بسم الله الرحمن الرحيم

Sudan University of Sciences and Technology

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Measurement of D-Dimer level and Blood Cell Count among Sickle Cell Anemia Patients in Khartoum State

قياس مستوى دي دايمر و حساب خلايا الدم بين مرضى انيميا الخلايا المنجلية بولاية الخرطوم

A dissertation submitted to partial fulfillment for the requirements of the degree of M.Sc. degree in medical laboratory sciences (hematology and immunohematology)

By

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بسم الله الرحمن الرحيم

((الله لا إله إلا هو الحي القيوم لا تاخذه سنة ولا نوم له ما في السموات وما في الارض من ذا الذي يشفع عنده إلا بإذنه يعلم ما بين ايديهم وما خلفهم ولا يحيطون بشئ من علمه إلا بما شاء وسع كرسيه السموات والارض ولا يؤده حفظهما وهو العلي العظيم))

صدق الله العظيم

سورة البقرة – الآية (255)

Dedication

I dedicate this work to my family, my friends, my colleagues and those people in my life who support me

Acknowledgment

I would thank my supervisor Dr. Mansour Mohamed Mansour for his valuable insights, and guiding instructions, Also colleague Maab Mousa, Khalid Fadul-ALseed, Husam Adam& Asia hospital laboratory staff for their support and effective assistance.

Abstract

Sickle cell anemia is one of common genetic condition in the world, it spreads in Sudan widely including all states of Sudan and spreads in some Sudanese tribes of African and Arab origins from western Sudan to eastern Sudan and from south to north in varying proportions depending on the spread of those tribes and factors of internal migration from the homeland The origin of these tribes to the different states of Sudan it is considered one of the most important health problem in the country, this disease occurs as a result of inheriting the mutated hemoglobin genes from both parents, this study aimed to determine the D-dimer level, total white blood cell count, Hemoglobin concentration and platelet count and correlation between these hematological parameters in Sudanese sickle cell anemia patients in steady state, the study was conducted in period from Jul 2019 to Sep 2020 in jaffar ibn auf hospital and Asia Hospital in Khartoum state include 100 subjects , 50 Sudanese patients with Sickle Cell Anemia(38 patients have hemoglobin SS and 12 patients have AS hemoglobin) in steady state include both sex(28 male and 22 female) and age groups (rang 2-19 year s) and 50 apparently healthy individual l(hemoglobin AA) both sex (30 male and 20 female)and age range(12-24 years) as control group,The blood count was performed by automated hematological analyzer (Sysmex XP 300), the D-dimer level was performed by Mispa I2™. Data was collected using a pre-structured questionnaire filed by face-to-face interview with participants and their medical supervisors, the Obtained results were analyzed through SPSS™ statistical software version 25.01. Control group and study group were compared using independent t-test for evaluating statistical significance (P-value<0.05), the parameter tested for correlation siginifcance using person-correlation. The resuls of D-dimer level was significantly increased in patients with SCA (0.88 pg/ml) with p value (0.00) and total white blood cell count and platelet count was significant higher in sickle cell anemia patients compared with control(mean (13.2×10^3/ul) and (527×10^3/ul), p-value (0.00) and (0.00) respectively) but hemoglobin significantly decreased (8.4 g/dl) p value (0.00),The elevation of D-dimer psitively correlate with increase of white blood cells count and platelet count R-values (0.523) and (0.298) respectively) and inversely correlate with hemoglobin concentration R-value (-0.476) in sickle cell anemia patients in steady state. The study showed increased in total white blood count, platelet ount and D-dimer level and showed significant decreased in hemoglobin concentration, D-dimer positvly correlated with total white blood count and negatively correlate with hemoglobin level.

مستخلص الدراسة

فقر الدم المنجلي من الحالات الوراثية الشائعة في العالم و ينتشر في السودان انتشارا واسعا يشمل جميع ولايات السودان وينتشر في بعض القبائل السودانية ذات الاصول الافريقية والعربية من غرب السودان وحتى شرق السودان ومن الجنوب حتى الشمال بنسب متفاوته اعتمادا على انتشار تلك القبائل عوامل الهجرة الداخلية من الموطن الاصلي لهذة القبائل الي ولايات السودان المختلفة ، وتعتبرانيميا الخلايا المنجليه من أهم المشاكل الصحية في البلاد، و يحدث هذا المرض نتيجه وراثه جيينات الهيموجلوبين الطافرة من كلا الابوين0 و هدفت هذه الدراسة إلى تحديد مستوى دي دايمر و إجمالي عدد خلايا الدم البيضاء ، تركيز الهيموجلوبين وعدد الصفائح الدموية والعلاقة بين هذه المحدات الدموية لدى مرضى فقر الدم المنجلي في السودان في الحالة المستقرة. أجريت الدراسة في الفترة من يوليو 2019 إلى سبتمبر 2020 بمستشفى جعفر بن عوف ومستشفى آسيا في ولاية الخرطوم ، وتضمنت(100) حالة ،50 مريضًا سودانيًا مصابًا بفقر الدم المنجلي(38 مريضا لديهم هيموجلوبين (SS) و12 مريضا لديهم هيوجلوبين AS) في الحالة المستقرة شملت كلا الجنسين (28 ذكرًا و22 أنثى) والفئات العمرية (تتراوح من 2 إلى 19 عامًا) و 50 فردًا سليمًا ظاهريًا (الهيموجلوبين AA) من كلا الجنسين (30 ذكرًا و 20 أنثى) والفئات العمرية (12-24 عامًا) كمجموعة تحكم، تم إجراء تعداد الدم بواسطة محلل الدم الآلي (Sysmex XP 300) ، وتم إجراء مستوى دي دايمر بواسطة Mispa I2 ™. تم جمع البيانات باستخدام استبيان منظم مسبقًا تم تقديمه عن طريق مقابلة وجهاً لوجه مع المشاركين والمشرفين الطبيين ، وتم تحليل النتائج التي تم الحصول عليها من خلال برنامج SPSS ™ الإحصائي الإصدار (25.01) وتمت مقارنة المجموعة الضابطة ومجموعة الدراسة باستخدام اختبارT المستقل لتقييم الدلالة الإحصائية (قيمة $P < 0.05$) ، حيث تم اختبار علاقة الارتباط باستخدام (Person-correlation) وقد كانت نتائج مستوى دي دايمر مرتفعه نسبيا لدى مرضى انيميا الخلايا المنجلية(0.884 pg/ml) و (0.00) p-value وكان إجمالي عدد خلايا الدم البيضاء وعدد الصفائح الدموية أعلى بشكل ملحوظ في مرضى فقر الدم المنجلي مقارنة مع مجموعة التحكم((13.2× 10^3/ul) و(527×10^3 /ul) و(Pvalue (0.00) and (0.00)) على التوالي و لكن الهيموجلوبين انخفض بشكل ملحوظ (8.4g/dl) و Pvalue (0.00) ويرتبط ارتفاع دي دايمر ارتفاعا موجبا بزيادة عدد خلايا الدم البيضاء وعدد الصفائح الدموية (0.523 R) و (0.298) على التوالي) ويرتبط عكسيًا مع تركيز الهيموجلوبين (0.476-) R-value) لدى مرضى فقر الدم المنجلي في الحالة المستقرة . أظهرت الدراسة زيادة في تعداد الدم الأبيض الكلي وعدد الصفائح الدموية ومستوى دي دايمر وأظهرت انخفاضًا معنويًا في تركيز الهيموجلوبين ، ارتبط دي دايمر ارتباطًا إيجابيًا بإجمالي تعداد الدم الأبيض ومرتبط سلبًا بمستوى الهيموجلوبين

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List of Abbreviation

Abbreviations	Terms
EDTA	Ethyldiaminetetraacetic acid
Hb	hemoglobin
LDH	Lactate dehydrogenase
MCV	Mean cell volume
NRBC	Nucleated red blood cell
PLT	Platelet count
RBC	Red blood cell
SCA	Sickle cell anemia
SCD	Sickle cell disease
WBCs	White blood cells

CHAPTER Ⅰ

Introduction

Sickle cell anemia (sickle cell disorder or sickle disease) is common genetic condition due to hemoglobin disorder inheritance of mutant hemoglobin gene, the first case of HbS gene was reported in 1950 Sudan . Later studies showed that sickle cell gene frequencies vary from region to another in Sudan as well as within the same region, in Khartoum Sickle cell disease is the major haemoglobinopathy seen in the Khartoum becase it is a multiethnic area, The rate is highest in Western Sudanese ethnic groups particularly in Messeryia tribes in Darfur and Kordofan regions, In the Blue Nile area, where groups of indigenous population live, the prevalence ranges from 0- 5% in addition to a rate of 16% among some immigrant tribes from western Sudan and West Africa in the area . The SCA presentation is usually severe and accompanied with major complications, and could be fatal in early childhood, the data about sickle cells gene in the north of Sudan is incomplete, it seems that this area shows a low frequency of SCA, in western Sudan: The presence of HbS is already well documented among Kordofan and Darfur region inhabitants, especially Albaggara, an Afro-Arab constellation of tribes with a predominantly African descent.(Mohammed Sabahelzain and Hamamy, 2014)

Sickle cell anemia (SCA) is an inherited autosomal recessive disease. SCD includes a set of quantitative and qualitative changes inside and outside the blood vessels that are responsible for the destruction of red blood cells. (Mombo *et al.*, 2019) Although SCD pathology is mainly due to RBCs, white blood cells (WBCs) also participate in obstructing blood vessels, a central phenomenon of vaso -occlusive crises in SCD .The hematological parameters in a complete blood count (CBC), which determines WBC abnormalities, may be a use ful tool to assess SCD severity. For instance, a high number of medical emergencies due to SCD are associated with leukocytosis .In many studies, a decrease in hematological parameters such as RBC count and hemoglobin and an increase in WBCs and platelets are associated with an increase in the number of vaso-occlusive crises in homozygous SCD patients (Mombo *et al.*, 2019)

CHAPTER Ⅱ

Literature Review

2.1. Geographic distribution

Hemoglobin S (sickle hemoglobin) is the most common hemoglobinopathy worldwide. Hemoglobin S is found most frequently in equatorial Africa and in people of African descent. In parts of Africa, approximately 10 to 40% of the population is heterozygous for hemoglobin S Approximately 8% of African Americans are heterozygous for hemoglobin S (sickle trait), and approximately 1 in 400 to 600 are homozygous (sickle cell anemia). There are separate pockets of hemoglobin S in Turkey, along the Mediterranean coast (Sicily, southern Italy, and northern Greece), in Saudi Arabia, and in India. These are areas where falciparum malaria is endemic suggesting that hemoglobin S arose as a protective mechanism against malaria. Based on genetic analysis, the sickle mutation appears to have arisen independently at least four different times.(Kern, 2002)

2.2. Molecular basis of sickling

The abnormality in Hemoglobin S is substitution of valine for glutamic acid at the sixth amino acid position. Deoxygenated hemoglobin S tends to polymerize into long rigid structures, which distort the cell into the characteristic sickle shape. Anything that causes deoxygenation of hemoglobin predisposes to sickling, including(hypoxia ,acidosis, and increased temperature) (Kern, 2002)

Deoxygenation of Hemoglobin S leads to a conformational change that exposes a hydrophobic patch on the surface of the βs-globin chain at the site of β6 valine . Binding of this site to a complementary hydrophobic site on a β-subunit of another hemoglobin tetramer triggers the formation of large polymers. The polymers consist of staggered hemoglobin tetramers that aggregate into 21-nm diameter helical fibers , with one inner and six peripheral double strands.(Hoffbrand *et al.*, 2016)

The polymerization proceeds after a delay, the length of which is extremely sensitive to the intracellular deoxy-Hemoglobin S concentration. Even a small increase in deoxy- Hemoglobin S concentration, such as might occur with cellular dehydration, profoundly shortens the delay time and augments sickling. The hemoglobin tetramers first aggregate into a nucleus, which rapidly

expands into a fiber The newly formed fiber provides nuclei on its surface for aggregation of hemoglobin tetramers to form several more fibers.(Hoffbrand *et al.*, 2016)

The polymerization of Hemoglobin S in the circulating red cells is influenced by the oxygenation status, the intracellular hemoglobin concentration and the presence of non-sickle hemoglobin S. Acidosis and elevated levels of 2,3-diphosphoglycerate (2,3- DPG) promote polymer formation by reducing the oxygen affinity of hemoglobin . The presence of Hemoglobin A within the red cells as in sickle trait, inhibits polymerization by diluting Hemoglobin S .(Hoffbrand *et al.*, 2016)

2.3. Effect on erythrocytes

Red cells acquire the sickle shape upon deoxygenation as results of intracellular polymerization of hemoglobin S a phenomenon that is reversible on reoxygenation (HOFFBRAND *et al.*, 2016), Normally shaped red cells, however, the presence of Hemoglobin S polymer reduces deformability, with consequent increase in blood viscosity. Repeated or prolonged sickling progressively damages the red cell membrane, which is a phenomenon of primary importance in the pathophysiology of SCD. Membrane damage causes movement of potassium ions and water out of the cell by the Gardos pathway and potassium-chloride co transport, leading to dehydration of red cells. The intracellular hemoglobin concentration rises (producing dense cells), which shortens the delay time to sickle polymer formation. A second key consequence of membrane damage is alteration of the chemistry of the red cell membrane. Perturbation of lipid organization causes negatively charged phosphatidylserine to appear on the red cell surface instead of its normal location in the inner monolayer. In addition, the red cells become abnormally adherent to the vascular endothelium through vascular cell adhesion molecule (VCAM)-1, thrombospondin and fibronectin.(Hoffbrand *et al.*, 2016)

2.4. Clinical manifestation and complication 0f sickle hemoglobin:

Heterozygosity for hemoglobin S (hemoglobin AS) is designated sickle cell trait. Sickle cell anemia is the preferred term for people who are homozygous for hemoglobin S (hemoglobin SS). The term sickle cell dis- ease indicates patients with clinical evidence of sickling and includes sickle cell anemia (hemoglobin SS), sickle/hemoglobin C (hemoglobin SC) .Since people with sickle trait are asymptomatic, sickle cell trait is not considered a sickle cell disease.(Kern, 2002)

2.5. Heterozygous Hemoglobin S (Sickle Trait)

People who are heterozygous for hemoglobin S (hemoglobin AS) are generally asymptomatic, have a normal blood hemoglobin level and complete blood count (CBC), and have a normal life span. Sickle cell trait is not incompatible with being a professional athlete .Microscopic hematuria is common due to infarction of the renal medulla (the very hypoxic, acidotic, and hyperosmolar environment in the renal medulla can cause even heterozygous cells to sickle). Rare cases of splenic infarcts at high altitudes and sudden death associated with strenuous physical exertion have been reported in people with sickle trait.(Kern, 2002)

2.6. Homozygous Hemoglobin S (Sickle Cell Anemia)

The severity of illness in sickle cell anemia (hemoglobin SS) is highly variable and can vary even within families. Many children become symptomatic in infancy after 3 to 4months of age (before that time they are protected by the high levels of hemoglobin F). Other people have very mild disease and may not be diagnosed until adulthood. The reasons for this variability are not clear; the level of hemoglobin F is a factor (an increase in hemoglobin F decreases the severity of sickle cell disease), but other factors also appear to be important(Kern, 2002)

2.7. Sickle Cell Crises

Acute vaso-occlusive (painful) crises are the most common type of crisis and are believed to be caused by occlusion of small blood vessels, with consequent infarction of tissues. Pain can occur in the abdomen, bones, joints, or muscles. Young children often present with pain involving the hands or feet (the hand-foot syndrome or dactylitis); long bones and the abdomen are more common sites of pain in adults.(Kern, 2002)

Sequestration crisis can occur during childhood, usually during the first 3 to 4 years, before the spleen has become infarcted. The spleen suddenly becomes enlarged and engorged with blood; this can sequester a major portion of the total blood volume and can be fatal.(Kern, 2002)

Acute aplastic crises this occurs as a complication of infections, usually but not always due to parvovirus B19. Acute parvovirus B19 infection causes a transient halt in production of erythrocytes, which usually lasts about 5 to 7 days. In normal individuals, in which erythrocytes are being replaced at the rate of about 1% per day, the transient drop in hemoglobin is not significant. Since red cell survival is greatly decreased in patients with sickle cell anemia (10–20

days, compared with 120 days in normal individuals), the hemoglobin drops much more quickly (up to 1 g/dL per day), and without transfusions, the marked exacerbation of anemia can be fatal. Recovery of hematopoiesis usually occurs after about 7 days.(Kern, 2002)

Infections are the most common cause of death in sickle cell disease. The risk of death from infection is highest during the first year of life but remains very high during the first 5 years. Overwhelming pneumococcal sepsis is the major risk, due to impaired splenic function and splenic auto infarction. Starting in infancy, children with sickle cell anemia are usually maintained on penicillin prophylaxis against pneu- mococcal sepsis. Gram-negative rods are the most common type of infectious agent in adults. Osteomyelitis is common in patients with sickle cell anemia; sickle cell disease has a particular association with osteomyelitis due to Salmonella.(Kern, 2002)

Cerebrovascular accidents Strokes are a major cause of morbidity in sickle cell disease, occurring in 5 to 8% of patients by the age of 14 years. They usually occur in young patients, with a median age below 10 years. Hemiplegia is a common presenting manifestation. Imaging studies indicate that many children have had subclinical undiagnosed strokes. Younger children usually have thrombotic or ischemic strokes; older teens and adults often have hemorrhagic strokes. There is a high risk of stroke recurrence (up to 50–70% within 3 years after the initial event). Chronic transfusion therapy to maintain the hemoglobin S concentration below 30% is recommended for at least 3 to 5 years after the first stroke, to prevent recurrence.(Kern, 2002)

Acute chest syndrome (acute lung syndrome)The acute chest syndrome is the second most common cause of hospitalization (after vaso- occlusive crises) and causes approximately 25% of the deaths from sickle cell disease. Manifestations include pulmonary infiltrates on chest radiograph, fever, chest pain, hypoxemia, tachypnea, cough, and dyspnea. Infection, fat embolism from infarcted bone marrow, other pulmonary embolism or vascular occlusions, hypoventilation and atelectasis due to rib infarcts or surgery, and pulmonary edema are all possible causes of the acute chest syndrome. Pulmonary fat embolism and other vasoocclusive events are probably the most common causes; infection may be a precipitating factor in children but is probably not a common cause overall. The acute chest syndrome may occur after surgery or general anesthesia. Patients with recurrent episodes of acute chest syndrome may develop chronic debilitating pulmonary disease.(Kern, 2002)

Altered splenic function and splenic infarct Infants and young children with sickle cell anemia often have mild splenomegaly. Later, sickling in the spleen leads to progressive splenic infarction, and by adulthood, the spleen is typically reduced to a small fibrous nodule (auto-splenectomy). Splenic filtering function appears to be impaired even before the spleen has become infarcted. This predisposes to overwhelming sepsis with Streptococcus pneumoniae and other encapsulated bacteria. Auto-splenectomy is uncommon in patients with hemoglobin SC and S thalassemia; they typically have mild splenomegaly, even as adults.(Kern, 2002)

Renal disease Infarction of the medulla of the kidney is common, resulting in hematuria (gross or microscopic) and loss of concentrating ability. This can occur in people with sickle trait, as well as those with homozygous disease. Progressive glomerular fibrosis with chronic renal insufficiency or renal failure may also occur in patients with sickle cell disease but is less common.(Kern, 2002)

Priapism is a common problem in males with sickle cell anemia, due to infarction of the corpora cavernosa of the penis. Repeated episodes result in impotence.(Kern, 2002)

Gallstones Bilirubin (pigment) gallstones are common in sickle cell disease, due to increased hemoglobin turnover and bilirubin production; however, serious complications due to gallstones are uncommon.(Kern, 2002)

Leg ulcers chronic cutaneous leg ulcers are a common complication of sickle cell disease, usually beginning in late adolescence and early adult- hood. They can be a major cause of morbidity and lost time at school and work. Healing of cutaneous ulcers is extremely slow, often requiring years. (Kern, 2002)

Aseptic necrosis of the femoral heads is common in patients with sickle cell anemia and sickle/hemoglobin C (hemoglobin SC). (Kern, 2002)

Retinopathy proliferative vascular retinopathy resembling diabetic retinopathy occurs occasionally in patients with sickle cell anemia. Interestingly, it is more common in patients with the relatively milder syndromes of hemoglobin SC and S/ thalassemia. The retinopathy can cause vitreous hemorrhages, with consequent retinal detachment and blindness. Laser phototherapy decreases the incidence of vitreous hemorrhages and blindness(Kern, 2002)

Complications of pregnancy the maternal mortality rate is increased (~1% for each pregnancy) in women with sickle cell anemia. They also have an increased rate of spontaneous fetal loss and low birth weight infants. Pulmonary complications are a common cause of maternal morbidity in pregnancy.(Kern, 2002)

2.8. Laboratory Diagnosis of Sickle cell anemia

Patients with sickle cell anemia will have a lifelong normochromic, normocytic anemia with decreased hemoglobin (between 6 and 8 g/dL), hematocrit, and red cell count. The reticulocyte count is always elevated leading to a slightly increased MCV in many cases. Bilirubin and LDH are increased, while haptoglobin is decreased, indicating extravascular hemolysis. (Ciesla, 2007)

2.8.1. Peripheral smear

The peripheral smear will show marked polychromasia, many NRBCs, target cells, and the presence of irreversible and reversible sickle cells. Peripheral smears from sickle cell patients not in crisis show minimal changes, a few oat-shaped reversible sickle cells, and some polychromasia (Ciesla, 2007)

2.8.2. Solubility test

A solubility test based on the principle that hemoglobin S precipitates in highmolarity buffered phosphate solutions. The amount of hemoglobin S is insignificant in this screening procedure because the purpose of this procedure is to detect the presence of hemoglobin S in the test sample. The end point is easy to read as a turbid solution in the presence of hemoglobin S and a clear solution if hemoglobin S is not present. (Ciesla, 2007)

2.8.3. Sickling test

The sickling phenomenon may be demonstrated in a thin wet film of blood (sealed with a petroleum jelly/paraffin wax mixture or with nail varnish). If Hemoglobin S is present, the red cells lose their smooth, round shape and become sickled. This process may take up to 12 hours in Hemoglobin S trait, whereas changes are apparent in homozygotes and compound heterozygotes after 1 hour at 37°C. These changes can be hastened by the addition of a reducing agent such as sodium dithionite.(Lewis *et al.*, 2006)

2.8.4. Hemoglobin Electrophoresis

Hemoglobin electrophoresis is a time-honored quantitative procedure for isolating hemoglobin bands. This technique is based on the principle that hemoglobin migrated to different positions depending on pH, time of migration, and media used. Cellulose acetate and citrate agar are the media most often selected. Hemoglobin is isolated from a patient sample using a variety of lysing agents such as saponin or water. A small amount of sample is applied to the media and electrophoresed for the prescribed amount of time, and then each band is quantified using densitometry.(Ciesla, 2007)

2.9. Treatment of Sickle Cell Disease

The patients with sickle cell disease should be referred to a center that has experience treating hemoglobinopathies. General supportive measures, including folic acid supplementation and prophylactic penicillin, are important. Psychological counseling is an important adjunct. It decreases anxiety related to having a chronic and potentially lethal disease, can help patients deal with chronic pain and increase their level of function, and improves compliance with therapy.(Kern, 2002)

2.10. Fibrin degradation products D-dimer

The final step in the regulation of fibrin deposition is the prevention and/or rapid removal of insoluble fibrin by the fibrinolytic system, once sufficient fibrin is generated, it binds tissue plasminogen activator (tPA), leading to the increased activation of plasminogen (PLG). This results in the formation of plasmin at the site of the fibrin clot, which breaks down fibrin into soluble fibrin degradation products (Hoffbrand *et al.*, 2016)

Plasmin can hydrolyse a variety of substrates, including FV and FVIII, but its major physiological targets are fibrin and fibrinogen, which are split progressively into a heterogeneous mixture of small soluble peptides (plasmin attacks at least 50 cleavage sites in fibrinogen) known collectively as fibrin degradation products (FDPs). The first stage in the proteolysis of fibrinogen involves the removal of several small peptides (fragments A, B and C) from the C-terminus ofthe A α-chains, each involving cleavage after a lysine residue. This is rapidly followed by removal of the first 42 amino acids from the N-terminal end of the B β-chain (the Bβ1–42 fragment). The large residual portion,which is known as fragmentX, and which still contains fibrinopeptide A, remains thrombin-clottable and will agglutinate some species of

staphylococcus spp. Assay of the Bβ1–42 fragment released from fibrinogen by plasmin gives a sensitive index of fibrinogenolytic activity. Asymmetrical digestion of all three pairs of chains of fibrinorfibrinogen then occurs with the release of the D fragment, in which the chains remained linked by disulfide bonds. The residue, known as fragmentY,is again attacked by plasmin, cleaving a second fragment D and leaving the disulfide- linked N-terminal ends of all six chains, which are referred to as fragment E. Fragments Y, D and E are not thrombin- clottable and do not agglutinate staphylococci. Their presence can be detected immunologically using an antibodycoated latex bead agglutination assay, which provides a simple test for most FDPs, although carefully prepared serum must be used to pre vent crossreactivity of the antibody with fibrinogen in plasma. These assays detect the degradation products of both fibrin and fibrinogen indiscriminately. Following thrombin generation and consequent activation of FXIII, inter molecular or intramolecular transamidation of the α-or β-chains by FXIIIa occurs and then the action of plasmin yields characteristic D-dimer, D-dimer–E fragments and oligomers of fragments X and Y (collectively known as cross- linked FDP or XDP), in addition to X, Y, D and E. These XDPs can be detected very simply using monoclonal-antibody-coated latex beads. Because the monoclonal antibodies to XDPs do not cross-react with fibrinogen, they can be detected directly in citrated plasma. The presence of D-dimers in blood samples can be used in a clinical algorithm that predicts the likelihood ofthe presence of venous thrombosis ,Furthermore, plasmin-induced cleavage of the N-terminal end of the β-chain of fibrin (the Bβ1–14 fibrinopeptide B frag- ment having been removed by thrombin) produces a β15–42 fragment, the detection of which indicates fibrin (as opposed to fibrinogen) degradation. Consequently, assays for the Bβ1– 42 and β15–42 fragments used in combination may be clinically useful by indicating whether fibrinogen or fibrin has been degraded, and thus whether fibrinolytic activity is primary or secondary to fibrin formation. However, clinically, FDP assays are used to detect DIC, when mixed fibrin/fibrinogen degradation products appear in the circulation.(Hoffbrand *et al.*, 2016)

D-Dimer (DD) is one of the protein fragments that are produced when blood clot dissolves in vivo. The antigen fibrin DD is the primary enzymatic degradation product of cross linked fibrin by plasmin. Systemic values of DD constitute an index of fibrin turn over in the circulation and a single measurement may be adequate to assess the fibrinlytic status, several studies have reported that systemic DD values are raised in a variety of clinical conditions including SCA in crisis and

in steady state and inclusion of DD testing may provide cost effective diagnostic strategies for some of these clinical condition. (Ekwere *et al.*, 2014)

2.11. Previous Studies

A Study conducted at the university of North Carolina, included African- Americans participants three groups, group one or control (AA) subject without hemoglobinopathy, group two subjects with sickle cell disease (SCD) (SS) and group three subject with sickle cell trait (AS). the results showed significant elevation in D-dimer levels in both Sickle cell (SS) and (AS) in compared with control (AA) (P-values (0.001) and (0.017) respectively).(Amin *et al.*, 2015)

A Study in Sudan aimed to determine the D-Dimer level in Sudanese children with SCA in a steady state and to correlate it with the hematological parameters (white blood cell count, hemoglobin concentration and platelets count), included 60 healthy subject (AA) as control and 40 subject with sickle cell anemia (SCA) . Mean D-Dimer level was significantly higher among SCA cases when compared with the controls (p value 0.00). Mean TWBC count and mean platelets count were significantly higher in the SCA patients than in controls (p value 0.000 and 0.005 respectively). There was no significant correlation between D-Dimer level and all hematological parameters.(Abdalla, 2014)

A study conducted in Nigeria involved 38 patients with homozygous sickle cell anemia (SS) and 78 adults with the (AA) phenotype, This study demonstrated a significant increase in the D-dimer levels of patients with (SS) in the steady state, when compared with those of individuals with (AA) of the same age and sex distribution. P-value (0.001) (Fakunle, Eteng and Shokunbi, 2012)

2.12. Rationale:

In Sudan, Sickle cell anemia it is a broad topic with insufficient published data, especially in relation to D-dimer, white blood cell count, hemoglobin concentration and platelet count. Furthermore, sickle cell patients threatened with high mortality rates than other anemia. Thus, presence of sufficient data will enhance patient prognosis and diagnosis practice among sickle cell anemia patients. At last, prediction of hypercoagulability in steady state patients will aid the development of effective protection measures.

2.14.Objective

2.14.1. General objective

To measure D-dimer level and blood cell count among patients with sickle anemia in steady state

1.14.2.Specific objective

1. To measure D-dimer level, total white blood cells, hemoglobin concentration and platelet count among patient with sickle anemia and control group

2. To compare D-dimer level, total white blood cells, hemoglobin concentration and platelets count between study group and control grou

3. To correlate D-dimer level with total white blood cell count, hemoglobin concentration and platelet count in study group.

Chapter Ⅲ

Materials and Methods

3.1. Study design This case-control study

3.2. Study population

The study was carried out at Khartoum State in the Sickle Cell Clinic of Gaffer Ibin Auf Children Specialized Hospital, Teaching Hospital and Asia hospital. In a period from the Jul 2019 to Sep of 2020, the study population comprises two groups in different age-groups, both sexes. Were included 100 subjects, 50 subjects as case group (38 patients who are known to have sickle cell anemia (Hemoglobin SS) &12 of patients who are known to have sickle cell trait (Hemoglobin AS)) and 50 subjects healthy appeared (Hemoglobin AA) as control group. The diagnosis of both groups was confirmed by hemoglobin electrophoresis and written medical reports.

3.2.1. Inclusion Criteria

Case group was defined as Known patients with SCA (confirmed by hemoglobin electrophoresis and written medical reports). Healthy appearance individuals with no known medical history counted as Control group

3.2.2. Exclusion criteria

In study, Any Patient known with medical history of coagulation disorder. Also, individuals who had received a blood transfusion during past three months were excluded.

3.3. Data collection

Data was collected using a pre-structured questionnaire filed by face-to-face interview with participants and their medical supervisors.

3.4. Methodology

The Complete blood count executed using automated cell analyzer Sysmex™ (XP 300) and D-dimer level estimated by Mispa I2™ immuno-fluorescence reader.

3.4.1. Samples collection

1.8 ml of venous blood samples was collected in (0.38 % trisodium citrate) container for D-dimer test & EDTA container for complete blood count

The Complete blood count executed using automated cell analyzer Sysmex™ (XP 300) and D-dimer level estimated by Mispa I2™ immuno-fluorescence reader.

3.4.2. Principles of hematology auto cell counter

This is the method used by all the analyzers that are currently in the market and that has been used for many years. It works very well when no interferences are present. With an impedance measurement, cells are passing one after the other through a capillary opening the aperture. There are electrodes on each side of the aperture – and direct current passes through these electrodes. The passing cells produce a change in the direct current resistance and thus an electronic signal which is proportionate to its volume. Hence, the cells are identified based on their size and get represented in a volume distribution curve, the so-called 'histogram' which is defined by the sum of impulses within a certain size distribution(Sysmex Europe GmbH, 2016)

This measuring principle was further improved in newer analyzers through hydrodynamic focusing. This centered stream principle will 'jacket' the stream of particles by a sheath flow so that the particles are passing centrally and one after the other through the measuring capillary This almost excludes interference factors such as double passages by coincidence, recirculation, etc. and cells will therefore be counted with greater precision.(Sysmex Europe GmbH, 2016)

The automated hematology analyzers with 3-part differentiation functionality rely on impedance technology to count and separate white blood cells on the basis of size. The red blood cells are lysed using chemical reagents whilst the white blood cells remain intact. Impedance technology involved a stream of cells in suspension passing through a small aperture across which an electrical current is applied. Each cell that passes alters the electrical impedance and can thus be counted.

The degree of change is in direct proportion to the size of the cell. The principle of hydrodynamic focusing further enhances the accuracy by ensuring that cells pass through the aperture in single file and eliminate false size estimates if, for example, 2 cells pass through together.(Münster and Sysmex Europe GmbH, 2012)

The reagent lyses red blood cells and white blood cells in the sample. The chemical reaction begins by altering the globin and then oxidizing the heme group. Now the SLS' hydrophilic groups can bind to the heme group and form a stable, colored complex (SLS-Hemoglobin), which is analyzed using a photometric method a light emitting diode (LED) sends out monochromatic light and by moving through the mixture light is absorbed by the SLS-Hemoglobin complexes.

The absorbance is measured by a photo sensor and is proportional to the hemoglobin concentration of the sample. Absorption photometric methods are usually influenced by the turbidity of the sample itself. In blood samples, turbidity can be caused due to lipaemia or leukocytosis. By using the SLS- Hemoglobin method these interferences can be minimized due to the effect of the reagent.

3.4.3. D-Dimer measurement

This D-dimer assay is a Nephelometric assay that utilizes antibody coated latex particles. In the presence of D-dimer, the particles aggregate and light scattering increased, The increase in scattering is proportional to the amount of D-dimer in sample.

Procedure:

.1. The card was inserted to reader slot.

2. 180 µL of Reagent1 and 6 µL of sample were pipetted and added to cuvette and placed the cuvette holder.

3. Incubation.

4. 60 µL of Reagent2 was added to cuvette. The result showed in display or print out.

3.5. Data analysis

The collected data and obtained results were analyzed through SPSS™ statistical software version 25.01. Control group and study groups were compared using independent t-test for

evaluating statistical significance (P-value<0.05). the observed parameter tested for correlation siginifcance using person-correlation.

3.6. Ethical consideration:

All participants were informed about the research objectives and procedures during the interview period and before blood sampling. A permission of conduction was obtained from Jafar Ibin Auf hospital and Asia Hospital managements, together, with Sudan University of science and technology research committee.

CHAPTER Ⅳ

Result

4.1. General characteristic of study population:

This case-control study was conducted in a period from Jul 2019 to Sep 2020 in Khartoum state in sickle cell clinic Jaffar Ibn Auf specialized hospital and Asia hospital. The study was conducted on 100 subjects .50 patients with sickle cell anemia (38 patients Hemoglobin SS& 12 have Hemoglobin AS) in steady state (28 male and 22 female) ages range (2-19 years old) and controls were 50 healthy individuals (Hemoglobin AA) (30 male 20 female) age range (12-24 years old) as control group. The D-dimer, hemoglobin level, white blood cells count and platelet count was measured for the subjects under study.

4.2. D-dimer and hematological parameters among study and control groups

The mean of D-dimer in case group (0.884 pg/ml) , white blood cells count (13.2× 10/ul) mean of hemoglobin (8.4 g/dl) and mean of platelet count(523×10/ul) . The mean of control group of D-dimer (0.191pg/ml), white blood cells count (6.0 ×10/ul), hemoglobin level (14.1 g/dl) and platelet count (267×10/ul), The patient with sickle cell anemia (case group) had higher plasma D-dimer concentration than healthy individuals (control group) were significant p value (0.00). The hemoglobin concentration was significantly lower in case than control with p value (0.00) But total white blood cells count and platelet count were significantly increased in compared with control(P values (0.00) and (0.00)) .

4.3. Correlation between D-dimer and hematological parameters

The D-dimer level is negatively correlate with hemoglobin concentration (p value (0.00) and R-value (-0.476)) and positively correlate with total white blood cells count and platelet count(p-values (0.00) and (0.036) and R-values (0.523) and (0.298) respectively)

Table: (4-1): Age in case and control groups

group	minimum	maximum	mean
case	**2**	**19**	**9.1**
control	**12**	**24**	**17.2**

Table (4-2):Comparison between case and control in mean of D-dimer level and hematological parameters.

variable	subjects	mean	SD	P-value
D-dimer	case	0.884	0.497	0.01
	control	0.191	0.o64	
Withe Blood cell count	case	13.2	5.82	0.01
	control	6.0	1.5	
Hemoglobin concentration	case	8.4	0.95	0.01
	control	14.1	1.0	
Platelet count	case	523	118	0.01
	control	267	66.9	

CHAPTER V

Discussion, Conclusion and Recommendation

5.1. Discussion

This is case-control study was conducted in a period from Jul 2019 to Sep 2020 .The study was carried out at Khartoum State in the Sickle Cell Clinic of Gaffer Ibn Auf Children Specialized Hospital, Teaching Hospital and Asia hospital. The study was conducted on 100 subjects .50 patients with sickle cell anemia (38 patients Hemoglobin SS & 12 have Hemoglobin AS) in steady state (28 male and 22 female) and 50 healthy individuals (Hemoglobin AA) (30 male 20 female) as control group.

This study show significant elevation in D-Dimer level in sickle cell anemia patients in steady state (0,884 pg/ml) with p-value (0.00). the study was agreed with anther study conducted in Nigeria involved 38 patients with homozygous sickle cell anemia (SS) and 78 adults as control, shows a significant increase in the D-dimer levels P-value (0.001) (Fakunle, Eteng and Shokunbi, 2012) ,also other study conducted at the university of North Carolina, included African- Americans participants (SS and AS) the result shows significant elevation in D-dimer levels in both Sickle cell (SS) and (AS) P-values (0.001) and (0.017) (Amin *et al.*, 2015) also this study agreed our study and other study conducted in Sudan shows D-Dimer level was significantly higher among sickle cell anemia pateints p value(0.00). also this study agreed with our finding (Abdalla, 2014)

The study show significant increase in total white blood cells count(13.2×10/ul) with p-value (0.00) and significantly increased in platelet count (527 ×10/ul) p-value (0.00) and show significant decreased in hemoglobin concentration (8.4 g/dl) in sickle cell anemia patients in steady state this was agreed with a study conducted in Sudan to determine the D-Dimer level in Sudanese children with SCA in a steady state p-values((0.00) ,(0.005) and(0.00) respectively)(Abdalla, 2014)

In this study the D-dimer level negatively correlate with hemoglobin concentration (p value (0.00) and R-value (-0.476)) and positively correlate with total white blood cells count and platelet count(p-values (0.00) and (0.036) and R-values (0.523) and (0.298) respectively) .these results disagree with that study conducted in sudan which showed no significant correlation between D-dimer level and hemoglobin concentration, total white blood count and platelets count .

Conclusion

- The D-dimer level, total white blood cells count and platelet count are significantly increased in patients with sickle cell anemia in steady state.
- D-dimer level showed positive correlation with total white blood cells count and platelet count and inverse correlation with hemoglobin concentration in sickle cell anemia patients in steady state.

Recommendation

- D-dimer use as indicator for prognosis of sickle cell anemia patients
- Further studies could explain more details about relations between studied parameters.
- Further study need to include different ethnicity in Sudan.

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Appendix

بسم الله الرحمن الرحيم

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Measurement of D-Dimer level and Blood Cells Count among Sudanese Patients with Sickle Cell Anemia in Khartoum State

Questionnaire

Date …….

Name:
……

Subject No ( )

Demographic data

Age …….

Gender: Male ( ) Female ( )

Hemoglobin genotype:

Hemoglobin AA ( ) Hemoglobin AS ( ) Hemoglobin SS ( )

History of blood Transfusion:

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History of crisis:

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History of other diseases:

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