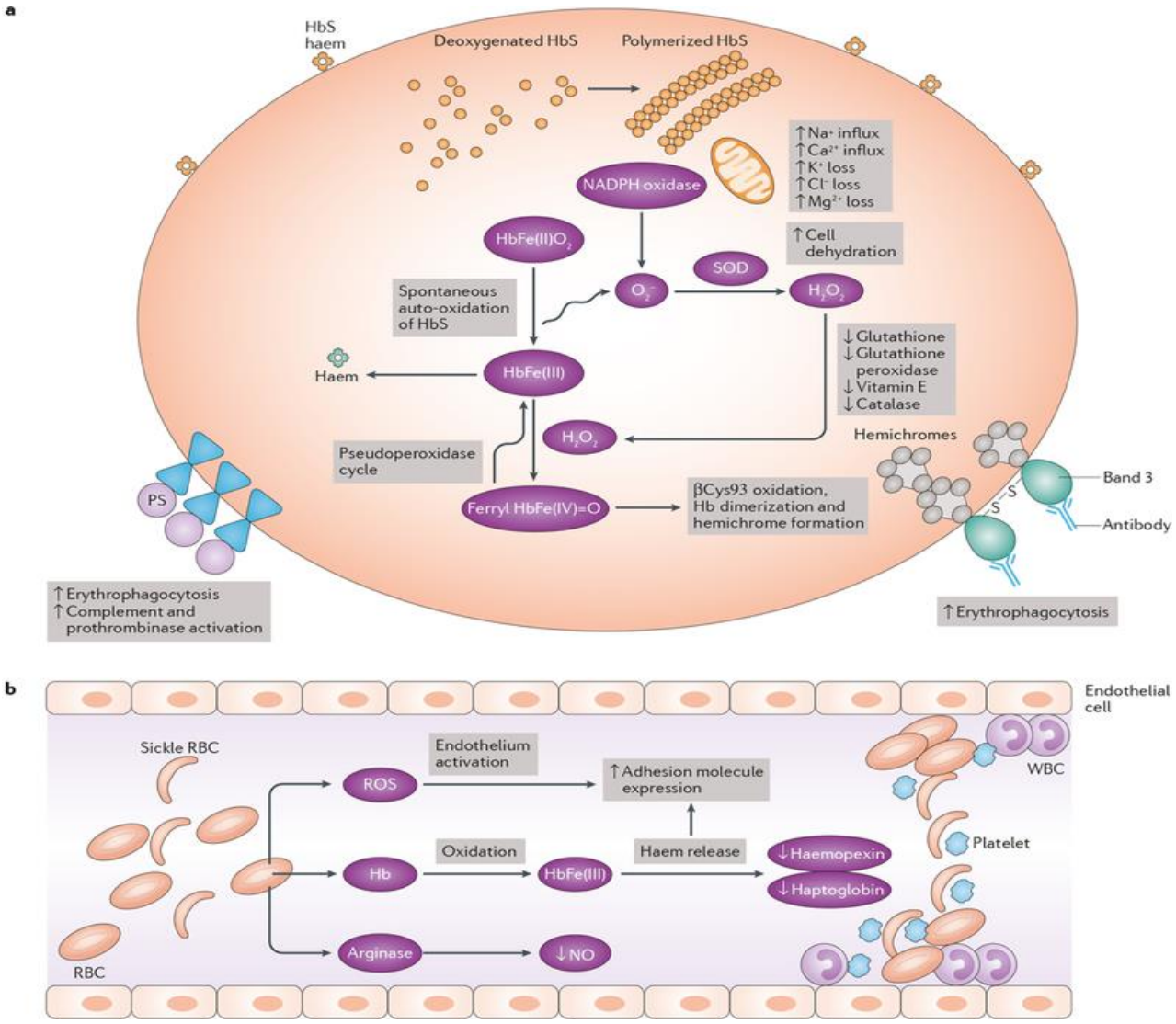

Figure (2-2): (Telen *et al*, .2019).

2.6.5. Clinical features:

The clinical presentation of sickle cell disease changes as life advances**.** Presence of HbF in the first 6 months of life has a protective role. Symptoms appear after 6 months of age as the HbF disappears (Nayak *et al*., 2011). There is great variability among affected individuals, but many patients are in good health most of the time. In children, most problems are related to pain, infection, or inflammation. In adults, clinical manifestations are likely to be more chronic, related to organ damage (Lichtman *et al*, 2017). Complications may include an enlarged heart, progressive loss of pulmonary or renal function, stroke, arthritis, liver damage, and other complications (Turgeon, 2012). Early complications include dactylitis and death may result from the acute chest syndrome and abnormal splenic function rendering the infant prone to overwhelming septicemia and to acute splenic sequestration (Serjeant, 2013). Nearly all patients with sickle cell disease will experience vaso-occlusive episodes during their lifetime. The first episode may occur in infancy, often presenting as dactylitis. Classical localizations for vasoocclusive crises in both children and adults are legs and arms, chest, and back (Houwing *et al*, 2019). Ulcers of the lower legs are common, the spleen is enlarged in infancy and early childhood but later is often reduced in size as a result of infarcts (autosplenectomy), pulmonary hypertension is detected and chronic damage to the liver may occur, Pigment (bilirubin) gallstones are frequent. Kidneys necrosis and Osteomyelitis may also occur. (Hoffbrand and Moss, 2011).

2.6.6. Common morbid complications:

2.6.6.1. Vaso-occlusive:

Acute recurrent painful sickle cell crises are caused by vaso-occlusion and ischaemic damage due to obstruction of post-capillary venules, but also due to ischaemia-reperfusion injury (Houwing *et al*, 2019). Through the years, the cumulative damage from vascular occlusion can lead to organ and tissue failure (Turgeon, 2012).

2.6.6.2. Infection:

Bacterial infections are a major cause of morbidity and mortality in children with sickle-cell disease. Several organisms, including *S pneumoniae, H influenza*, and non-typhi *Salmonella* species, have been identified as important causes of infection in developed countries (Rees *et al*, 2010).

2.6.6.3. Acute chest syndrome:

In a patient with sickle cell disease it is generally defined by the development of a new pulmonary infiltrate that is consistent with alveolar consolidation but not atelectasis, involving at least one complete lung segment (Gladwin and Vichinsky, 2008). Thoracic bone infarction is common in patients with SCD who are hospitalized with acute chest pain. The cause of acute chest syndrome is uncertain (Turgeon, 2012).

2.6.6.4. Central Nervous System:

Thrombotic strokes occur more commonly in children, usually without warning. Risk is highest during first decade of life. Recurrence is common (in at least two thirds), usually within 3 years. Older adults may have increased risk of hemorrhagic stroke (Lichtman *et al*, 2017). Cerebral infarction in SCD is associated with an occlusive vasculopathy involving the distal intracranial segments of the internal carotid artery as well as the proximal middle and anterior cerebral arteries (Turgeon, 2012). The vasculopathy seems to start in infancy, with a first-stroke incidence of 1·02 per 100 patient-years between the ages of 2 years and 5 years, and 11% of

patients with sickle-cell disease have had a stroke by the age of 20 years (Rees *et al*, 2010).

2.6.6.5. Renal dysfunction:

Renal dysfunction is almost inevitable in sickle cell disease and starts very early in life with impaired urine concentrating ability and glomerular hyperfiltration. There is a strong tendency for HbS to polymerise in the renal medulla, due to low partial pressure of oxygen, low pH, and high osmolality (Houwing et al, .2019). Complications such as retinopathy, avascular necrosis, neurological decline, leg ulcers, and recurrent priapism are associated with morbidly and impaired quality of life, but renal dysfunction and cardiopulmonary disease are the most lethal (Ware *et al*, .2017).

2.6.7. Sickle-cell crisis:

2.6.7.1. Vaso-occlusive crisis:

The vaso-occlusive crisis is caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ resulting in ischaemia, pain, necrosis, and often organ damage (Vasaikar et al, .2015).

2.6.7.2. Haemolytic crises:

Characterized by an increased rate of haemolysis with a fall in haemoglobin but rise in reticulocytes and usually accompany a painful crisis (Hoffbrand and Moss, 2011). Intravascular haemolysis in SCD results from complement recognition of sickling-induced membrane changes, cell dehydration and direct membrane damage by rigid haemoglobin polymers (Schnog et al, 2004).

2.6.7.3. Aplastic crises:

Occur when erythropoiesis is suppressed. Because red cell survival is greatly shortened in sickle cell disease, even temporary reduction in erythropoiesis is rapidly manifested by a dramatic fall in blood hemoglobin concentration (Lichtman *et al*, 2017).

2.6.7.4. Sequestration crisis:

Usually occurs in children with chronically enlarged but normal functioning spleen. Sudden trapping of blood in spleen or liver causes rapid enlargement of the organ with resultant drop in hematocrit and hypovolemic shock (Nayak *et al*., 2011).

2.6.8. Laboratory diagnosis:

Sickle cell syndromes are suspected on the basis of hemolytic anemia, RBC morphology (Fig 2-3), and intermittent episodes of ischemic pain. (Longo, .2010).

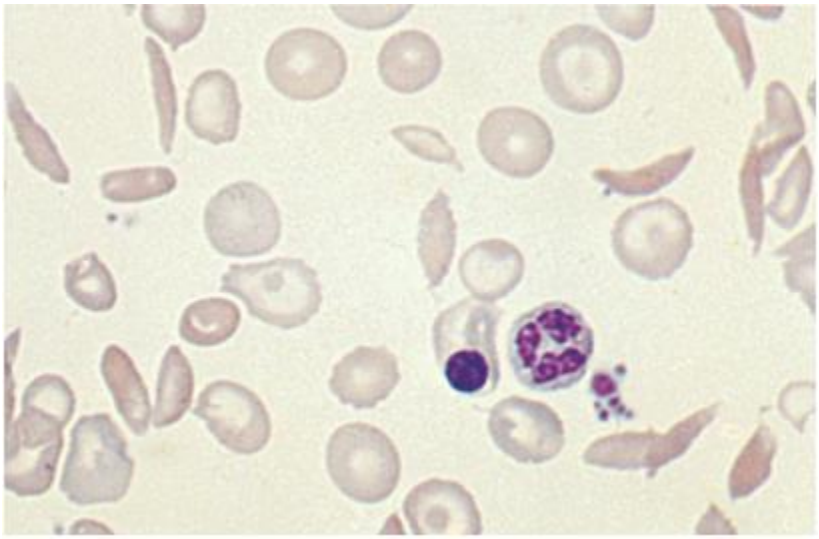

Figure (2-3):

Sickle cell anemia. The elongated and crescent-shaped red blood cells seen on this smear represent circulating irreversibly sickled cells. Target cells and a nucleated red blood cell are also seen. (Longo, .2010).

The Hb level is usually between 5 and 11 g/dL. Anemia is normochromic and normocytic, but considerable variation in red cell size and shape is noted **(Fig 2-4)**. Reticulocytosis is almost always present, Leukocytosis and thrombocytosis are common, even in patients without acute problems (Lichtman *et al*, 2017). Diagnosis of haemoglobinopathies is based on the detection of HbS in relation to HbA and HbF. The most commonly used methods are electrophoresis (gel- or capillary-based), high-pressure liquid chromatography (HPLC), isoelectric focusing and molecular approaches such as PCR (Houwing et al, .2019). Screening tests for sickling are positive when the blood is deoxygenated (e.g. with dithionate andNa2 HPO4)

(Hoffbrand and Moss, 2011).

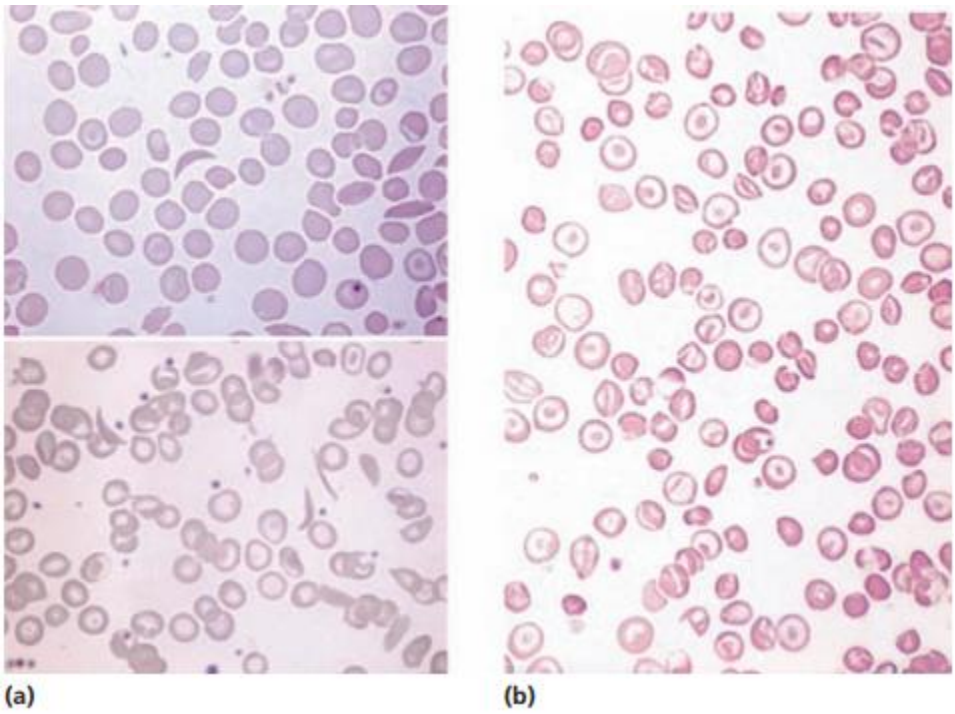

Figure (2-4): (a) Sickle cell anaemia: peripheral blood films showing deeply staining sickle cells, target cells and polychromasia. **(b)** Homozygous Hb C disease: peripheral blood fi lm showing many target cells, deeply staining rhomboidal and spherocytic cells (Hoffbrand and Moss, 2011)

2.6.9. Management:

The management of SCD begins by informing couples at high risk of conceiving children with SCD about the possibilities of prenatal diagnostic testing. Early detection of patients with SCD by newborn screening programmes enables early provision of comprehensive care, which in itself will improve the quality of life and survival of this patient population (Schnog *et al*, .2004).

- Management of Complications

Patients in vascular crises should be kept warm and given adequate hydration and pain control; oxygen is beneficial only for hypoxic patients. Overhydration should be avoided. The period of crisis usually resolves in hours to days. Hydroxyurea therapy may be considered for prevention or decreased frequency of recurrences. (Lichtman *et al*, 2017). The protective effect of sickle-cell trait does not apply to people with sickle cell disease; in fact, they are more vulnerable to malaria, since the most common cause of painful crises in malarial countries is infection with malaria. It has therefore been recommended that people with sickle cell disease living in malarial countries should receive anti-malarial chemoprophylaxis for life (Vasaikar et al, .2015). When an acute stroke is diagnosed, immediate exchange transfusion should be performed, followed by regular transfusion therapy to prevent stroke recurrence (Houwing et al, .2019). Prophylactic – avoid those factors known to precipitate crises, especially dehydration, anoxia, infections, stasis of the circulation and cooling of the skin surface (Hoffbrand and Moss, 2011). For severely affected patients, judicious use of red cell transfusions may be the most powerful therapeutic for preventing major SCD-related complications, and general transfusion guidelines for SCD have recently been published (Houwing et al, .2019). Chronic complications of SCD can involve most organs and organ systems during the lifespan of affected individuals. In addition, certain acute complications, such as stroke and priapism, often do not resolve completely but evolve into subacute or

chronic phases that require special approaches to management (Vichinsky et al, .1995). Many cytotoxic drugs increase fetal haemoglobin concentrations, which is potentially beneficial in patients with sickle-cell disease. Hydroxycarbamide was chosen for studies of sickle-cell disease because of its oral efficacy and low toxic effects (Rees *et al,* 2010). The mainstay of treatment for sickle cell disease is erythrocyte transfusions, with more than 90% of adults receiving at least one transfusion in their lifetimes (Ware *et al*, .2017). Erythrocyte transfusion has an established role in the management of both acute and chronic complications in sickle-cell disease (Rees *et al,* 2010). Bed rest, elevation, and zinc sulfate dressings are used to treat leg ulcers. Iron overload is managed by iron chelators: desferrioxamine given subcutaneously at a dose of 25 to 40 mg/kg per day or deferasirox given orally at a dose of 20 to 40 mg/kg per day (Lichtman *et al*, 2017). Bone marrow transplants have proven to be effective in children. However, bone marrow transplants are difficult to obtain because of the specific HLA typing necessary (Vasaikar et al, .2015). Urinary tract infection in these patients should be treated aggressively. Patients with ESRD should be on regular EBT especially if renal transplant is being planned. End stage renal disease is managed with repeated dialysis, erythropoietin therapy, and/or renal transplant (Adewoyin, 2015). Senicapoc, selectively blocks the calcium-activated, potassium efflux (Gardos) channel, and improves anemia and hemolysis in patients with SCD (Ataga and Desai, .2018).

2.7. Sickle Cell Trait:

In sickle cell trait, less than half of the Hb in each red blood cell is Hb S (approximately 40%) and the rest is normal Hb, principally A. This effectively protects against sickling except under special circumstances, such as severe hypoxia or the hyperosmolarity encountered in the renal circulation (Lichtman *et al*, 2017). Sickle cell trait provides a survival advantage over individuals with normal hemoglobin in regions where malaria, *P. falciparum,* is endemic. Sickle hemoglobin impairs malaria growth. Sickle cell trait does not provide absolute protection, but individuals are more likely to survive the acute illness (Turgeon, 2012).

2.8. Haemostasis:

Haemostasis, , comes from the Greek roots, haeme meaning blood and stasis meaning causing to stop, defined as the arrest of bleeding (Thornton and Douglas, 2010). Hemostasis is a complex and tightly regulated process whereby the body attempts to maintain a homeostatic balance to permit normal blood flow, without bleeding or thrombosis. (Bonar *et al*, .2017).

2.9. Component of haemostatic system:

The haemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process for fibrinolysis. The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels (Hoffbrand and Moss, 2011).

2.9.1. Platelets:

Platelets are extremely small and discoid, 3.0 × 0.5 μm in diameter, with a mean volume of 7 – 11 fL. The ultrastructure of platelets is represented in Figure (2-5). The glycoproteins of the surface coat are particularly important in the platelet reactions of adhesion and aggregation which are the initial events leading to platelet plug formation during haemostasis (Hoffbrand and Moss, 2011).

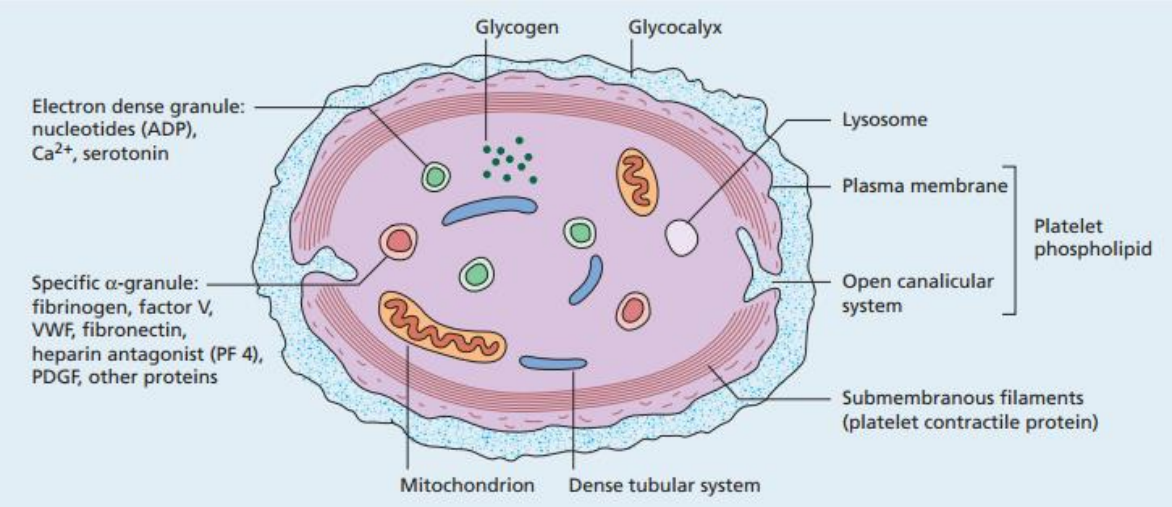

Figure (2-5): The ultrastructure of platelets. ADP, adenosine diphosphate; PDGF, platelet-derived growth factor; PF, platelet factor; VWF, von Willebrand factor (Hoffbrand and Moss, 2011).

The main steps in platelet function are adhesion, activation with shape change and aggregation. When the vessel wall is damaged, the subendothelial structures, including basement membrane, collagen and microfibrils, are exposed (Bain *et al*, .2011).

- **Platelet granules:**

Platelet activation leads to secretion of granule contents and to the formation of microvesicles by shedding of membranes from the cell surface. (Heijnen *et al*, 1999). **α Granules** are stained by Wright Giemsa stain and contain fibrinogen, fibronectin, factor V, vWF, platelet factor-4 (PF-4), platelet-derived growth factors (PDGF), transforming growth factor-β (TGF-β), and thrombospondin (Wahed and Dasgupta, .2015).

Dense granules (also known as dense bodies or δ granules) are electron dense due to the presence of calcium and appear as dark bodies under the electron microscope. Dense granules are present in low numbers (, 10 per platelet) and contain adenosine triphosphate (ATP), ADP, ionized calcium, histamine, serotonin (5-hydroxytryptamine (5-HT)), and epinephrine. **Lysosomes** contain acid hydrolases. The organelle zone of platelets also contains glycogen and mitochondria. (Wahed and Dasgupta, .2015).

The role of Platelets in haemostatic process:

Platelet adhesion to exposed subendothelium is a complex process that involves multiple adhesive ligands and receptors, Platelet activation is the process where platelets change shape, release the contents of alpha and dense granules, and express receptors on their surface to facilitate platelet aggregation (McMichael, 2005). Platelet aggregation occurs when platelets interact with one another to provide the catalytic surface necessary for thrombin generation and subsequently fibrin formation, which stabilizes the hemostatic plug (McMichael, 2005). The initial tethering of platelets at sites of vascular injury is mediated by glycoprotein Ib/V/IX, a structurally unique receptor complex expressed in megakaryocytes and platelets. Von Willebrand factor is the major ligand for one component of this complex, glycoprotein Ib, and the absence of the factor causes defects in primary hemostasis and coagulation **(**Davì and Patrono, .2007). Identification of the platelet receptors

for thrombin is critical for understanding thrombosis and haemostasis. Protease-activated receptor-1 (PAR1) is important for activation of human platelets by thrombin (Kahn et al, .1998). Fibrin, formed upon coagulation, stabilises the platelet plug during the haemostatic response. Also, in thrombosis, aggregated platelets and fibrin form the main constituents of intra-arterial thrombi (Heemskerk et al, .2002).

2.9.2. Blood vessels:

Blood vessels, have walls consisting of three concentric layers (tunicae). The intima (tunica intima) is the innermost layer. Its main component, the endothelium, lines the entire vascular tree, including the heart and lymphatic vessels. The media (tunica media) contains muscle cells, elastic fibers and collagen. While it is the thickest layer in arteries. The adventitia (tunica adventitia) is the outer coat of the vessel and consists of connective tissue, nerves and vessel capillaries (vasa vasorum) (Standring, 2016). Functions to transport vital materials such as oxygen, nutrients, and waste products, including carbon dioxide, hormones, defense elements, and cells involved in wound healing (Chung *et al*, .2015). The intimal surface of healthy endothelium is both anticoagulant and antithrombotic: endothelial cells secrete a variety of molecules important for the regulation of blood coagulation and platelet functions. The major antiplatelet agents secreted by endothelial cells are prostacyclin (PGI2) and nitric oxide (NO) both synergistically increase cAMP content in platelets, hence preventing their aggregation (Michiels, 2003). It also expresses ecto-adenosine diphosphatase, which degrades adenosine diphosphate and inhibits platelet aggregation; thrombomodulin, serves as a binding site for thrombin to activate protein C; and heparin-like molecules. Also secretes tissue plasminogen activator, which activates the fibrinolysis system. Endothelium secretes von Willebrand factor, which mediates platelet adhesion and shearstress-induced aggregation (Wu and Thiagarajan, 1996).

2.9.3. Coagulation factors:

These plasma glycoproteins, including factor XII, factor XI, factor IX, factor X, factor VII, and prothrombin, are zymogens of serine proteases. As a family, these proteins bear marked structural and functional homology to the digestive proteases trypsin and chymotrypsin. They are each converted from an inactive form to an active enzyme by limited proteolysis of one or two peptide bonds (Furie and Furie, 1988).

2.9.3.1 Classification of coagulation factors: Table (2-2)

Classification of coagulation factors :		
Fibrinogen family	**Vitamin K dependent**	**Contact family**
Fibrinogen	Factor II	Factor XI
Factor V	Factor VII	Factor XII
Factor VIII	Factor IX	HMWK
Factor XIIII	Factor X	Prekallikerin

HMWK – High molecular weight kininogen (Palta *et al*, .2014).

2.9.3.2. Coagulation cascade:

Blood coagulation involves a biological amplification system in which relatively few initiation substances sequentially activate by proteolysis a cascade of circulating precursor proteins (the coagulation factor enzymes) which culminates in the generation of thrombin; this, in turn, converts soluble plasma fibrinogen into fibrin (Hoffbrand and Moss, 2011). The initiation of clotting begins with the activation of two enzymatic pathways that will ultimately lead to fibrin formation: the intrinsic and extrinsic pathways. The common pathway is the point at which the intrinsic and extrinsic pathways come together (Ciesla, 2007).

Extrinsic pathway: It is considered as the first step in plasma mediated haemostasis. It is activated by TF, which is expressed in the subendothelial tissue. Under normal physiological conditions, normal vascular endothelium minimizes contact between

TF and plasma procoagulants, but vascular insult expose TF which binds with factor VIIa and calcium to promote the conversion of factor X to Xa (Palta *et al*, .2014).

Intrinsic pathway: Contact activation is initiated by changes induced by vascular trauma. Prekallikrein is required as a cofactor for the autoactivation of factor XII by factor XIIa. XI is activated and requires a cofactor of HMWK. XIa activates IX to IXa, which in the presence of VIIIa converts X to Xa. Also present are platelet phospholipids PF3. Calcium is required for the activation of X to proceed rapidly (Ciesla, 2007).

Common pathway: Activated factor X along with its cofactor (factor V), tissue phospholipids, platelet phospholipids and calcium forms the prothrombinase complex which converts prothrombin to thrombin. This thrombin further cleaves circulating fibrinogen to insoluble fibrin and activates factor XIII, which covalently crosslinks fibrin polymers incorporated in the platelet plug. This creates a fibrin network which stabilizes the clot and forms a definitive secondary haemostatic plug (Palta *et al,* .2014).

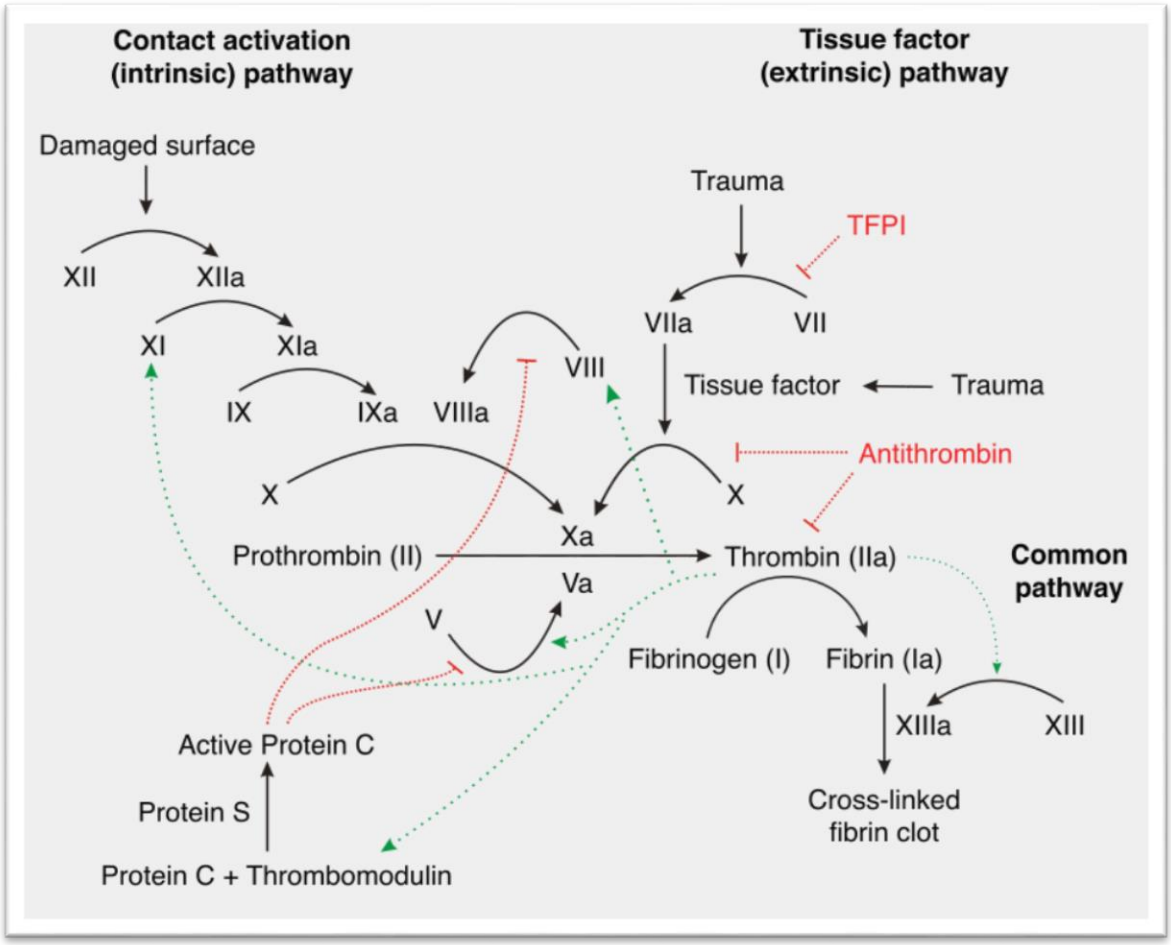

Figure (2-6): "coagulation cascade"

2.9.4. Fibrinolytic system:

The fibrinolytic system is responsible for the dissolution of a clot. Fibrin clots are not intended to be permanent. The purpose of the clot is to stop the flow of blood until the damaged vessel can be repaired (Ciesla, 2007). The plasminogen (fibrinolytic) system comprises an inactive proenzyme, plasminogen, that can be converted to the active enzyme, plasmin. Plasmin degrades fibrin and activates matrix metalloproteinases (MMPs) that, in turn, degrade the extracellular matrix (ECM) (Collen, 1993). Two immunologically distinct physiologic plasminogen activators (PA) have been identified: the tissue-type PA (t-PA) and the urokinase

type PA (u-PA). t-PA–mediated plasminogen activation is mainly involved in the dissolution of fibrin in the circulation. u-PA binds to a specific cellular receptor (u-PAR) resulting in enhanced activation of cell bound plasminogen (Lijnen, 2001). Poor control of fibrinolysis can result in excessive bleeding or alternatively accelerate thrombosis development. (Bonar et al, .2017).

2.9.5. Physiological limitation of blood coagulation (Natural Inhibitors):

Inhibitors are soluble plasma proteins that are natural anticoagulants. They prevent the initiation of the clotting cascade. There are two major inhibitors in plasma that keep the activation of coagulation under control.

These inhibitors are:

1. Protease inhibitors: inhibitors of coagulation factors, which include • Antithrombin • Heparin cofactor II • Tissue factor pathway inhibitor • Alpha-2 antiplasmin •C1

2. The protein C pathway: inactivation of activated cofactors, which includes • Protein C and protein S (Ciesla, 2007).

The anticoagulant system exerts a regulatory role over the procoagulant activity in blood thus localizing the thrombus formation (Colvin, 2004). The main anticoagulant mechanisms naturally present in the body include the following:

Antithrombin: (AT), previously known as AT III is the main inhibitor of thrombin. It is a serine protease inhibitor, which binds and inactivates thrombin, factor IXa, Xa, XIa and XIIa. The enzymatic activity of AT is enhanced in the presence of heparin. Other thrombin inhibitors are heparin cofactor II, α2 macroglobulin and α1-antitrypsin (Ezihe-Ejiofor and Hutchinson, 2013).

Tissue factor plasminogen inhibitor: It is a polypeptide produced by endothelial cells. It acts as a natural inhibitor of the extrinsic pathway by inhibiting TF-VIIa

complex (Price et al, .2004). Protein S enhances the interaction of factor Xa in the presence of calcium and phospholipids (Dahm *et al*, .2008).

Heparin Cofactor II: Heparin cofactor II is another coagulation inhibitor. It acts against thrombin, and it is heparin dependent. Heparin cofactor deficiency alone is not associated with thrombosis. (Ciesla, 2007).

Protein C: Protein C is a circulating vitamin K-dependent serine protease that is synthesized in the liver. It is converted to activated protein C (APC) by thrombin. APC is a potent anticoagulant and degrades FVa and FVIIIa (with protein S and phospholipid as cofactors), thereby limiting coagulation. The action of APC is limited by protein C inhibitor, a2- macroglobulin, and a1-antitrypsin. (Ezihe-Ejiofor and Hutchinson, 2013).

Protein Z dependent protease inhibitor/protein Z (PZI): It is a recently described component of the anticoagulant system that is produced in the liver. It inhibits Factor Xa in reaction requiring PZ and calcium (Corral *et al*, .2007).

Natural inhibitors of platelets:

Nitric oxide: Produced by the vascular endothelium. It is the most important vasodilator which prevents platelets aggregation (Bredt and Snyder, 1994). ADPase: Present on the endothelial cell surface, it metabolizes ADP released from activated plts leading to inhibition of plts aggregation (Safier and Gayle, 1997). Prostacyclin: Released from endothelial cells. It lowers free ionized Ca2+ thus prevents plts adhesion and aggregation to the intact blood vessel (Hoffbrand and Moss, 2016).

Natural inhibitors of fibrinolytic system:

Plasminogen Activator Inhibitor-1: Rapid inhibition of both t-PA and u-PA in normal human plasma (Lijnen, 2001). α2-Antiplasmin is the main physiological plasmin inhibitor in human plasma, whereas plasmin formed in excess of α2-antiplasmin may be neutralized by α2- macroglobulin (Holmes et al, .1987).

2.10. Screening tests of blood coagulation:

Screening tests provide an assessment of the ‘extrinsic’ and ‘intrinsic’ systems of blood coagulation and also the central conversion of fibrinogen to fibrin. (Hoffbrand and Moss, 2011).

2.10.1. The prothrombin time (PT):

Measures factors VII, X, V, prothrombin and fibrinogen (extrinsic and common coagulation pathways). Tissue thromboplastin (a brain extract) or [synthetic] tissue factor with lipids and calcium is added to citrated plasma. The normal time for clotting is 11 – 16 s. It may be expressed as the international normalized ratio (INR).

2.10.2. The activated partial thromboplastin time (APTT):

Measures factors VIII, IX, XI and XII in addition to factors X, V, prothrombin and fibrinogen (intrinsic and common coagulation pathways). Three substances – phospholipid, a surface activator (e.g. kaolin) and calcium – are added to citrated plasma. The normal time for clotting is approximately 30 – 40 s (Hoffbrand and Moss, 2011).

2.10.3. Other coagulation test: (Bain *et al,* .2012).

- Full blood count.
- Platelet count.
- Thrombin time.
- Fibrinogen assay.
- D-dimer.

2.11. Hypercoagulability state in sickle cell disease:

A large number of coagulation abnormalities have been described in SCD. Thus, activation of coagulation has been shown to be a feature of SCD, both in the steady state and during painful crisis, with rates of thrombin and fibrin formation constantly higher than normal. Levels of plasma fibrinogen and factor VIII were also found to be increased, while protein C and protein S were reduced in the steady state and further diminished during crisis (Nsiri *et al*, .1996). Chronic hypercoagulable or prothrombotic state is generally known to be one of the factors that contribute to vaso-occlusion and progressive end-organ damage in sickle cell disease (SCD) (Ataga and Orringer, .2003). Studies on SCD patients at steady state patients from different geographic and demographic origins have shown elevated level of markers of coagulation activation, and decreased natural anticoagulant proteins (Wright *et al*, .1997). Agents that physiologically activate platelets and coagulation in vivo include adenosine diphosphate (ADP), collagen, epinephrine, thrombin, serotonin, arachidonic acid and thromboxane A2 (Zhou and Schmaier, 2005). Sickle cell patients with coagulation disorders may have alteration of the APTT (Activated partial thromboplastin time), PT (Prothrombin time) or both. A patient with prolonged APTT and a normal PT is considered to have a defect in the "Intrinsic" coagulation pathway. The name indicates that all components of the APTT test except Kaolin are "Intrinsic" to the plasma. On the other hand, a patient with prolonged PT and a normal APTT has a defect in the "extrinsic" coagulation pathway. (Tissue factor is extrinsic to the plasma). Prolongations of both the APTT and PT suggest that the defect lies in a common pathway. Moreover, alteration of thrombin time (TT) also shows a defect in the common pathway *(Maduabuchi, 2010).*

2.12. Previous studies:

study on haemostatic alterations in patients of sickle cell trait and homozygous sickle cell disease: A hospital based case control study conducted by (*Nilesh*, et al., 2014) in India, aim was to determine the mean levels of platelet indices, Prothombin time (PT), activated Thromboplastin time (APTT) and fibrinogen in patients with homozygous sickle cell disease (HbSS), sickle cell trait (HbAS) and normal controls (HbAA) and their role as prognostic markers. The study included 321 cases of sickle cell haemoglobinopathies (118 HbSS and 203 HbAS) and 321 normal controls. Platelet indices were determined by automated cell counter. PT, APTT and fibrinogen levels were estimated by using commercial agents and BK coagulometer. The results showed mean fibrinogen levels were 275.56, 357.37 and 522.24 mg/dl respectively in HbAA controls, HbAS and HbSS patients. The fibrinogen levels in HbSS patients were found to be raised even more in those in crisis. Mean platelet volume (MPV), Platelet distribution width (PDW) and PT and APTT values were also significantly prolonged in these patients. In conclusions: Since, fibrinogen levels showed a higher increase in crisis, its estimation can be used as a parameter to monitor progression of sickle cell crisis. We obtained high MPV and PDW in HbSS patients as compared to controls; larger platelets are more thrombogenic, we propose a hypothesis that larger platelets in HbSS patients may predispose them to vaso-occlusive crisis (*Nilesh*, et al., 2014)

(Raffini *et al*.,2006) conducted a retrospective review of pre-operative coagulation studies in pediatric patients with SCD followed by a prospective study of 100 well children with SCD to determine the prevalence of abnormal coagulation screening tests.In the retrospective study, 38.1% had a prolonged prothrombin time (PT), compared to those in the prospective study. Prolongations of the activated partial thromboplastin time (APTT) were less common. Children in the prospective study with prolonged PTs had significantly lower levels of Factor V and VII compared to

those with normal PTs. They conclude that children with SCD admitted for surgical procedures were more likely to have prolonged PTs than those tested at a well visit. (Raffini *et al*., 2006)

Buseri FI, et al (2006) studied the plasma levels of some blood coagulation parameters; prothrombin time, (PT), partial thromboplastin time with kaolin (PTTK), thrombin clotting time, (TCT), fibrinogen and factor X assay were determined in 50 Nigerian homozygous (HbSS) patients and 50 HbAA healthy individuals for the purpose of assessing their baseline values and susceptibility of patients with sickle cell disease (SCD) to hypercoagulability. . The mean age of the study participants was 21.7 +/- 5.0 years. The mean PT of in HbSS patients was found to be significantly longer than the mean PT value in HbAA control subjects .The mean PTTK values of in HbSS patients was also found to be significantly higher than the recorded among the control subjects .The mean TCT in HbSS patients was however found to be significantly lower than the mean value obtained in the control group. Fibrinogen level in HbSS patients and in HbAA controls was also found to be significantly different .Factor X level in the sickle cell patients, was equally found to be significantly lower than that of the apparently healthy HbAA control individuals.

Chapter Three

Materials and Methods

Chapter Three

Materials and Methods

3.1. Study design:

This is a case control study conducted in the period from August to October 2019. The study was carried out at Khartoum State in the Sickle Cell Clinic of Gaffer Ibn Auf Children Specialized Hospital, and al-ribat university hospital.

3.2. Study population:

The study population comprises two groups of children in different age groups, both sexes was included. fourty one of patients included the children who are known to have sickle cell anemia (HbSS) and nine of patients included the children who are known to have sickle cell trait (HbAS) and fifty of healthy children (HbAA) as control group.

3.2.1. Inclusion criteria:

Children already confirmed SCA based on hemoglobin electrophoresis.

3.2.2. Exclusion criteria:

Patients with any type of infective illness.

Patient with recent blood transfusion during the preceding 3 months.

Patients who parents or caregiver refused to participate in the study.

3.3. Data collection tools:

Data was collected using questionnaire included age and sex.

3.4. Samples collection:

2.5 ml of venous blood samples was collected, and added slowly to 0.25 ml of 0.38% trisodium citrate for coagulation tests.

3.5. Methodology:

3.5.1. Prothrombien time (PT):

3.5.1.1. Principle:

The PT test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiecy of extrinsic clotting system. Although originally thought to measure prothrombin, the test is now known to depend also on reactions with factors V, VII, and X and on fibrinogen concentration of the plasma (Dacie *et al, .2006*).

3.5.1.2. Reagents and equipments:

Thromboplastin with calcium reagent.

System control (control plasma, commercial and pool plasma, normal and pathological levels). Parameters programming:

From the menu select test parameters by pressing the (C) key and confirm with (YES) Select PT by pressing (1) and confirm with (YES).

3.5.1.3. Procedure:

1. Centrifugation of blood sample at 2000 r for 15 min at 4c to prepare Platelet Poor Plasma (PPP).
2. Clean plastic cuvette was applied on the pre-warming area of the device.
3. 50 µl of ppp was pipetted into the cuvette, incubated for 2 minutes at 37c, and then transferred to the measuring area.
4. 100 µl of pre warmed BioMed- LIQUIPLASTIN reagent was added and the timer of the coagulometer was pressed to start.
5. When the clot was formed the timer stop and the result was recorded.

3.5.2. Activate partial thromboplastin time (APTT):

3.5.2.1. Principle:

The test measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin and so indicates the overall efficiency of

The intrinsic pathway. The test depends not only on the contact factors and on factors VIII and IX, but also on the reaction with factors X, V, prothrombin, and fibrinogen. It is sensitive to the presence of circulating anticoagulants (inhibitors) and heparin. (Dacie *et al*, .2006).

3.5.2.2. Reagents and equipments:

CaCl2 0.025 M

System control (control plasma, commercial and pool plasma, normal and pathological levels). Parameters programming:

From the menu select test parameters by pressing the (C) key and confirm with (YES) Select APTT by pressing (3) and confirm with (YES).

3.5.2.3. Procedure:

1. Centrifugation of blood sample at 2000 r for 15 min at 4c to prepare Platelet Poor Plasma (PPP).
2. Clean plastic cuvette was applied on the pre-warming area of the device.
3. 50 µl of ppp is pipetted into the cuvatte and 50 µl of pre warmed Bio-Med APTT reagent is added. The mixture was incubated at 37oc for 3 minutes, then transferred to the measuring area.
4. 50 µl of pre-warmed CaCl2is then added and simultaneously the timer was started.
5. When the clot was formed the timer stopped and the result was recorded.

3.5.3. Platelet count:

Platelets count obtained from records.

3.6. Ethical approval:

- Ethical clearance from the ethical committee of the Sudan University of Science and Technology, College of Graduate Studies.
- Consent was taken from parents /care givers of all children participated in the study.
- Permission was obtained from Administration of the study area to conduct the study.

3.7. Data analysis:

The data was extracted from the questionnaires and the lab records into a major spread sheet and then fed on the statistical software SPSS version 20. Descriptive statistics and probability testing was done by using independent T-test. The results obtained were presented in tables and figures. Level of significance was set at <0.05. The mean was calculated ±SD.

Chapter Four

Results

Chapter Four

Results

4. Results:

One hundred subjects of age between one to eighteen years were enrolled in this study divided into cases group (SCD steady state) and control group. Cases group composed of fifty SCD (41 HbSS and 9 HbAS) patients with mean of age 7.9 ± 4.5, 27 (54%) were males and 23 (46%) were females. Fifty were healthy subjects (HbAA) comprised the control group with mean of age 8.5±5, 54% were males and 46% were females (Table4-1). PT and APTT were measured using DIA-TIMER 2, platelets count were obtained from records.

Mean level of platelets count (cumm) were 389±204, 281±71 in the cases and controls respectively, *P. value* was 0.01. Statistically there was significant deferens between study group and control group in platelets count, increased in patients than controls. PT (seconds) mean and SD were 15.4±1.7, 14.6±0.8 in cases and healthy subjects respectively, *P. values* were 0.000 and APTT (seconds) mean and SD were 40.8±4.9, 37.1±3.3 in cases and healthy subjects respectively, *P. values* were 0.000 (Table 4-2).

PT mean of males and females of the cases group were 15.9±1.5 and 15.5±1.9 respectively, *P. value* was 0.491 and mean of APTT in males and females were 39.7±4.6 and 41.9±5 respectively, *P. value* was 0.112 (Figure 4-1).

PT mean of males and females of control group were 14.4±0.8 and 14.7±0.8 respectively, *P. value* was 0.349 and mean of APTT in males and females were 36.3±2.7 and 38.1±3.5 respectively, *P. value* was 0.043.

There was no statistical correlation between PT and age in cases *P. value* 0.60 but there was statistical correlation between APTT and age *P. value* 0.038 (Figure 4-6).

In controls there was no statistical correlation in PT and APTT *P. value* 0.177 and 0.578 respectively.

The age grouped in three age groups and age group (7-12year) high frequent 22(44%), followed by age group (1-6 year) 20(40%) and age group (13-18year) was lowest 8(16%). One way ANOVA test showed comparison between age groups and PT, APTT in both cases and controls P. value 0.064 and 0.098. Respectively 0.551 and 0.790 (Figure 4-3), (Figure 4-4).

The mean level of PT in SS Hb type 15.6±1.8 and in AS Hb type 16.4±1.1 P. *value 0.227* And mean level of APTT in SS Hb type 41.2±5 and in AS Hb type 38.5±6 P. value 0.140 (Table 4-3).

Table (4-1): Mean ± SD of age and gender distribution among the study groups:

Study group	Mean ± SD of age	Male	Female	Total
Case group	7.9 ± 4.5	27(54%)	23(46%)	50
Control group	8.5±5	27(54%)	23(46%)	50
Total		54	46	100

Table (4-2): comparison of PT, APTT, platelets count between case and control groups:

Subject	case group (N = 50)	control group (N = 50)	*P. value*
PT	15.4±1.7	14.6±0.8	0.000
APTT	40.8±4.9	37.1±3.3	0.000
Platelets count	389±204	281±71	0.01

Table (4-3): Mean ± SD of PT and APPT in case group according to Hb type:

Hb type	AS	SS	*P. value*
PT	16.4±1.1	15.6±1.8	0.227
APTT	38.5±6	41.2±5	0.140

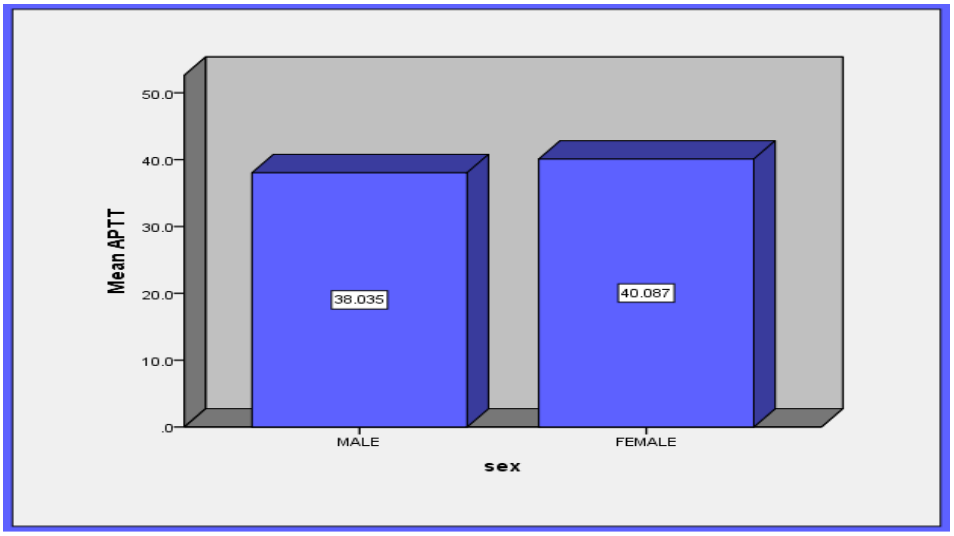

Figure (4-1): Comparison mean level of PT in males and females in case and control group.

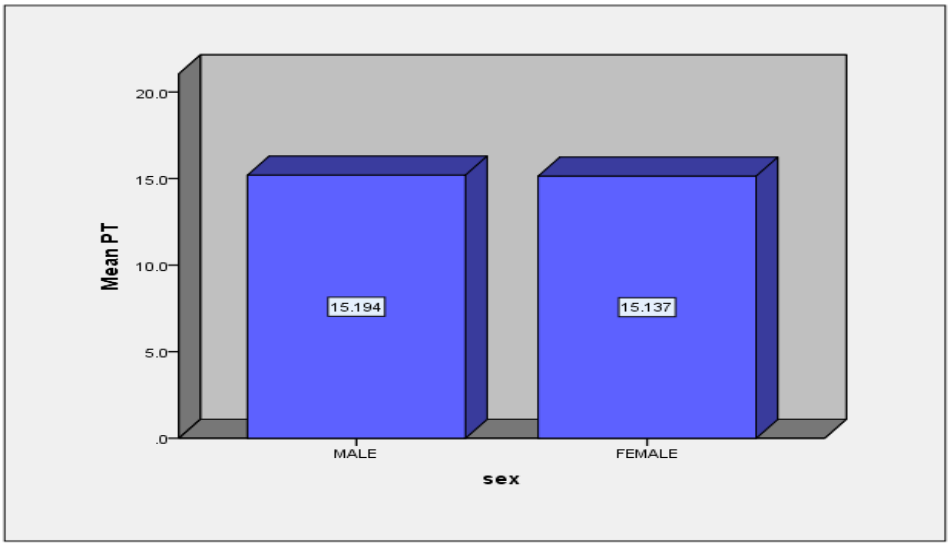

Figure (4-2): Comparison mean level of APTT between males and females in case and control group.

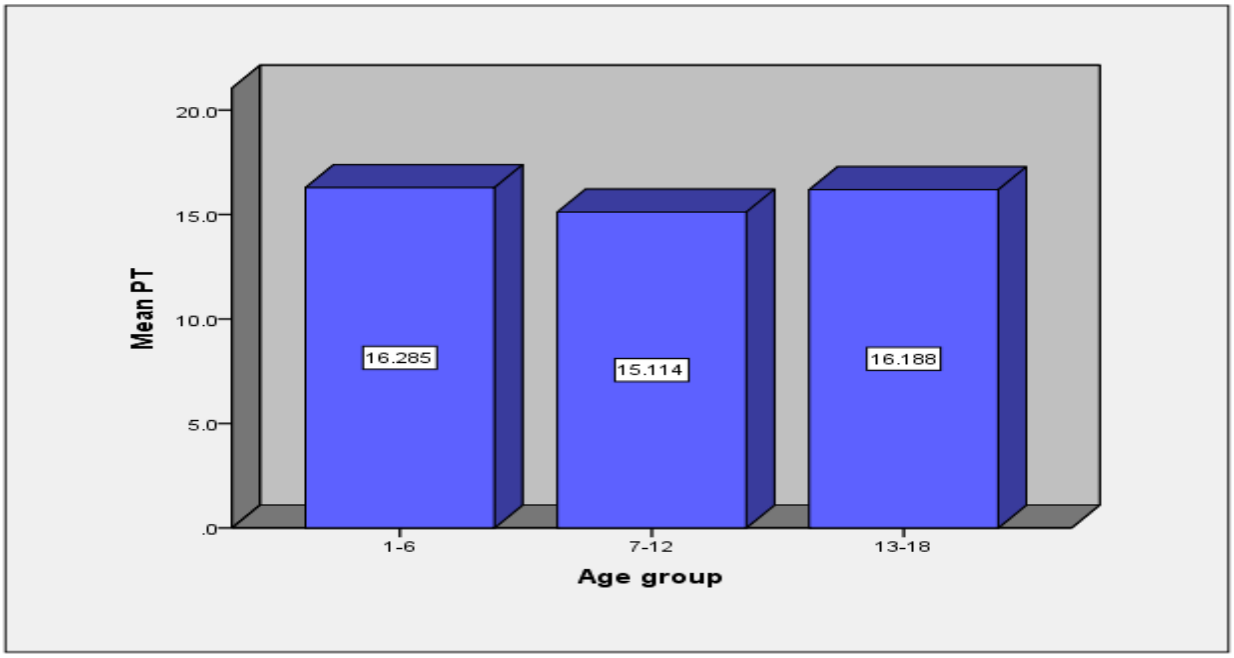

Figure (4-3): Mean level of PT according to age group in case group (P. value 0.064).

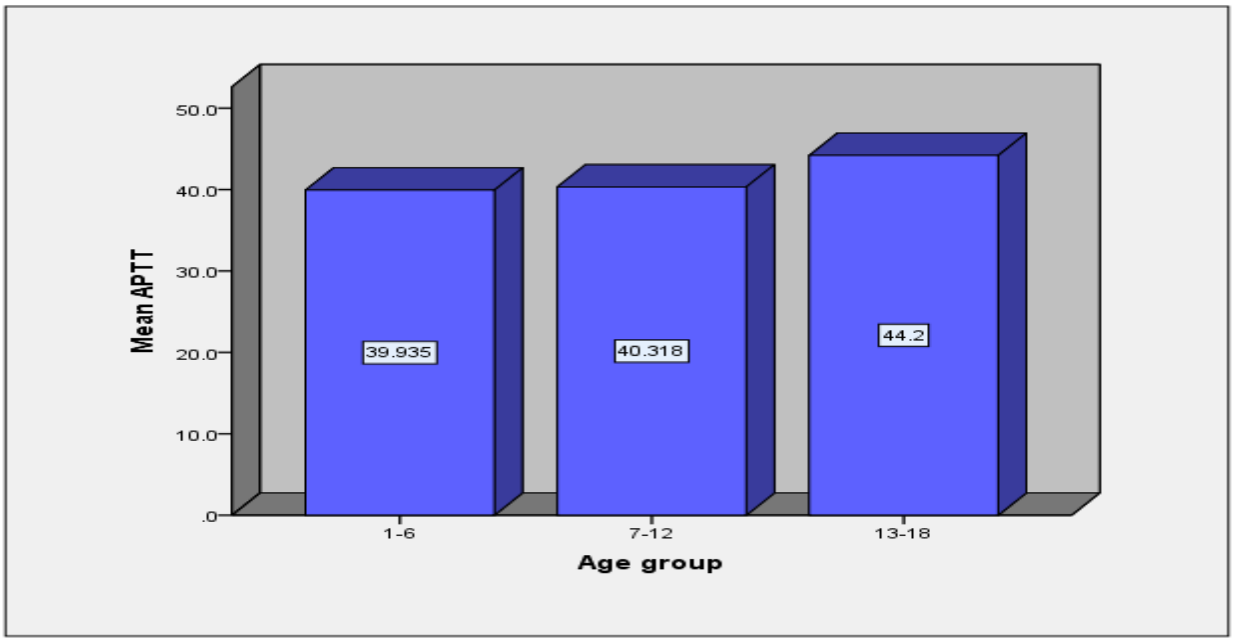

Figure (4-4): Mean level of APTT according to age group in case group (*P. value* 0.098).

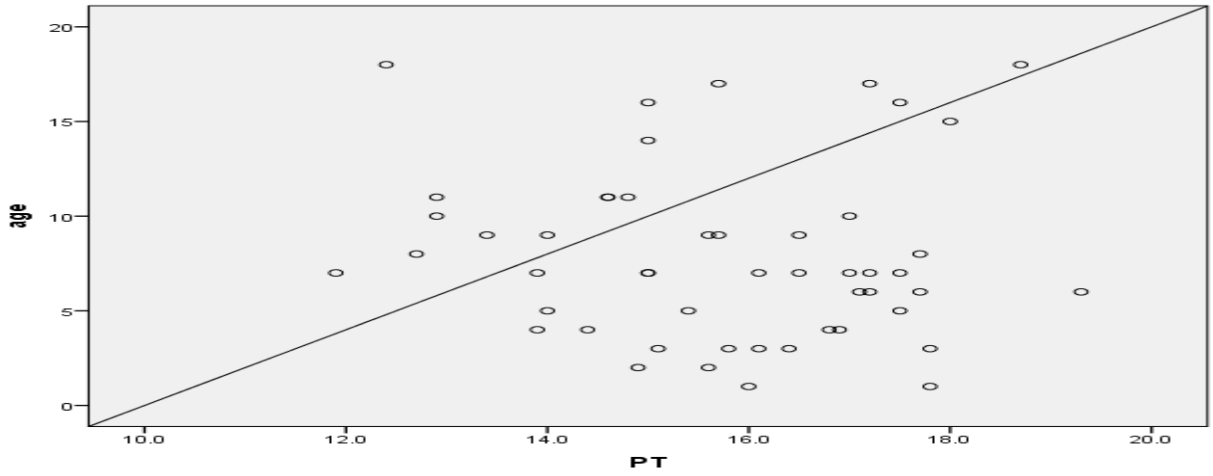

Figure (4-5): correlation between PT level and age of cases. There was no correlation between PT level and age of cases *P. value* 0.60.

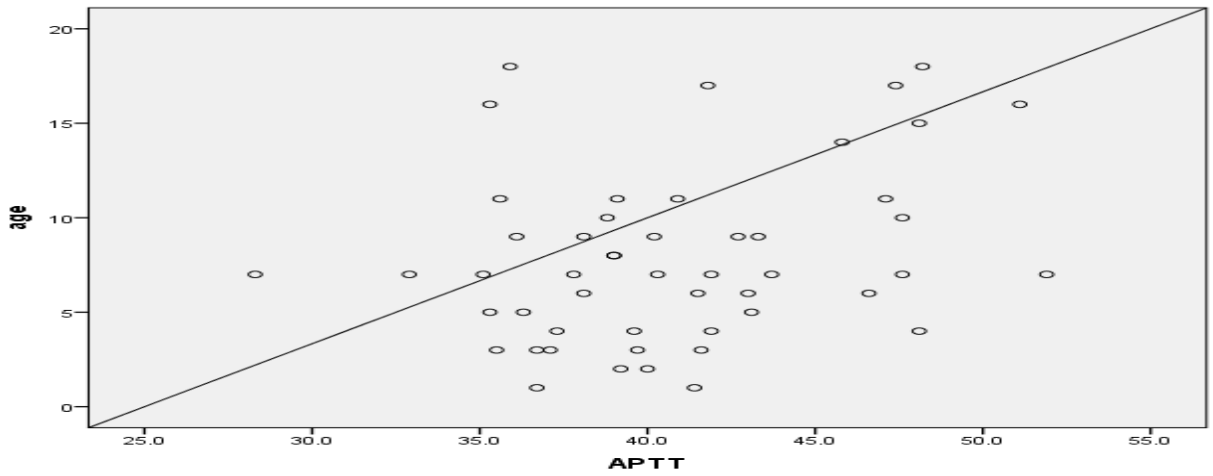

Figure (4-6): correlation between APTT level and age of cases. There was correlation between APTT level and age of cases *P. value* 0.038

Chapter Five

Discussion, Conclusions and Recommendations

Chapter Five

Discussion, Conclusions and Recommendations

5.1. **Discussion**:

Sickle cell anaemia is considered to be a hypercoagulable state that contributes to the morbidity associated with the disease. Numerous mechanisms can attribute to this hemostatic activation among these patients, and its results in a myriad of metabolic nutritional, haematological and clinical effect. The study evaluated the effect of sickle cell anaemia on blood constituents, estimating mean of prothrombin time (PT), activated partial thromboplastin time (APTT) and mean of platelet count in children with sickle cell anaemia. These parameters were compared with those in normal HbAA controls. In the present study PT and APTT were measured in fifty SCA patients (during steady state) (82% are HbSS and 8% are HbAS) and in fifty apparently health subjects (HbAA). Higher levels of platelet count in cases compared to normal controls, with statistically significant difference (*P.value*s were 0.01). This finding agrees with Abdullahi, *et al*, Fasola and Adekanmi who showed significantly elevated levels of platelet count in cases versus controls (Abdullahi, *et al*., 2014; Fasola and Adekanmi, .2019). The results indicated that platelet count increased in anaemic patients. Elevated platelet could be associated with splenic sequestration. The increase of platelet activation may associate with high haemolytic rate in anaemic patients (Setty *et al.,* 2001).

In the present study both PT and APTT were measured and appear to be significantly higher in cases than normal controls (*P.value* 0.000, 0.000) respectively. Findings of higher PT and APTT among the SCD participants could be as a result of reported decrease in the plasma levels of factor V, total factor VII, and factor VII zymogen in SCD patients (Ajuwon et al, .2014). This result was supported by Nilesh et al,

Saud et al, Chinawa et al, Antwi-Baffour et al, who demonstrated that significantly higher PT and APTT in the cases versus controls (Nilesh, *et al*, .2014; Saud, *et al*, .2017; Chinawa, *et al,* .2013; Antwi-Baffour, *et al*., 2019). The increase in APTT and PT might have resulted from defective liver due to blockage by sickle cells as they traverses the capillary as seen in the study by Raffini et al , where they reported hepatic dysfunction amongst SCD patients. Since most of the coagulation factors are synthesized in the liver, a defective liver might result in decrease synthesis and as such may result in coagulopathy Raffini et al. Decreased synthesis and increased consumption of coagulation factors was also observed by Wright et al, Raffini et al., where the increased consumption was thought to be caused by the hypercoagulability seen in SCD patients (Raffini et al., 2006; Wright et al., 1997).

5.2. Conclusions:

5.2.1. There were significant differences in PT and APTT between patients with sickle cell diseases and normal controls, higher in patient than controls.

5.2.2. There was statistical correlation with age and APTT in cases group.

5.2.3. Platelets count had higher value in patients with sickle cell anaemia compared with normal controls, there was statistically significant.

5.3. Recommendations:

5.3.1. Larger sample size should be tested in the future to increase the accuracy of results.

5.3.2. Establishment of studies on the markers of haemolysis (haemoglobin level, reticulocyte count, LDH, and bilirubin concentration) with the changes in coagulation in SCD patients.

5.3.3. Further researches on D dimmer and VWF and platelet aggregation and activation to study the platelet function and hyperactivity in SCD.

5.3.4. More advanced researches (including molecular technique) should be conducted to evaluate the effect of SCD on haemostasis.

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Appendixes

Appendix (1)

Questionnaire

Measurement of prothrombin time and activated partial thromboplastin time in sickle cell disease patient in Khartoum State.

حساب زمن البروثرومبين وزمن الثرمبوبلاستين الجزئي النشط لدى مرضى الأنيميا المنجلية السودانيين في ولاية الخرطوم

SECTION A: BIODATA

1. Name ----------. Study I.D No. ----------.
2. Age ---------- (Yrs),
3. Sex ---------- M=1, F = 2
4. Genotype ----------.

Clinic details

1 When were you diagnosed? ----------
2 When last did you have a crisis? ----------
3 When last were you transfused? ----------

SECTION B: COAGULATION PROFILE

1. Prothrombin time (PT) ----------
2. Activated Partial thromboplastin time (APTT) ----------
3. Platelet Count ----------

Appendix (2)

استمارة موافقة مشاركة
حساب زمن البروثرومبين وزمن الثرمبوبلاستين الجزئي النشط لدى مرضى الأنيميا المنجلية السودانيين في ولاية الخرطوم

أنيميا الخلايا المنجليه هي أحد أنواع فقر الدم وهي تصيب كريات الدم الحمراء وهي من أشهر أمراض الدم الوراثيه الإنحلاليه التى تسبب تكسر كريات الدم الحمراء. من مضاعفات المرض السكتة الدماغية، متلازمة الصدر الحادة، إرتفاع الضغط الدموي وتضرر الاعضاء.

هل توافق على المشاركة في البحث العلمي عن مرضى أنيميا الخلايا المنجلية، التابع لجامعة السودان للعلوم والتكنولوجيا كلية علوم المختبرات الطبية، وأنت على علم تام بمحتوى البحث، مشاركه عن طريق التبرع بعينه من دمك، مشاركه اختيارية من دون إكراه ومن دون أي مقابل مادي؟

.....
.....
.....

اسم المشارك والتوقيع:

.....

رقم تلفون المشارك.....

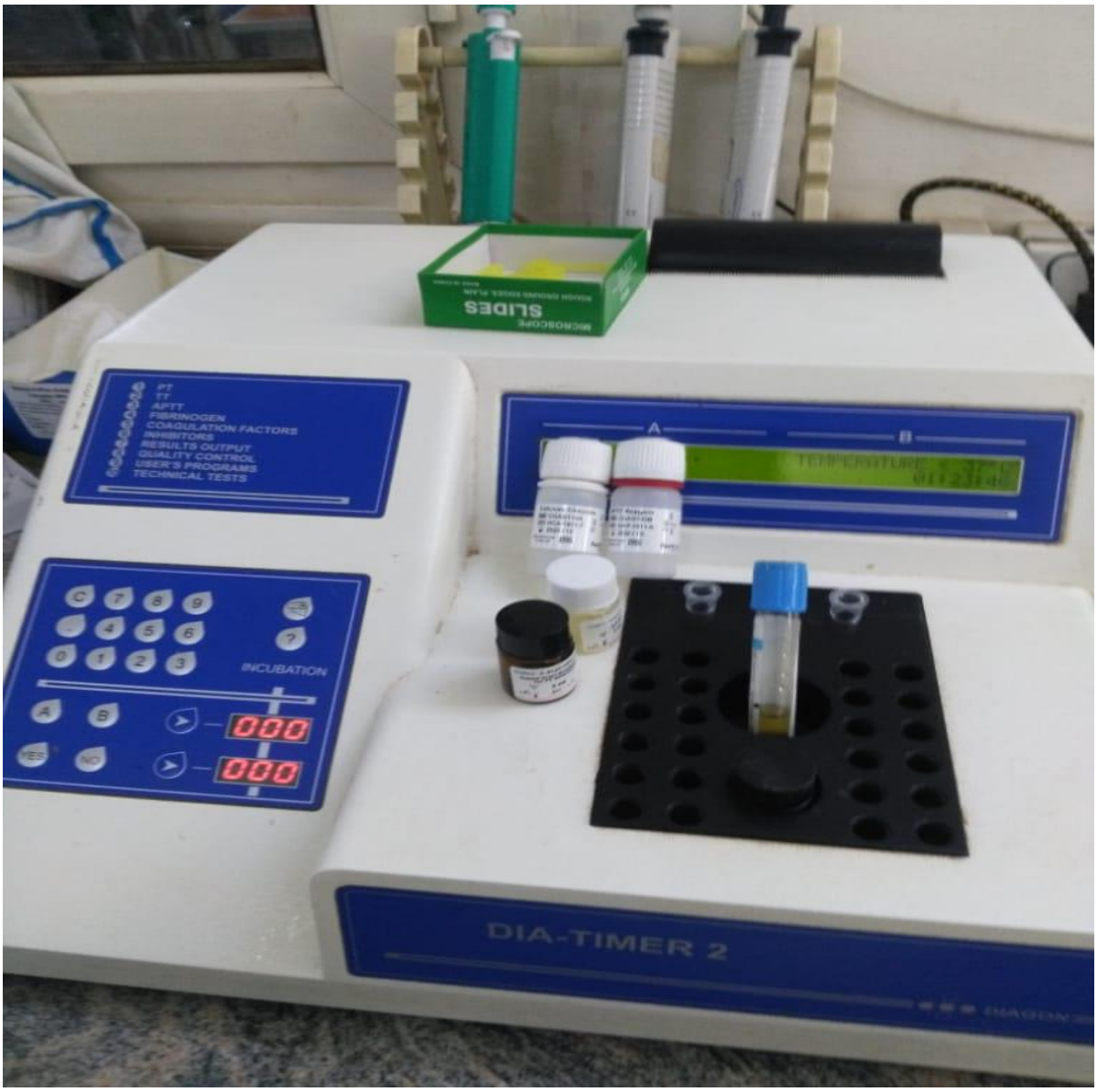
هذه المعلومات لغرض البحث العلمي فقط، وتتمتع بالسرية الكاملة.

اسم الباحث والتوقيع: .....

رقم تلفون الباحث: .....

Appendix (3)

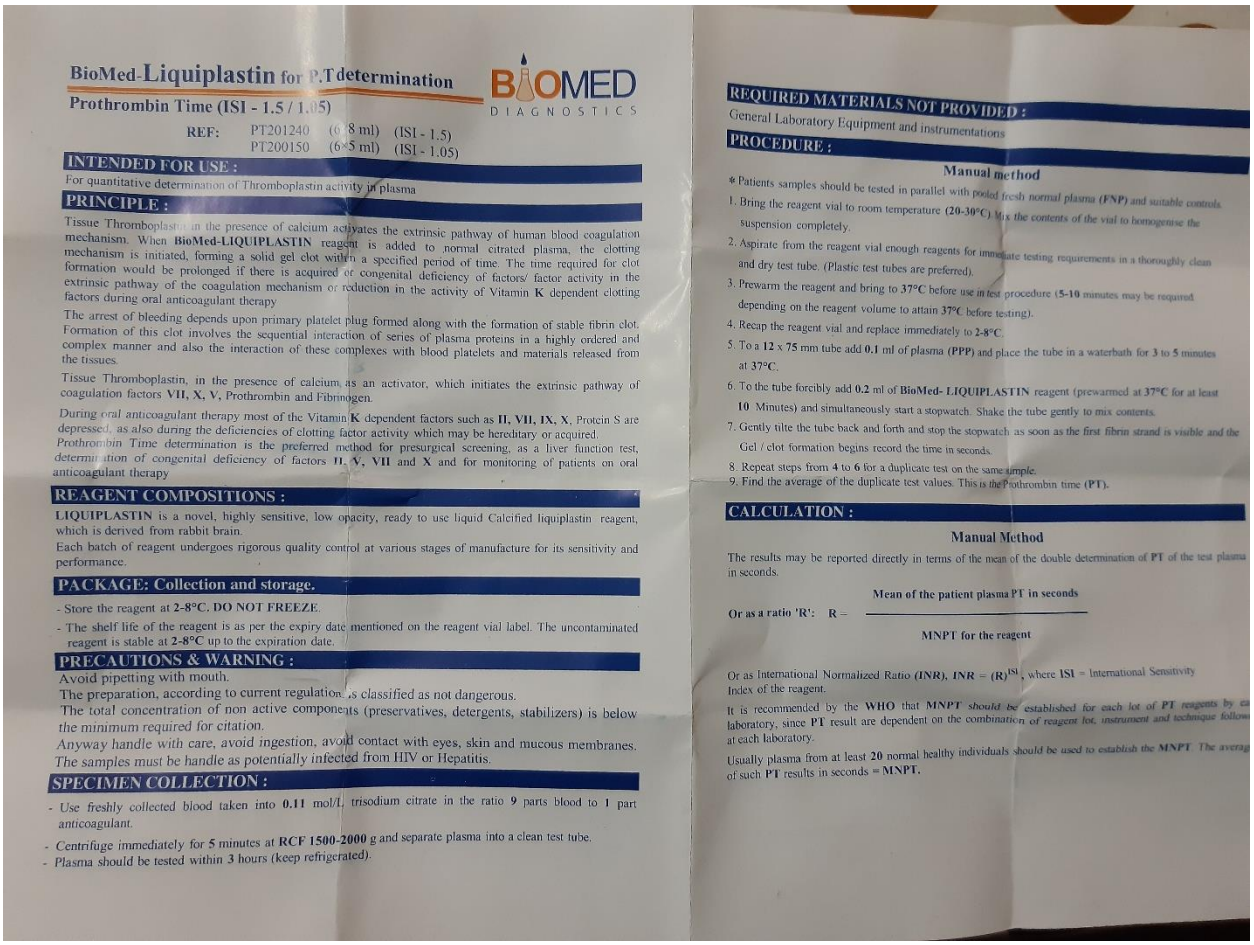
“DIA TIMER 2”



Appendix (4)

Reagents

(PT)



BioMed-Liquiplastin for P.T determination

Prothrombin Time (ISI - 1.5 / 1.05)

BIOMED DIAGNOSTICS

REF: PT201240 (6×8 ml) (ISI - 1.5)
PT200150 (6×5 ml) (ISI - 1.05)

INTENDED FOR USE :

For quantitative determination of Thromboplastin activity in plasma

PRINCIPLE :

Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When **BioMed-LIQUIPLASTIN** reagent is added to normal citrated plasma, the clotting mechanism is initiated, forming a solid gel clot within a specified period of time. The time required for clot formation would be prolonged if there is acquired or congenital deficiency of factors/ factor activity in the extrinsic pathway of the coagulation mechanism or reduction in the activity of Vitamin **K** dependent clotting factors during oral anticoagulant therapy

The arrest of bleeding depends upon primary platelet plug formed along with the formation of stable fibrin clot. Formation of this clot involves the sequential interaction of series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues.

Tissue Thromboplastin, in the presence of calcium as an activator, which initiates the extrinsic pathway of coagulation factors **VII, X, V,** Prothrombin and Fibrinogen.

During oral anticoagulant therapy most of the Vitamin **K** dependent factors such as **II, VII, IX, X**, Protein S are depressed, as also during the deficiencies of clotting factor activity which may be hereditary or acquired.
Prothrombin Time determination is the preferred method for presurgical screening, as a liver function test, determination of congenital deficiency of factors **II, V, VII** and **X** and for monitoring of patients on oral anticoagulant therapy

REAGENT COMPOSITIONS :

LIQUIPLASTIN is a novel, highly sensitive, low opacity, ready to use liquid Calcified liquiplastin reagent, which is derived from rabbit brain.
Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

PACKAGE: Collection and storage.

- Store the reagent at **2-8°C. DO NOT FREEZE.**
- The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The uncontaminated reagent is stable at **2-8°C** up to the expiration date.

PRECAUTIONS & WARNING :

Avoid pipetting with mouth.
The preparation, according to current regulations, is classified as not dangerous.
The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.
Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes.
The samples must be handle as potentially infected from HIV or Hepatitis.

SPECIMEN COLLECTION :

- Use freshly collected blood taken into **0.11** mol/L trisodium citrate in the ratio **9** parts blood to **1** part anticoagulant.
- Centrifuge immediately for **5** minutes at **RCF 1500-2000** g and separate plasma into a clean test tube.
- Plasma should be tested within **3** hours (keep refrigerated).

REQUIRED MATERIALS NOT PROVIDED :

General Laboratory Equipment and instrumentations

PROCEDURE :

Manual method

* Patients samples should be tested in parallel with pooled fresh normal plasma **(FNP)** and suitable controls.

1. Bring the reagent vial to room temperature **(20-30°C)**. Mix the contents of the vial to homogenise the suspension completely.
2. Aspirate from the reagent vial enough reagents for immediate testing requirements in a thoroughly clean and dry test tube. (Plastic test tubes are preferred).
3. Prewarm the reagent and bring to **37°C** before use in test procedure (**5-10** minutes may be required depending on the reagent volume to attain **37°C** before testing).
4. Recap the reagent vial and replace immediately to **2-8°C**.
5. To a **12** x **75** mm tube add **0.1** ml of plasma **(PPP)** and place the tube in a waterbath for **3** to **5** minutes at **37°C**.
6. To the tube forcibly add **0.2** ml of **BioMed- LIQUIPLASTIN** reagent (prewarmed at **37°C** for at least **10** Minutes) and simultaneously start a stopwatch. Shake the tube gently to mix contents.
7. Gently tilte the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the Gel / clot formation begins record the time in seconds.
8. Repeat steps from **4** to **6** for a duplicate test on the same simple.
9. Find the average of the duplicate test values. This is the Prothrombin time **(PT)**.

CALCULATION :

Manual Method

The results may be reported directly in terms of the mean of the double determination of **PT** of the test plasma in seconds.

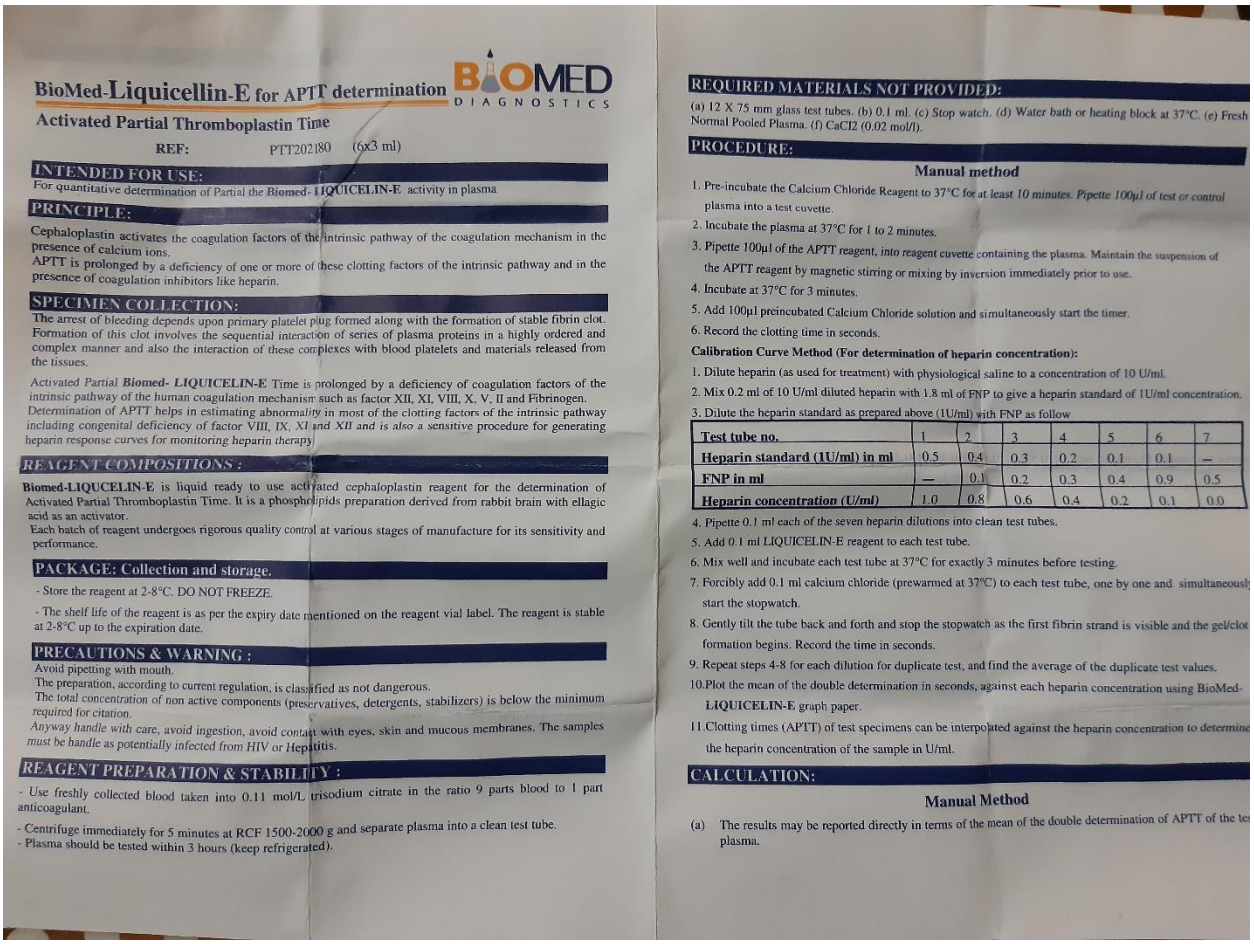
$$\text{Or as a ratio 'R':} \quad R = \frac{\text{Mean of the patient plasma PT in seconds}}{\text{MNPT for the reagent}}$$

Or as International Normalized Ratio **(INR)**, $INR = (R)^{ISI}$, where **ISI** = International Sensitivity Index of the reagent.

It is recommended by the **WHO** that **MNPT** *should be established for each lot of* **PT** *reagents by* laboratory, since **PT** result are dependent on the *combination of reagent lot, instrument and technique follow* at each laboratory.

Usually plasma from at least **20** normal healthy individuals should be used to establish the **MNPT**. The averag of such **PT** results in seconds = **MNPT.**

(APTT)



BioMed-Liquicellin-E for APTT determination

BIOMED DIAGNOSTICS

Activated Partial Thromboplastin Time

REF: PTT202180 (6x3 ml)

INTENDED FOR USE:

For quantitative determination of Partial the Biomed- LIQUICELIN-E activity in plasma

PRINCIPLE:

Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions.

APTT is prolonged by a deficiency of one or more of these clotting factors of the intrinsic pathway and in the presence of coagulation inhibitors like heparin.

SPECIMEN COLLECTION:

The arrest of bleeding depends upon primary platelet plug formed along with the formation of stable fibrin clot. Formation of this clot involves the sequential interaction of series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues.

Activated Partial **Biomed- LIQUICELIN-E** Time is prolonged by a deficiency of coagulation factors of the intrinsic pathway of the human coagulation mechanism such as factor XII, XI, VIII, X, V, II and Fibrinogen. Determination of APTT helps in estimating abnormality in most of the clotting factors of the intrinsic pathway including congenital deficiency of factor VIII, IX, XI and XII and is also a sensitive procedure for generating heparin response curves for monitoring heparin therapy.

REAGENT COMPOSITIONS :

Biomed-LIQUCELIN-E is liquid ready to use activated cephaloplastin reagent for the determination of Activated Partial Thromboplastin Time. It is a phospholipids preparation derived from rabbit brain with ellagic acid as an activator.

Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

PACKAGE: Collection and storage.

- Store the reagent at 2-8°C. DO NOT FREEZE.
- The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The reagent is stable at 2-8°C up to the expiration date.

PRECAUTIONS & WARNING :

Avoid pipetting with mouth.

The preparation, according to current regulation, is classified as not dangerous.

The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.

Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis.

REAGENT PREPARATION & STABILITY :

- Use freshly collected blood taken into 0.11 mol/L trisodium citrate in the ratio 9 parts blood to 1 part anticoagulant.
- Centrifuge immediately for 5 minutes at RCF 1500-2000 g and separate plasma into a clean test tube.
- Plasma should be tested within 3 hours (keep refrigerated).

REQUIRED MATERIALS NOT PROVIDED:

(a) 12 X 75 mm glass test tubes. (b) 0.1 ml. (c) Stop watch. (d) Water bath or heating block at 37°C. (e) Fresh Normal Pooled Plasma. (f) CaCl2 (0.02 mol/l).

PROCEDURE:

Manual method

1. Pre-incubate the Calcium Chloride Reagent to 37°C for at least 10 minutes. Pipette 100µl of test or control plasma into a test cuvette.
2. Incubate the plasma at 37°C for 1 to 2 minutes.
3. Pipette 100µl of the APTT reagent, into reagent cuvette containing the plasma. Maintain the suspension of the APTT reagent by magnetic stirring or mixing by inversion immediately prior to use.
4. Incubate at 37°C for 3 minutes.
5. Add 100µl preincubated Calcium Chloride solution and simultaneously start the timer.
6. Record the clotting time in seconds.

Calibration Curve Method (For determination of heparin concentration):

1. Dilute heparin (as used for treatment) with physiological saline to a concentration of 10 U/ml.
2. Mix 0.2 ml of 10 U/ml diluted heparin with 1.8 ml of FNP to give a heparin standard of 1U/ml concentration.
3. Dilute the heparin standard as prepared above (1U/ml) with FNP as follow

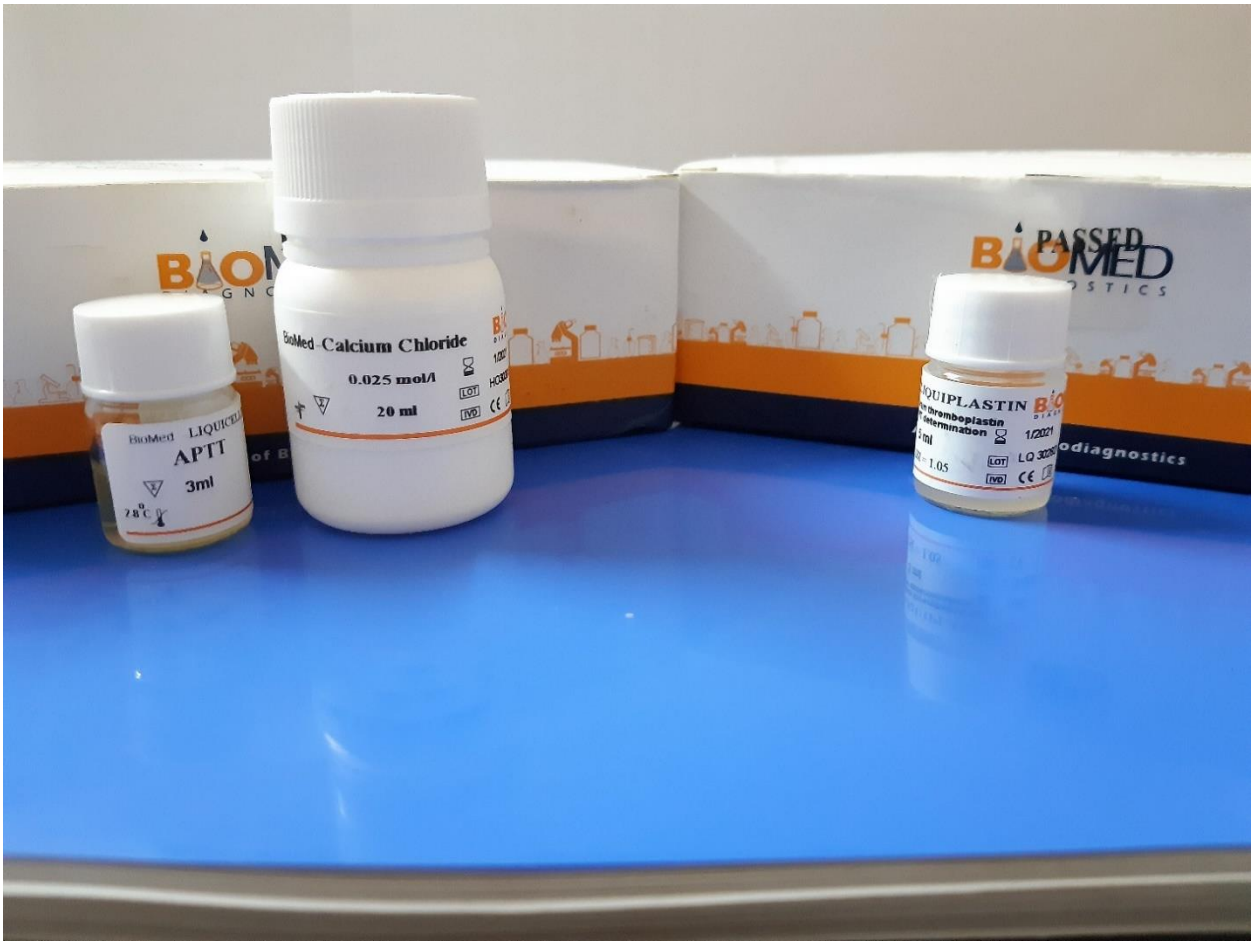
Test tube no.	1	2	3	4	5	6	7
Heparin standard (1U/ml) in ml	0.5	0.4	0.3	0.2	0.1	0.1	–
FNP in ml	–	0.1	0.2	0.3	0.4	0.9	0.5
Heparin concentration (U/ml)	1.0	0.8	0.6	0.4	0.2	0.1	0.0

4. Pipette 0.1 ml each of the seven heparin dilutions into clean test tubes.
5. Add 0.1 ml LIQUICELIN-E reagent to each test tube.
6. Mix well and incubate each test tube at 37°C for exactly 3 minutes before testing.
7. Forcibly add 0.1 ml calcium chloride (prewarmed at 37°C) to each test tube, one by one and simultaneously start the stopwatch.
8. Gently tilt the tube back and forth and stop the stopwatch as the first fibrin strand is visible and the gel/clot formation begins. Record the time in seconds.
9. Repeat steps 4-8 for each dilution for duplicate test, and find the average of the duplicate test values.
10. Plot the mean of the double determination in seconds, against each heparin concentration using BioMed-**LIQUICELIN-E** graph paper.
11. Clotting times (APTT) of test specimens can be interpolated against the heparin concentration to determine the heparin concentration of the sample in U/ml.

CALCULATION:

Manual Method

(a) The results may be reported directly in terms of the mean of the double determination of APTT of the test plasma.


BioMed Calcium Chloride
0.025 mol/l
20 ml
APTT
3ml
PASSED
BIOMED
diagnostics