

Chapter one

Introduction and Literature Review

1.1 Platelets:

Platelets are cytoplasmatic fragments of bone marrow megakaryocytes, with a diameter of 3-5 μm and a volume of 4.5–11 fL. A single megakaryocyte releases 1500–2000 of them to the bloodstream, where they circulate for 7–10 days. Inactivated platelets in the blood are discoid shaped and do not contain a nucleus. Their cytoplasm contains three different types of granules (i.e. alpha granules, dense granules, and lysosomal granules), secretory vesicles that contain preformed molecules, and a complex membranous system (Hoffbrand, 2006).

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

Platelets plays an important role in physiological and pathological processes such as coagulation, thrombosis, inflammation and maintenance the integrity of vascular endothelial cells (Gardiner and Andrews , 2014).

Platelets (PLT) are membrane bound discoid structures that play a Central role in hemostasis. Normal platelet count range from 1,50000/ mm^3 to 4,50000/ mm^3 (Drew *et al*, 2007) Platelets are the first line of defense in preventing blood loss due to micro and macro vascular injury. Abnormal platelet function and counts can result in bleeding typically characterized by mucocutaneous hemorrhage

(Hackner , 2009). Platelet are versatile fragments of cytoplasm whose major function is to arrest bleeding (Zucker and Nachmias , 1985).

1.1.1 Platelets structure:

Structurally platelet can be divided into four zone, from peripheral to innermost peripheral zone – is rich in glycoproteins required for platelet adhesion, activation, and aggregation. For example, GPIb/IX/X; GPVI; GPIIb/IIIa ,sol – gel zone – is rich in microtubules and microfilaments, allowing the platelets to maintain their discoid shape., organelle zone – is rich in platelet granules. Alpha granules contain clotting mediators such as factor V, factor VIII, fibrinogen, fibronectin, platelet-derived growth factor, and chemotactic agents. Delta granules, or dense bodies, contain ADP, calcium, serotonin, which are platelet-activating mediators , membranous zone – contain membranes derived from megakaryocytic smooth endoplasmic reticulum organized into a dense tubular system which is responsible for thromboxane A₂ synthesis. This dense tubular system is connected to the surface platelet membrane to aid release of thromboxane A₂ (Machluset *al*, 2014). Platelet structure is classified into four general areas: The platelet surface, The membranous structure, The cytoskeleton (sol-gel-zone) and The granules (Greer, 2004).

1.1.2 Platelets Production:

Platelets are produced in the bone marrow, the same as the red cells and most of the white blood cells. Platelets are produced from very large bone marrow cells called megakaryocyte which develop into giant cells; they undergo a process of fragmentation that results in the release of over 1,000 platelets per megakaryocyte. The dominant hormone controlling megakaryocyte development is thrombopoietin (often abbreviated as TPO) (Andrews *et al*,2013). Production of platelets depends on the proliferation and differentiation of a hemopoietic stem and progenitor cells to a cell committed to the large megakaryocyte lineage, its maturation to a large, polyploidy megakaryocyte , and its final fragmentation into platelet (Bonneyfoy *et al*, 2001)

The external influences that impact megakaryopoiesis and thrombosis are a supportive marrow stroma consisting of endothelial and other cells, matrix, glucoseaminoglycans, and a family of protein hormones and cytokines, including thrombopoietin, stem cell factor, interleukin-6, interleukin-11 and stromal-cell derived factor-1 (Bennett *et al*,2009)

1.1.3 Platelet Function:

Primarily, platelet activity is associated with the initiation of coagulation cascades. Damage in blood vessel makes the sub endothelial surface the primary target site of platelet action, where it establishes the hemostasis. Various proaggregatory stimuli also known as platelet agonists promote the action of platelet adhesion to the sub endothelial surfaces. During this process, platelet changes its shape, release its granule contents, and gradually forms aggregates by adhering with each other. Thus its primary activity remains associated with minimizing blood loss (Vinik *et al*, 2001).

The main function of platelets is the formation of mechanical plugs during the normal hemostatic response to vascular injury. In the absence of platelets spontaneous leakage of blood through small vessels may occur. Central to their function are platelets activation, adhesion, secretion, aggregation, fusion and procoagulant activity (Hoffbrand *et al*, 2011). Platelets cause leucocytes to accumulate around the platelet plug; that is, they may release chemotactic substances, release vasoactive amines, release hydrolytic and proteolytic enzymes directly into the intimal and subintimal structures provoking changes that may eventually lead to atheroma, act to transport serotonin from sites of synthesis to other sites of function (Hemker *et al*,1983), also play important role in innate immunity as well as regulation of tumor growth and extravasation in the vessel (Holinstat,2017). and play important role in inflammatory and proliferative events. Also have critical role for tissue remodeling and wound healing (Platelet Research Laboratory, 2015).

1.1.3.1 Adhesion:

Thrombus formation on an intact endothelium is prevented by nitric oxide, prostacyclin and CD39. Endothelial cells are attached to the subendothelial collagen by von Willebrand factor (vWF) which these cells produce vWF and stored in the Weibel-Palade bodies of the endothelial cells and secreted constitutively into the blood. Platelets store vWF in their alpha granules, when the endothelial layer is disrupted collagen and vWF anchor platelets to the subendothelium and platelet GpIb-IX-V receptor binds with vWF and GPVI receptor and integrin $\alpha_2\beta_1$ bind with collagen (Wang *et al*, 2016).

1.1.3.2 Activation :

The intact endothelial lining inhibits platelet activation by production nitric oxide, endothelial-ADP ase, and PGI₂, endothelial-ADP ase degrades the platelet activator ADP, resting platelets maintain active calcium efflux via a cyclic AMP activated calcium pump, intracellular Platelet activation begin seconds after adhesion occurs, it is triggered when collagen from the subendothelium binds with its receptors on the platelet. GPVI is associated with the Fc receptor γ - chain and leads via the activation of a tyrosine kinase cascade finally to the activation of PLC- γ_2 PLCG2 and more calcium release, tissue factor also binds to factor VII in the blood which initiates the extrinsic coagulation cascade to increase thrombin production, thrombin is a potent platelet activator acting through Gq and G12, these are G protein coupled receptors and they turn on calcium mediated signaling pathways within the platelet, overcoming the baseline calcium efflux, families of three G proteins (Gq, Gi, G12) operate together for full activation, thrombin also promotes secondary fibrin-reinforcement of the platelet plug (Gupta *et al*, 2010).

calcium concentration determines platelet activation status as it is the second messenger that drives platelet conformational change and degranulation (Hegazai *et al*, 2010).

1.1.3.3 Trigger (Induction)

Aggregation begins minutes after activation and occur as a result of turning on the GPIIb/IIIa receptor allowing these receptors to bind with VwF or fibrinogen, when any one or more of at least nine different plt surface receptors are turned on during activation, intraplatelet signaling pathways cause existing GpIIb/IIIa receptors to change shape and thus capable to binding. Since fibrinogen is a rod-like protein with nodules on either end capable of binding GpIIb/IIIa can bind fibrinogen to aggregate. GpIIb/IIIa may also further anchor the plt to subendothelial vWF for additional clot structural stabilization (Nurden, 2014).

1.2 Disorder of platelet function:

1.2.1 Thrombocytopenia:

Reduce production of platelet: Infection, Aplastic anemia, Drugs and Deficiency of folate or vitamin B12. Increase destruction or consumption of platelet: DIC, Hypersplenism and Immune destruction of platelets (ITP, SLE, CLL, CTD).

1.2.2 Thrombocytosis:

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

1.3 Platelet indices:

Definition of platelet indices: PLT indices are a group of parameters that are used to measure the total amount of PLTs, PLTs morphology and proliferation kinetics (Guclu *et al*, 2011). The commonly used PLT indices include PLT count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) (Zhang *et al*, 2014). The MPV refers to the ratio of PCT to PLT count. PDW is numerically equal to the coefficient of PLT volume variation, which is used to describe the dispersion of PLTs volume (Zhang *et al*, 2014).

1.3.1 Platelet Count:

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

1.3.2 Plateletcrit (PCT):

Plateletcrit is a measure of total platelet mass, values vary depending on mean platelet volume resulting in overlap between normal platelets, thrombocytopenia and thrombocytosis, plateletcrit is an effective screening tool for detecting platelet quantitative abnormalities. Normal platelet count has aplateletcrit with in the range of 0.20 – 0.36% (Chandrashekhar, 2013).

1.3.3 Mean Platelet Volume (MPV):

MPV is a reflection of megakaryocyte ploidy, it's increased in conditions associated with increased platelet turnover. The platelet mass remain constant in normal individuals (Bowles *et al*, 2005).

1.3.4 Platelet Distribution Width (PDW):

PDW is a marker of differences in the platelet size, which can be an indicator of active platelet release. These platelet parameters are estimated routinely by automated blood counters (Gurney *et al*, 2002). Is usefulness for distinguishing between reactive thrombocytosis and thrombocytosis associated with the myeloproliferative disorder (Bhutta *et al*, 2013).

1.3.5 Platelet Large Cell Ratio (P-LCR):

Means platelet large cell ratio with normal range (13.0 -43.0%) and it's calculated in automated blood analyzers. Increased percentage of large platelets (P-LCR) is observed in patients with hyper-lipidemia and suggest possible risk

thrombosis .an increase in P-LCR+MPV+PDW has been observed in autoimmune thrombocytopenic purpura (Chandrashekar, 2013).

1.4 Anemia's:

Anemia is define as quantitative or qualitative deficiency of hemoglobin,a molecule found inside red blood cell (RBCs). Since hemoglobin normally carries oxygen from the lung to the tissues, anemia leads to hypoxia (lack of oxygen) in organs. Since all human cells depend on oxygen for survival, varying degree of anemia can have a wide range of clinical consequences. Anemia also caused by a lack of iron in the body (Bush, 1997). The three main classes of anemia include excessive blood loss (acutely such as a hemorrhage or chronically through low-volume loss), excessive blood cell destruction (hemolysis) or deficient red blood cell production (ineffective hematopoiesis). Anemia is the most common disorder of the blood. There are several kinds of anemia, produced by a variety of underlying causes. Anemia can be classified in a variety ways, based on the morphology of RBCs. Underlying etiologic mechanisms (Vincent,2002)

1.4.1 Classification of Anemia:

Morphological classification of anemia's include: Microcytic hypochromic anemia:In this group both Mean cell volume (MCV) less than 80 FL (normal 80-100) and Mean cell hemoglobin (MCH) less than 27 pg. (normal 27-32 pg).These includes: iron deficiency anemia, thalassemia, sideroblastic anemia, lead poisoning and anemia of chronic diseases (Vincent, 2002). Macrocytic normochromic anemia:In this group Mean cell volume (MCV) more than 100 FL (normal 80-100 FL), these include: megaloblastic anemia, drug-induced macrocytic anemia, alcohol, liver disease and Aplastic anemia. Normocytic Normochromic anemia's :These include: hypoplasti-canemia's resulting from bone marrow suppression by drugs, alcohol, and infection, replace of bone marrow by tumor or fibrosis (Vincent, 2002).

1.4.2 Etiological Classification of Anemia's:

Increased destruction: (hemolytic disease) either due to intracellular defects include: heredity spherocytosis, G-6pD involve destruction of red blood cells by parasitic infection, antibodies, chemicals, and physical agents Increased blood loss: (anemia that cause by acute or chronic loss). Inadequate production of blood cell: This group can be divided in to nutritional anemia (reducing or lacking essential substance required for hemopoiesis). Bone marrow atrophy (Aplastic anemia secondary to the action of chemicals agent, physical agent, drugs and viral infections).and bone marrow infiltrate by tumor or fibrosis (Corwin, 2004)

The hereditary disorders of hemoglobin called hemoglobinopathies which are characterized by the production of structurally defective hemoglobin due to abnormalities in the formation of the globin moiety of the molecule. The thalassemia are characterized by a reduced rate of a production of normal hemoglobin due to absent or decreased synthesis of one or more types, consisting of two pairs of polypeptide chains. Human hemoglobin exists in anumber of types, consist of two pairs of polypeptide chains to each of which aheam molecule is attached, but differ slightly in the structure of their globin moiety (Vincent, 2002). Normal adult hemoglobin contain the following components: Hb A (95% to 97%), HbA₂ (2% to 3%), and Hb F (less than 1%). The major fraction is HbA. typically, the individual with ahemoglobinopathy will demonstrate alternation in this pattern (Vincent, 2002).

The hemoglobinopathies are inherited diseases, some of these disorders are caused by the inheritance of an autosomal dominant gene that will produce a hemolytic disease in its heterozygous state. Others are autosomal recessive genes are needed to be in homozygous state to produce the disease. The distinction between the disease state and the trait condition is made, the disease is defined as either homozygous occurrence of the gene for the abnormality or the possession of a heterozygous dominant gene that produces a hemolytic condition. A trait is described as the heterozygous and normally asymptomatic

a state. The major hemoglobinopathies have been identified as originating as mutation in Africa, Asia and Europe. Two frequent hemoglobinopathies are Sickle cell anemia/sickle cell trait and thalassaemia. The sickle mutant has the highest frequency of occurrence in Central Africa. Thalassaemia major can be traced back to Mediterranean. The Middle East, South Asia, and the Orient have α -thalassaemia as prevalent hemoglobinopathy (Sauntharajah, 2005).

1.5 Sickle Cell Anemia:

Sickle cell disease, one of the world's commonest single gene disorders, was first described by Herrick in 1910, who linked his patient's symptoms to abnormally shaped erythrocytes in the blood. Pauling and colleagues in 1949 detected abnormal hemoglobin, that has subsequently called sickle hemoglobin (HbS), and was shown by Ingram to contain a valine residue in place of glutamic acid as the 6th amino acid of the β -hemoglobin chain; the mutation was subsequently confirmed as GAG to GTG in codon 6. A higher hemoglobin level causes increased blood viscosity, unless the additional red cells have a high content of fetal hemoglobin (HbF). homozygosity for the HbS mutation and is the most common other genotypes of sickle cell disease are due to compound heterozygosity for HbS and other hemoglobin variants like HbC, HbE, and HbD, or the many different genotypes of HbS- β thalassaemia. These disorders, with the exception of HbS- β^0 thalassaemia, are when considering each genotype, usually less clinically severe than sickle cell anemia. Nevertheless, within each genotype there is great clinical and hematological heterogeneity that is poorly explained by the effects of the other variant hemoglobin. Usually, HbS- β^+ thalassaemia is a milder disorder than HbS- β^0 thalassaemia but this depends in part on the concentration of HbA found in HbS- β^+ thalassaemia (Steinberg and Habara, 2016)

1.5.1 History of Sickle Cell Anemia:

This collection of clinical findings was unknown until the explanation of the sickle cell in 1904 by the Chicago cardiologist and professor of medicine James B. Herrick (1861-1954) whose intern Ernest Edward Irons (1877-1959) found

"peculiar elongated and sickle shaped" cells in the blood of Walter Clement Noel, a 20 years old first year dental student from Grenada after Noel was admitted to the Chicago Presbyterian Hospital in December 1904 suffering from anemia. Noel was readmitted several times over the next three years for "muscular rheumatism" and "bilious attacks" .Noel completed his studies and returned to the capital of Grenada (St. Georges) to practice dentistry. He died of pneumonia in 1916 and is buried in the Catholic cemetery at sauteurs in the north of Grenada the disease was named "sickle cell anemia" by Vernon Mason in 1922. In retrospective some elements of the disease had been recognized earlier. A paper in the Southern Journal of Medical pharmacology in 1846 described the absence of a spleen in the autopsy of a runaway slave. The African medical literature reported this condition in the 1870s where it was known locally as ogbanjes (children who come and go) because of the very high infant mortality rate caused by this condition. A history of the condition tracked report back to 1670 in one Ghanaian family (Desai, 2004).

Linus Pauling and colleagues were the first, in 1949, to demonstrate the sickle cell disease occurs as a result of an abnormality in the hemoglobin molecule. This was the first time a genetic disease was linked to a mutation of specific protein, a milestone in the biology. The origin of the mutation that led to the sickle cell gene was initially thought to be in the Arabian Peninsula, spreading to Asia and Africa. It is now known, from evaluation of chromosome structures, that there have been at least four independent mutational events, three in Africa and a fourth in either Saudi Arabia or central India. These independent events occurred between 3,000 and 6,000 generation ago, approximately 70 – 150,000 years (Desai, 2004).

1.5.2 Etiology of Sickle Cell Anemia:

A point mutation in the beta globin chain of the hemoglobin causes sickle cell disease. Specifically, it occurs when a single base from A to T in the codon for glutamic acid at position 6 is changed to valine of the beta globin. If this mutation affects both of the beta globin chains, sickle cell anemia occurs; if

only one chain is affected, it results in the sickle cell trait (Willam,2016). The sickle cell disease is an autosomal recessive disorder of a gene mutation on chromosome 11, nucleotide mutation leads to substitution of glutamic acid to valine at position six on the β -globin subunit.This lead to change in the physical properties of the globin chain.Many inciting factors lead to this physical property change of red blood cells hypoxia, dehydration, exposure to cold or weather changes, stress and infections (Hiran, 2005).

1.5.3 Classification and Variant of Sickle Cell Anemia:

The sickle cell anemia can be classified according to genetic defect in to three main types:

1.5.3.1 Sickle Cell Trait (Heterozygote):

Sickle cell trait does not cause health problems. In fact, sickle cell trait is protective against malaria, a disease caused by blood-borne parasites transmitted through mosquito bites. Survivors pass the mutation on to their offspring, and the trait became established throughout areas where malaria was common. The malaria parasite has a complex life cycle and spends part of it in red blood cells. In a carrier, the presence of the malaria parasite causes the red cell to rupture, making the plasmodium unable to reproduce. Further, the polymerization of Hb affects the ability of the parasite to digest Hb in the first place. Therefore, in areas where malaria is a problem, peoples chances of survival actually increase if they Cary sickle-cell trait (selection for the heterozygote). In the USA, where there is no endemic malaria, the prevalence of sickle-cell anemia amongst African Americans is lower (about 0.25%) than in West Africa (about 4.0%) and is failing without endemic malaria (Kwiatkowski, 2005).

Individual have been shown to resist invasion by lethal plasmodium falciparum form of the disease. there is no anemia or hemolysis and physically normal sickle trait erythrocyte are capable of sickling , particularly under condition of significant hypoxemia and a number of clinical abnormalities have been linked which includes splenic infection or sequestration , hypothermia, painless

hematuria, bacteriuria, Under intense, stressful condition, exhaustion, hypoxia (low oxygen) , and/or severe infection, the sickling of the defective hemoglobin may occur and result in some complications associated with the sickle cell disease. Hemoglobin electrophoresis shows about 60% hemoglobin A and about 40% hemoglobin S with normal levels of hemoglobin A2 and hemoglobin F. A confirmatory solubility test excludes other abnormal hemoglobin with similar electrophoretic mobility. Neonatal screening programs for the detection of infants with sickle cell disease are widely established. These disorders can also be determined antenatally using amniocyte or chorionic villus DNA sampling. Each child has a 1 in 4 (25%) chance of having sickle cell anemia if both parents carry the sickle cell trait (Kwiatkowski, 2005).

People with sickle cell trait usually do not have any of the symptoms of the disease. However, it is possible for a person with sickle cell trait to have complications of the disease under extreme conditions, such as: high altitude (flying, mountain climbing, or cities with a high altitude), increased pressure (scuba diving), low oxygen (mountain climbing or exercising extremely hard, such as in military boot camp or when training for an athletic competition), dehydration (too little water in the body). In addition, a person with sickle cell trait can pass the disease on to their children (Kwiatkowski, 2005).

1.5.3.2 Homozygous sickle cell disease (Hb- SS):

The most common form (>70% of SCD worldwide) results from the homozygous inheritance of the β^S -mutation and is usually referred to as either 'SCD SS' or as 'sickle cell anaemia' (SCA) and is the most severe form of sickle cell anemia (Williams and Chakravorty, 2015)

In homozygous hemoglobin S (HbSS), a valine for glutamic acid substitution occurs on both β - globin chain genes from both parents. The condition is described as "Sickle cell anemia or Sickle cell disease" because of the sickle cell shaped red blood cells that occur when there is a "Sickle cell crisis" (Ogundunmade and Jasper, 2014) .

1.5.3.3 Variant of Sickle Cell Anemia (double heterozygous):

Several genetic variants of SCD result from the interaction of different mutations of the human β -globin genes. When the β^s gene interacts with the β^c gene, the resulting sickling disorder known as HbSC disease is typically very mild. When a β^S gene interacts with a β -thalassemia gene (a mutant β -globin gene that either fails to produce normal β -globin mRNA or produces it at markedly decreased levels), the severity of the resulting sickling disorder depends on the severity of the coinherited β -thalassemia mutation. When the coinherited β -thalassemia gene is completely inactive i.e. β^0 -thalassemia), the resulting sickling disorder known as $S\beta^0$ thalassemia tends to be of severity similar to that of homozygous HbSS disease.

In contrast, when the coinherited β -thalassemia gene is partially active (i.e., β^+ thalassemia), the resulting sickling disorder known as $S\beta^+$ thalassemia can have spectrum of clinical severity.

If the β^+ thalassemia mutation is mild, as is commonly the case in people of African descent, the resulting $S\beta^+$ thalassemia tends to be clinically mild. In contrast, if the β^+ -thalassemia mutation is severe, as is commonly the case in the Mediterranean populations, the clinical sickling disorder tends to be moderate (George and Paul, 2007).

1.5.4 Genetic and Inheritance of Sickle Cell Anemia:

Hb is the oxygen-carrying protein in blood. It is a tetramer of 4 proteins, 2 α -globins and 2 β -globins. Each globin has an associated oxygen-binding heme group. The α -globins and β -globins are encoded by genes on different chromosomes. The Hb S mutation (β^S) is a single nucleotide substitution in the sixth codon of the β -globin gene (HBB). This yields a protein with a hydrophobic valine residue, instead of the normal hydrophilic glutamic acid at the sixth position, that is prone to polymerization on deoxygenation. Heterozygosity for β^S , called sickle cell trait, occurs frequently in individuals of African ancestry (sub-Saharan, equatorial Africa), but it also occurs commonly in the eastern provinces of Saudi Arabia, central India, and parts of the

Mediterranean. It is now found throughout the world due to migration. Heterozygosity for β^S provides some protection against severe malarial infection, which is the generally accepted explanation for the maintenance of this balanced polymorphism. The inheritance of SCD is often referred to as autosomal recessive. However, one can consider β^S to be inherited in an autosomal codominant fashion, because even a single β^S gene is expressed and produces phenotypic changes in the Hb profile (and rare clinical complications). Moreover, homozygous inheritance (β^S/β^S) or compound heterozygosity with certain other mutant β -globins, such as Hb C (β^S/β^C), produce different types of SCD (Quinn, 2013).

1.5.5 Pathogenesis of Sickle Cell Anemia:

The polymerization of Hb S within red blood cells (RBCs) (“sickling”) on deoxygenation underlies all the pathophysiology of SCD. As Hb S-containing RBCs traverse the circulation undergoing cycles of oxygenation and deoxygenation, rigid polymers of Hb S repeatedly form and damage the RBC membrane, drastically shortening the RBC life span. RBCs also become dehydrated, relatively inflexible, and abnormally adhesive. Consequently, they are prone to adhere to the endothelium of blood vessels, in concert with leukocytes and platelets, impeding the flow of blood. This microvascular obstruction, called vaso-occlusion, leads to ischemia, infarction, and ischemia-reperfusion injury of multiple organs and tissues. This pathophysiology produces an ongoing inflammatory response and endothelial dysfunction. Some complications of SCD can be considered to be primarily a consequence of either hemolysis or vaso-occlusion. For example, chronic hemolysis predisposes to bilirubinate cholelithiasis, whereas vaso-occlusive ischemia and infarction of bone marrow is thought to cause the acute painful event crisis (Quinn, 2013).

A point mutation in the beta globin chain gene, where T replaces A which changes the codon for glutamic acid to valine, causes sickle cell anemia. This mutation presence in both the chain causes a change in hemoglobin. Normal

hemoglobin is soluble, and it does not precipitate in the presence of hypoxia, low pH, and dehydration. The polymerization of the sickle cell hemoglobin molecules inside RBCs is responsible for the sickling. This polymerization with subsequent aggregation takes place when the sickle cell hemoglobin is deoxygenated. Initial aggregation and polymerization is a reversible process. It occurs while the hemoglobin is deoxygenated and reverses with oxygenation. The sickle or holly leaf shape of the polymerized hemoglobin. However, multiple cycles of sickling cause damage to the RBCs cell membranes, making them prone to phagocytosis by the macrophages, this phagocytosis leads to the destruction and reduction of the RBC count and hence results in anemia. Reversibly sickled cell revert to normal shape with oxygenation and continue to perform their functions, but when the hemoglobin is irreversibly polymerized oxygenation cannot reverse the pathology (Romana, 2018).

1.5.6 Sign and Symptoms of Sickle Cell Anemia:

Sickle-cell disease may lead to various acute and chronic complication, several of which are potentially lethal which include:

1.5.6.1 Vaso-occlusive crisis:

is caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ, resulting in ischemia, pain, and organ damage. The frequency, severity, and duration of these crisis vary considerably (Smithet *al*, 2008). Because of its narrow vessels and function in clearing defective red blood cells, the spleen is frequently affected. It is usually infuncted before the end of childhood in individuals suffering from sickle-cell anemia. This auto splenectomy increases the risk of infection from encapsulated organisms. (Platt, 1994). Preventive antibiotics and vaccinations are recommended for those with such asplenia. A recognized type of sickle crisis is the acute chest syndrome, a condition characterized by fever, chest pain, difficulty breathing, and pulmonary infiltrate on a chest X-ray (Smithet *al*, 2008).

1.5.6.2 A plastic crisis:

are acute worsening of the patients' anemia producing pallor, tachycardia, and fatigue. This crisis is triggered by parvovirus B19, which directly affects erythropoiesis (production of red blood cells). Parvovirus infection nearly completely prevents red blood cell production for 2-3 days. In normal individuals this is of little consequence but the shortened red cell life of sickle-cell patients' results in an abrupt, life threatening situation. Reticulocyte counts drop dramatically during the disease and the rapid turnover of red cells leads to the drop in hemoglobin. Most patients can be managed supportively, some need blood transfusion (Smith, 2008).

1.5.6.3 Splenic Sequestration Crisis:

Are acute, painful enlargements of the spleen. The abdomen becomes bloated and very hard. Management is supportive, sometimes with blood transfusion (Smith, 2008).

1.5.6.4 Hemolytic crisis:

are acute accelerated drop in hemoglobin level. The red blood cells break down at a faster rate. This is particularly common in patients with co-existent G6PD deficiency. Management is supportive, sometimes with blood transfusion (Smith, 2008).

1.5.7 Complication of Sickle Cell Anemia:

Sickle-cell anemia can lead to various complications, including: Overwhelming post- (auto) splenectomy infection (OPSI) is due to functional asplenia, Stroke, Cholelithiasis and cholecystitis, A vascular necrosis, Decreased immune reactions due to hyposplenism (malfunctioning of the spleen), Osteomyelitis, leg ulcers, Pulmonary hypertension and Chronic renal failure.

1.6 Laboratory Diagnosis of Sickle Cell Anemia:

The diagnosis is based on clinical features, family history and laboratory findings. A complete blood count with a peripheral picture is the initial test. A reduced RBC number, variable mean corpuscular volume, increased leucocyte count, reduced ESR, and the presence of sickle shaped cells in the periphery

usually indicate the diagnosis of sickle cell disease. The presence of Howell-Jolly bodies indicates functional asplenia in the patient (Goonsekera, 2018).

Abnormal hemoglobin form can be detected on hemoglobin electrophoresis, a form of gel electrophoresis on which the various types of hemoglobin move at varying speed (Hb-A is absent and both Hb-F and Hb-A2 are increased. Sickle cell hemoglobin (Hb-S) and hemoglobin C with sickling (Hb-SC), the two most common forms-can be identified from there. The diagnosis can be confirmed with high performance liquid chromatography and polymerase chain reaction (PCR) (Clarke, 2000).

1.7 Literature Review:

Many studies found the platelet count and indices were increased in sickle cell disease, in (Michael and Simon, 2018) found the platelet count elevated 1.7-fold and megathrombocyte number 2.3-fold during a symptomatic periods, suggesting elevated result from lack of splenic sequestration.

Another study (Nilesh *et al*, 2014) found mean values for Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) significantly higher in sickle cell patients compared to controls. (Curits *et al*, 2015) found the platelet counts significantly increased ($P < .001$) in 432 adult sickle cell patients.

Whereas (Kenney *et al*, 1980) found significant increased in platelet number and microaggregate formation in sickle patients when compared with healthy controls, also suggested the platelet hyperactivity of the sickle cell reflects an increased circulating population of young, metabolically active platelets resulting from previous autosplenectomy.

1.8 Justification:

Sickle cell disease is an inherited blood disorder, when diagnosed early it will increase life expectancy and decrease the risk of infection. Platelets are housed in spleen at any given time if the spleen is stop functioning properly (functional asplenia) as in sickle cell disease, thrombocytosis result. Thus, this work was intended to evaluate the effect of sickle cell disease regarded to points above.

1.9 Objectives:

1.9.1 General objective:

To determine platelet count and indices, hemoglobin electrophoresis and sickling test among sickler patients in Khartoum state.

1.9.2 Specific objectives:

- To measure the platelets count, platelets indices, sickling test and hemoglobin electrophoresis in sickle cell trait and sickle cell disease in the sickler patient and control.
- To detect the percentage of different types of hemoglobin's by using Hb electrophoresis.
- To compare platelet count and indices between case and control groups.

Chapter Two

Materials and Methods

2.1 Study design:

Case Control analytical study.

2.2 Study Area and Time:

This study was carried out at Khartoum state in Alzaytouna specialist hospital and Private laboratory from Sep-2018 to April 2019.

2.3 Study Population:

Fifty patients (26 Males and 24 females) with Sickle cell anemia and fifty healthy individuals as controls (25 Males and 25 females) from Khartoum state were enrolled in this study.

2.4 Inclusion Criteria:

For a case group , any patients with sickle cell disease were included in the study. Healthy individuals with no sickle cell disease were included in the control group.

2.5 Exclusion Criteria:

Patients who were not diagnosis as sickle cell disease, also patients who did not agree to give information and exposed to blood transfusion or any medications affected on results were excluded.

2.6 Sample Size:

Fifty samples were collected from patients with Sickle cell anemia and fifty samples were collected from normal individual.

2.7 Ethical consideration:

Written informed consent was obtained from all patients and controls included in this study

2.8 Data collection:

2.8.1 Self-administrated pre-coded questionnaires:

Personal information included:-Name, Age, Sex, Telephone number.

2.8.2 Data processing:

All data was written in master sheet and then computerized by medical statistician then analyzed by using SPSS (Statistical Package for Social Sciences ,version22). Continuous data was expressed as mean \pm Standard Deviation and Categorical data was expressed in frequencies and percentages, t-sample test was used for analysis data in this study .Data was presented in form of tables and figures.

2.9 Methodologies:

2.9.1 Collection of Blood Specimens:

Two ml of blood was drawn from anti-cubical vein on the side of the elbow or the back of the hand. The puncture site was cleaned with an antiseptic (70% ethanol), and an elastic band was placed around the upper arm to apply pressure and restrict blood flow through vein. A needle was inserted in to the vein, the blood was collected in a K3 EDTA fluid anticoagulant plastic blood container, and during the puncture the tourniquet was removed to restore the circulation. Once the blood has been collected the needle was removed, and the puncture site was covered to stop any bleeding (European Committee for Clinical Laboratory Standards, 1984).

2.9.2 Laboratory Analysis:

2.9.2.1 Platelets counts and indices from (CBC) :

Materials:

Reagents:

Diluents:Cell pack.

WBC/HBG lysis reagents: stomatolyser- WH

Detergent: -Cell clean.

2.9.2.2 Platelet count and Indices:

Platelets and indices were performed using full automated hematology analyzer (sysmex). Sample analysis performed by aspiration from the sample probe into the sample rotor valve, and then mixed the diluted sample immediately before counting, then a 4.0 ML of blood measured by the sample rotor valve (SRV) and diluted with 1.996 mL of diluents (1:500) and brought to the mixing chamber as diluted sample (1st step dilution) and out of 1:500 dilution sample ; 40 ML is measured by the sample rotor valve, then diluted into 1:2500 with 1.960 ML of diluents then transferred to the RBC/PLT transducer chamber (2nd step dilution) then 250ML of the sample in the RBC/PLT transducer chamber was aspirated through the aperture, the RBC and PLT were counted by the DC detection method and out putted Analysis result.

Table 2.1 Reference value (Golwala, 2016)

Parameter	Males	Females
PLT count	$5.5 \times 10^{10}/\text{unit} <$	$5.5 \times 10^{10}/\text{unit} <$
MPV fl	8.1-12.4 fl	8.5-12.4 fl
PDW fl	9.8-18.0 fl	9.4-18.1 fl
PCT %	0.19-0.39%	0.22-0.40%

2.9.3 Sickling Test:

2.9.3.1 Principle of Sickling Test:

The principle of sickling test was based on microscopical observation of sickling red blood cells when exposed to a low oxygen tension .the slide preparation were left in a humidified chamber for 15min at RT and then examined under the microscope (10x). further observation were taken after 30min , 1 and 2hrs. This process may take up to 12 h in Hb-AS, whereas changes are apparent in homozygote and compound heterozygote after 1 h at 37c. These changes can be hastened by the addition of a reducing agent such as sodium dithionate.

2.9.3.2 Reagents:

Sodium dithionate prepare from:

Solution "A" disodium hydrogen phosphate (Na_2HPO_4) 0.114 mol/l (16.2 g/l).

Solution "B" sodium dithionate ($\text{Na}_2\text{S}_2\text{O}_2$) 0.114mol/l (19.85 g/l) prepared freshly just before use. Working solution prepare by mix 3 volumes of A with 2 volumes of B to obtain a pH of 6.8 use immediately.

2.9.3.3 Method and Result:

Five drops of the freshly prepared reagent was added to 1 drop of anticoagulated blood on a slide. Sealed between slide and cover with a petroleum jelly/ paraffin wax mixture. Sickling takes place almost immediately in Hb S disease and should be obvious in HbS trait within 1 h. a positive control should be included (Hunstman, 1970).

2.9.4 Cellulose Acetate Electrophoresis at Alkaline PH:

For routine work, electrophoresis at pH 8.4-8.6 using a cellulose acetate membrane as substrate is simple rapid and sensitive.

2.9.4.1 Principle of Cellulose Acetate Electrophoresis:

At alkaline pH hemoglobin, a negatively charged protein and in an electric field would be migrated towards the anode (+). Structural variants with surface charge differences would be separated from HbA, those without a change in charge would not (International Committee for Standardization in Hematology, 1978).

2.9.4.2 Equipment:

Electrophoresis tank any horizontal electrophoresis tank which will allow abridge cap of 7cm. Power back capable of delivering 350 V at 80 mA. Wicks of filter or chromatography paper, blotting paper and Applicators. Cellulose acetate paper membranes (plastic backed membranes 7.6 x6.0 cm). Staining equipment and drying oven. PH meter.

2.9.4.3 Reagents:

Electrophoresis buffer. Tris /EDTA/ borate (TEB) pH 8.5, the buffer should be stored at 4c and can be used repeatedly without deterioration. Distaining solution 5 % (v/v) acetic acid 50ml, water to 1 liter. Absolute methanol. Clearing solution. Glacial acetic acid 125 ml, methanol 375 ml, polyethylene glycol 20 ml (International Committee for Standardization in Hematology, 1978).

2.9.4.4 Method:

Prepared a lysate (lyse 1 volume of washed packed cells in 4 volumes of lysing reagent consist of 3.8 g EDTA +0.7 g of potassium cyanide to 1 liter of water).

With the power supply disconnected, fill the components of the electrophoresis tank were filled with TEB buffer. Sock and position the wicks. In a separate dish the cellulose acetate membrane was socked in TEB buffer for at least 5 minutes membrane was immersed slowly, to saturation of the membrane. Blotted the membrane between the two pieces of absorbent paper, but do not let it dry out before sample application. Placed small volume (10ul) of each diluted sample in to a sample well. Dip the applicator in to the sample wells. Applied the samples to the cellulose acetate approximately 3 cm from one end of the membrane.

Allowed the applicator tips to remain in contact with the membrane for 3s.Placed the membrane upside down across the bridge of the tank so that the cellulose acetate surface in contact with the buffer, with the line of application at the cathode end. Connected the power supply and run at 250-350 V for 20 min or until a visible separation is obtained. Disconnected the power supply, remove the membrane and stain in ponceau S for 3-5 min. Dehydrated in absolute methanol for 2-3 min. Immersed in a clearing solution for 6-8 min. Dried at 65c for 4-6 min. Labeled the membranes and store a protective plastic envelope. (International Committee for Standardization in Hematology, 1978).

2.9.4.5 Interpretation and Comment:

Hb S appears in form of band between Hb-A and Hb-A₂. When abnormal Hb is found it is useful to measure the percentage of Hb variant, quantification of Hb-S is particularly important in transfusion regime aimed at suppressing the production of Hb-S is being undertaken, also is important in diagnosing the different interactions of Hb-S with α and β -thalassemia's (International Committee for standardization in Hematology, 1978).

Chapter Three

Results

3.1 Data processing:

One hundred individuals were selected for this study, 50 were sickle cell patients (26 males and 24 females) and 50 matched by age and gender considered as controls (25 males and 25 females). Statistical analysis performed by using SPSS program, the results were expressed as mean \pm SD. Mean of age in sickle cell disease, sickle cell trait and controls was (6.25), (11.68) and (19.04) respectively. Student's t-test was used to compare mean differences between cases and controls. Differences between means of all control and case variables (platelet count, mean platelet volume, platelet distribution width and platelet crit except the platelet large cell ratio) were significantly increased at P value of ≤ 0.05 or lower.

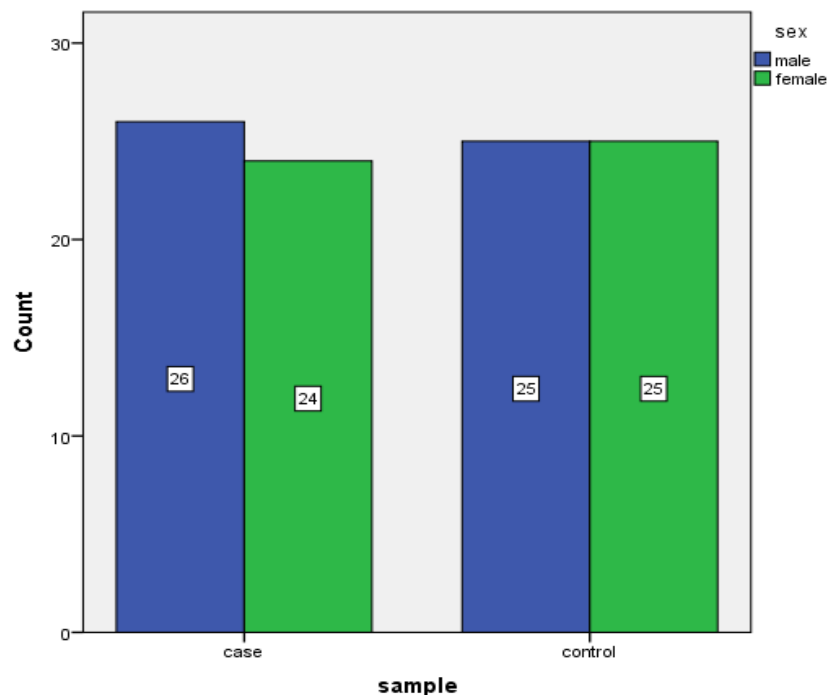


Figure (3.1): Frequency between Sample and sex

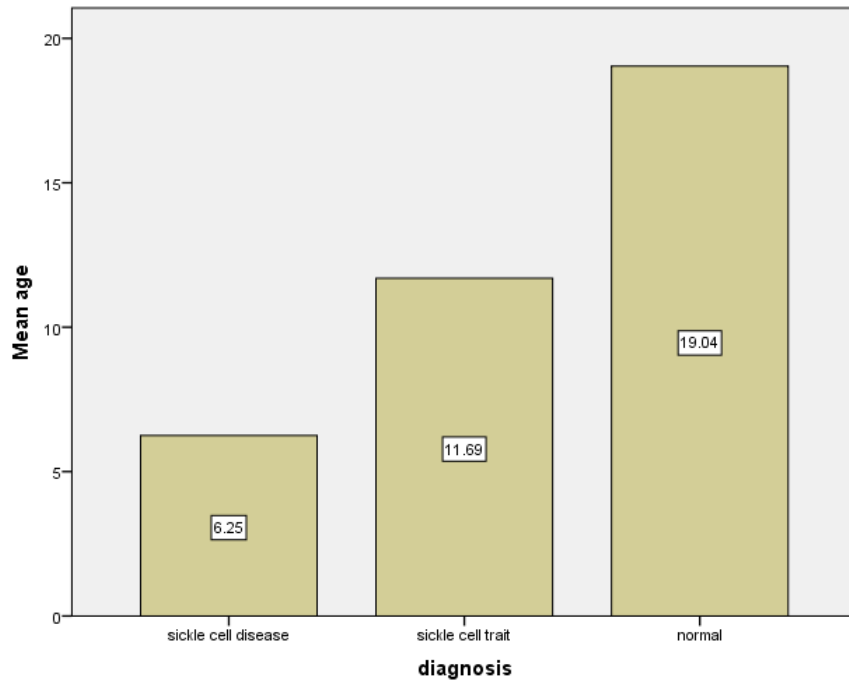


Figure (3.2): Age group among different types of sickle cell and control

Table (3.1): Levels of (HbA, HbA², HbF, HbS) in case and control.

Diagnosis		hbA	hbA ²	hbF	HbS
sickle cell disease	Mean	.00	3.08	20.12	75.38
	Std. Deviation	.000	.654	14.253	15.251
sickle cell trait	Mean	34.92	3.19	17.45	42.88
	Std. Deviation	28.302	.801	27.134	17.294
Normal	Mean	97.34	2.66	.00	.00
	Std. Deviation	.479	.479	.000	.000

Table(3.2): Comparison of (PLTCount, MPV, PDW, PCT and PLCR) in case and control.

Variable	Sample	N	Mean	Std.Deviation	P-value
PLT Count	Case	50	425.64	171.250	0.000
	Control	50	283.78	59.283	
MPV	Case	50	10.250	1.2706	0.000
	Control	50	9.410	1.0148	
PDW	Case	50	10.474	2.0546	0.005
	Control	50	11.542	1.6375	
PCT	Case	50	.3966	.15175	0.000
	Control	50	.2632	.05468	
PLCR	Case	50	20.458	5.9672	0.666
	Control	50	21.014	6.8568	

Chapter Four

Discussion, Conclusion, Recommendation

4-1 Discussion:

Sickle cell disease it is a chronic blood disorder and congenital hemoglobinopathy lead to pain syndrome, infections and organ damage. Results of the present study indicate that platelet count and indices (MPV, PDW and PCT) increased significantly in sickler patients compared with control ($p < 0.05$) while the platelet indices (P-LCR) was not changed ($p > 0.05$). The significant increase of platelet count was in agreement with (Kenny *et al*, 1980), (Curits *et al*, 2015), (Doku *et al*, 2018) and (Michael and Simon, 2018) they reported similar results. The significant increase of platelet indices (MPV and PDW) agree with (Nilesh *et al*, 2014), (Amin *et al*, 2004) and (Celik *et al*, 2015) which founds mean platelet volume (MPV) and platelet Distribution Width (PDW) significantly higher for sickle cell patients compared to controls.

(Nilesh *et al*, 2014) explain that the platelet hyperactivity of the sickle cell reflect an increased circulating population of young, metabolically active platelets, resulting from inflammation, spleen malfunction or previous autosplenectomy and lead to thrombocytosis, Platelet play an active inflammatory role in sickle cell disease by secreting increased amounts of cytokines, also mediate inflammation as well as thrombosis. (Khandekar *et al*, 2018) suggested the use of Mean Platelet Volume (MPV) as a potential marker to identify patients at a greater risk of developing sickle cell crisis.

Our study found The level of HbF highest in SS, few in AS and not found in AA individuals. The highest level of HbS was seen in SS, moderate in AS and also not found in AA persons. The level of HbA was found to be highest in AA individual, moderate in AS and not found in SS individuals. HbA₂ was observed in little amount in SS, AS and AA individuals. (Mahajan *et al*, 2014) found the Level of HbF was to be highest in SS, negligible in AS and not

found in AA individuals. Similarly, the highest level of HbS was seen in SS, moderate in AS and not seen in AA individuals. However, the level of HbA was found to be highest in AA individuals , moderate in AS and negligible in SS individuals .whereas, HbA₂ was observed in minute quantity in SS , AS and AA individuals. Also (Hoffbrand, 2011), (Thachil and Bates, 2014), and (Jawarker and Bhatia, 2018) reported similar results.

4.2 Conclusion:

- Increased of platelet counts, MPV, PDW and PCT were found significantly higher among sickler patients than in normal person.
- Platelet indices (P-LCR)was not changed.
- Increased levels of HbS and HbF in sickler patients compared to control.
- Increased level of HbA in control group and not found in sickler patients.
- HbA₂ was found in little amount in both group case and control.

4.3 Recommendation:

- Detection of Hb-S by using Hemoglobin Electrophoresis will be help in fixing the dose of the treatment.
- Future study should be done on large number of sample to more accurate results.
- Use of Anti-platelet drugs to avoid the risk of crisis.

Reference

- Aldirch T, Nagel R (1998). Pulmonary Complication of Sickle Cell Disease in Bone , editors: Pulmonary and Critical Care Medicine, 6th edition , pp.1-10.
- Amin MA, Amin AP, Kulkani HR (2004). Platelet distribution width(PDW) is increased in vaso-occlusive crisis in sickle cell disease. *Ann Hematol*;83(6):331-5.
- Bart J. B, Ewout R. E, Erfan N and Charlotte F.J. Van T (2018). High Incidence and Recurrence Rate of Venous Thromboembolic Events in Patients with Sickle Cell Disease. *Blood journal* ;132:2381
- Bennett J, Berger B and Bllings C (2009).The structure and function of platelet integrins. *ThrombHemost J* ;7(1):200-5.
- Bhutta R, NaheedA ,Asad A and ShehperKha (2013). Platelet-Platelet indices. *www.Lappedia.net*.
- Bonnefoy A, Harsfalvi J and Pfliegler G (2001).The subendothelium of the HMEC-1 cell line supports thrombus formation in the absence of von Willebrand factor and collagen types I,III and V. *ThrombHaemost J*;85:552-559.
- Bowles KM, CookeL J, Richards EM, Baglin TP (2005) "Platelet size has diagnostic predictive value in patients with thrombocytopenia". *Clinical Lab Hematology* 27 (6):370-3.
- Bowman JE, Murray RF Jr. (1990) Genetic variation and disorders in peoples of African origin. Baltimore: Johns Hopkins University Press: 196-201.
- Bush RL, Pevac WC, Holcroft JW (1997) .A concept of anemia *Am. J. Surg.* 174(2): 143-8.
- Celik T, Unal S, Eknici O, Ozer C, Ilhan G, Oktay G (2015). Mean platelet volume can predict cerebrovascular events in patients with Sickle Cell Anemia. *Pak J Med Sci* ;31(1):203-208.

- Chandrashekhar V (2013). plateletcrit as Screening Tool for Detection of Platelet Quantitative Disorders. *British Journal of Haematology* ;2 (1),pp: 22-26.
- Cheesbrough Monica (2000).District Laboratory Practice in Tropical Countries, Part 2. 4th Edition, Cambridge University Press, 341.
- Clarke GM, Higgins TN (2000) . Laboratory investigation of hemoglobinopathies and thalassemias: review and update .*ClinicalChemistry* . **46** (8) : 1284-90.
- Corwin HL, Gttinger A, Pearl R (2004). The CRIT Study: Anemia and blood transfusion in the critically ill_current clinical practice in the united state. *Crit. Care Med.* **32** (1): 39-52.
- Curtis SA,DandaN,EtzionZ,Cohen HW, Desai and Hiren D (2004). Sickle Cell Disease: History And Origin. *TheInternetJournal of Haematology* 1540-2649.
- Doku A, Dzudzor B, Dankawah G, Sey F (2018). Hematological parameter in Ghanaian sickle cell disease patients. *Journal of blood medicine* ;**30**(9):203-209.
- Drew P, Adrian N, Philip R. Primary immune thrombocytopenias. In: Victor Hoffbrand, DanealCatovsky, eds (2007). Postgraduate Hematology. 4th ed. US: Wiley-Blackwell; p:928-938.
- European committee for clinical laboratory standard(1984). Standard for specimen collection part 1:ECCLS Document **1**(1):1-6.
- Gardiner EE, Andrews RK (2014). Structure and function of platelet receptors initiating blood clotting. *AdvExp Med Biol*;**844**:263-75
- George F, Paul S (2007). Sickle cell disease: old discoveries, New concept and future promise. *The journal of clinical investigation*;**117**(4):850-858
- Gill FM, Sleeper LA, Weiner SJ, Brown AK, Bellevue R, Grover R, Pegelow CH, Vinchinchinsky E (1995). Clinical events in the first

decade in a cohort of infant with sickle cell disease. Cooperative study of sickle cell Disease. **86**:776-783.

- Gladwin MT, Sachdev V, Jison ML (2004). Chronic renal as a risk factor for death in patients with sickle cell disease. *N. Engl. J. Med.*, **350** (9):886-95.
- Golebiewska EM, Poole AW (2015). Platelet secretion: From hemostasis to wound healing and beyond. *Blood Rev*; 29:15362.
- Golwala ZM. (2016) Mean Platelet Volume, Platelet Distribution Width, Platelet Count, and Plateletcrit as Predictors of in-hospital pediatric Mortality. **16**(2)p:356-362.
- Goonsekera HW, Paththinige CS, Dissanayake VHW (2018). Population Screening for Hemoglobinopathies. *Annu Rev Genomics Hum Genet*; **19** (8):355-380
- Greer J.P, Forester J, Lukens J. N and Rodgers G. (2004). Wintrobe's Clinical Haematology. 11th edition. Lippincott Williams and Wilkins: Philadelphia, London, New York, Tokyo, Hong Kong: 619-620.
- Guclu E, Durmaz Y, Karabay O (2013). Effect of severe sepsis on platelet count and their indices. *Afr Health Sci* **13**:333-8.
- Gurney D, Lip GY, Blann AD (2002). "A reliable plasma marker of platelet activation: does it exist?" *Am J Hematol*; **70**:139-44.
- Gupta A, Chandra T, Kumar A (2010). Evaluation of random donor platelets at different temperatures for an extended shelf life. *Biomed Res.* **21**:433-6.
- Hackner SG (2009). Bleeding disorders. In Small Animal Critical Care Medicine. Silverstein DC, Hopper K, Saunders. Philadelphia, USA, pp: 507-514.
- Hegazai M, Qari M (2010). The influence of exposure to light and dark environment on platelet concentrates during storage. *Alexandria Bull Fac Med.* **43**:709-16.

- Hemker HC, van Rijn JL, Rosing J. (1983). Platelet membrane involvement in blood coagulation. *Blood Cells*; **9**:303-17
- Hoffbrand A.V., Catovsky D., Tuddenham E.G.D. and Green A.R. (2011) Postgraduate Haematology. 6th Ed. Oxford, UK: willey-Blackwell: 773.
- Hiran S (2005). Multiorgan dysfunction syndrome in sickle cell disease *.J Assoc physicians India*; **53**(1):19-22.
- Hoffbrand AV, Moss PAH, Pettit JE, eds (2006). Essential Haematology. 5th ed. Carlton, Australia: Blackwell publishing Ltd.
- Holinstat M, (2017). Normal Platelet Function. *Cancer and Metastasis Reviews* **36**, Pp:195-198.
- Huntsman .R.G, Barclay G.P, Canning .E (1970). Rapid whole blood sickling test to differentiate the sickle cell trait from sickle cell anemia. *journal of clinical pathology*, **23**, 781.
- International Committee for Standardization in Hematology (1978). Simple electrophoretic system for presumptive identification of abnormal hemoglobin. **53**, 1058.
- Jawarker A, Bhatia V (2018). A study of HPLC patterns in patients of sickle cell anemi. *Int J Res Med Sci*; **6**(7):2390-2395.
- Jennifer D, Deepa M, Ljiljana V, Mauro A, Joan U, Karen I and Mitchell W (2015). A novel in inflammatory role for platelets in sickle cell disease ;**26**(8);p:726-729
- Khandeker A, Acharya S, Shukla S (2018). Mean Platelet Volume as a Prognosis Indicator in Sickle Cell Anemia. *Int J Recent Surg Med Sci* ; **4**(1)p:5-9.
- Kenny MW, George AJ, Stuart J (1980). Platelet hyperactivity in sickle cell disease: *a consequence of hyposplenism from clinical pathology journal* ;**33**p:622-625.
- Kwiatkowski DP (2005). How malaria has affected the human genome and what human genetics can teach us about malaria. *Am. J. Hum, Genet*; **77** (2): 171-92.

- Lopez E, Bermejo N, Berna-Erro A, Alonso N, Salido GM, Redondo PC (2015). Relationship between calcium mobilization and platelet α - and δ -granule secretion. A role for TRPC6 in thrombin-evoked δ -granule exocytosis. *Arch Biochem Biophys*; **585**:75-81.
- Machlus KR, Thon JN, Italiano JE (2014). "Interpreting the developmental dance of the megakaryocyte: A review of the cellular and molecular processes mediating platelet formation". *British Journal of Haematology* ;**165**(2): 227-36.
- Mahajan AR, Zade VS, Chade SM and Klukarni KM (2014). Fetal Hemoglobin Level in Sickle Cell Anemic Individuals. *British Biomedical Bulletin*. pp:430-436
- Michael L. F, Simon K (2018). Elevated Platelet Count and Mega thrombocyte Number in Sickle Cell Anemia; the American Society of Hematology: Blood journal ; **86**(2): pp 778-783.
- Nilesh T, Deepti J, Ingole NS, Nitin G (2014) Hemostatic alterations in patients of sickle cell trait and homozygous sickle cell disease – A hospital based case control study. *Indian Journal of Basic and Applied Medical Research*; **3**(6); p. 264-274
- Nurden AT (2014). Platelet membrane glycoprotein: a historical review. *Semin Thromb Hemost*; **40**: 577-84
- Ogundunmade M, Habara A (2016). Genetic basis of heterogeneity and severity in sickle cell disease. *Experimental Biology and Medicine* ;**241**:689-696
- Platelet Definition Online. 2013, Platelet Count. <http://medical-dictionary.thefreedictionary.com/platelet>.
- Platelet Research Laboratory. 2015, last update 2015. www.platelet-research.org/1/f
- Platt OS, Brambilla DJ, Rosse WF, et al (June 1994). Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N. Engl. J. Med.* **330** (23):1639-44.

- Powers DR, Elliott-Mills DD, Chan L, *italic* (1991). Bacterial infection in sickle cell disease: risk factors, clinical course, and mortality. *Ann. Intern. Med.* **115** (8):614-20.
- Quinn C (2013). Sickle Cell Disease in Childhood. *Pediatr Clin North Am*; **60**(6):1363-1381
- RomanaM, Connes P, Key NS (2018). Microparticles in sickle cell disease. *Clin. Hemorheol. Microcirc.***68**(2-3):319-329
- Salam A, Anwaar A, SamahAand Anil P (2018). Thromboembolic Complications in Patients with Sickle Cell Disease. *Blood journal* ; 132:4913
- Smith WR, Penberthy LT, Bovbjerg VE, *italic* (2008).Daily assessment of pain in adults with sickle cell disease. *Ann. Intern. Med.* **148**(2):94-101.
- Steinberg M, Habara A (2016). Genetic basis of heterogeneity and severity in sickle cell disease .*Experimental Biology and Medicine*; **241**:689-696.
- ThachilJ,BatesI (2014). Mansons Tropical Infectious Disease.Hematological Disease in the Tropics, 23^{ed}; 894-932.
- Vincent JL, Baron JF, Reinhart K (2002). Classification of anemia and blood transfusion in critically ill patients. *JAMA* **288** (12): 1499-507.
- Vinik A. I, Erbas T, Sun T, Nolan R, and Pittenger G. L (2001) "Platelet dysfunction in type2 diabetes "Diabetes Care; **24**(8): 1476-1485.
- Wang Y, Gallant RC, Ni H (2016). Extracellular matrix protein in the regulation of thrombus formation. *Curr Opin Hematol.* **23**:280-7.
- Wenk R (1976). Comparison of five methods for preparing blood smears. *American journal of medical technology*, 42, 71.
- Williams T, Chakravorty S (2015). Sickle cell disease: a neglected chronic disease of increasing global health importance. *ArchDisChild* , **100**:48-53

- Williams TN (2016).Sickle Cell Disease in Sub-Saharan Africa. *Hematol.Oncol. Clin. North Am*; **30**(2):343-58
- Witekend, D, H (1979). On the nature of Romanowesky dyes and the RomanoweskyLieshman dye. *Clinical and Laboratory hematology*, 1.247
- Wong W, Powars D, Chan L, Haiti A, Johns C, Overturf G, (1992). Cholelithiasis infection in sickle cell anemia: a thirty year epidemiologic experience. *Am J Hematol* **39** (3): 176-82.
- Wun T, Pagleroni T, Pangaswami A, Franklin PH, Wellborn J, Cheung A, Tablin F. (1997) Platelet activation in Patients with sickle cell disease. *BritishJournal of Haematology* **100**(11),741-749.
- Zhang Z, Xu X, Ni H, Deng H (2014). Platelet indices are novel predictors of hospital mortality in intensive care unit patients. *J Crit Care*; 29:885.
- Zucker MB and Nachmias V T. (1985) Platelet activation *ArteriosclerThromb Vase Bio J*; **5**:2-18.

Appendice (1)

Questionnaire

Date:

Name:

Specimen No:

Age:

Telephone No:.....

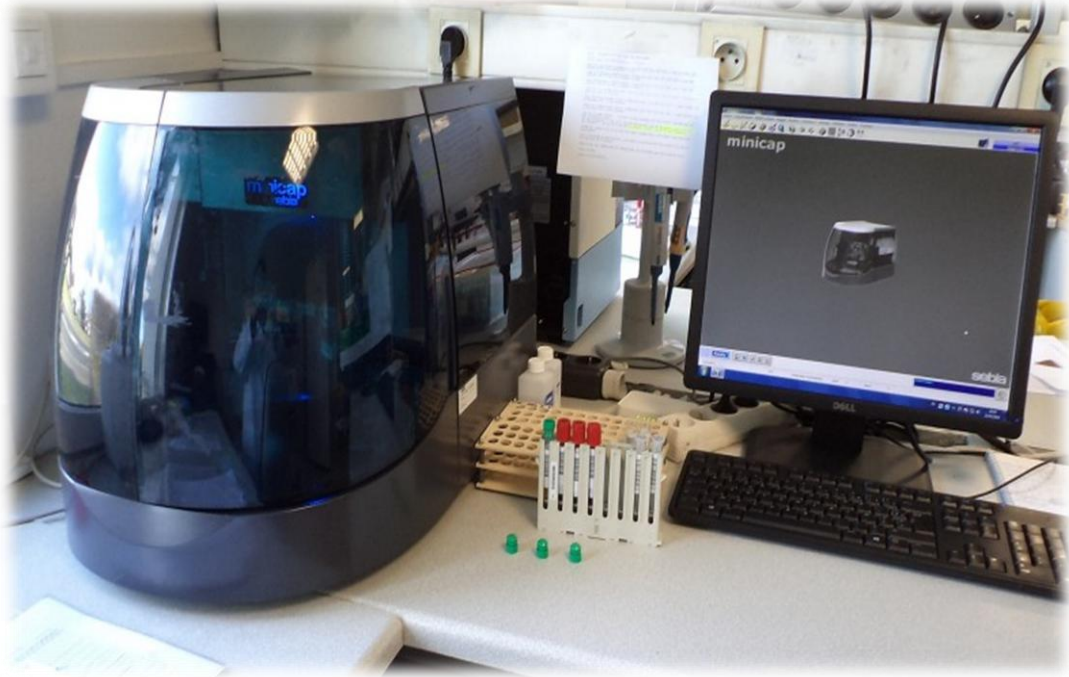
Sex: Male (.....) Female (.....)

Appendice (2)



Hematology Analyzer device
(Sysmex kx-21N)

Appendice (3)



Machine for Hb Electrophoresis
(minicap)

Appendice (4) :

استمارة طلب موافقة

من الشخص المشارك فى البحث او من ينوب عنه

أنا الباحث : ملاذ نصر الدين بابكر

المؤسسة : جامعة السودان للعلوم والتكنولوجيا – السودان

القسم : أمراض الدم

المؤهل المطلوب : الماجستير

أقوم ببحث ودراسة عن (تحديد تعداد الصفائح الدموية ومؤشراتها، رحلان الخضاب الكهربائى واختبار المنجلة للمصابين بالانيميا المنجلية) .

عزيزي المشارك :

لقد تم اختيارك لتشارك فى هذا البحث أنت ومعك عدد آخر من المرضى من مرضى الانيميا المنجلية الذين لم يخضعوا للعلاج ..

نتوقع بمشاركتك أنت والمرضى الآخرين أن نتحصل على نتائج تفيد مرضى الانيميا المنجلية للتأكد من سلامة الدم بتقنيات بسيطة وغير مكلفة مثل محلل الدم الكلى والرحلان الكهربائى.

خلال هذه الدراسة سنقوم بأخذ المعلومات الشخصية والمرضية وعينة من الدم مع خلو هذا الفحص من أية مخاطر تذكر.

ونحن اذ نأمل فى مشاركتك معنا فى هذا البحث ، نؤكد لك على سرية المعلومات والوثائق الخاصة بك ، وانه لن يطلع عليها إلا الباحث .

ونود أن نشير كذلك الي أن المشاركة فى البحث طوعية وان رفضك للمشاركة فى البحث لا تفقدك الحق فى أي فوائد من البحث ، وانه بمشاركتك ستكون احد المتطوعين والذين يشملهم البحث وعددهم حوالي 50 مشارك متطوع .

.....
إذا كان لديك أي سؤال أو استفسار يخص البحث ، المشاركين معك فى البحث ، أو حقوقك كمشارك أثناء تنفيذ البحث يمكنك تقديم السؤال مباشرة دون تردد .

فورم إقرار موافقة المشارك في البحث موقعا

إقرار المشارك :

لقد اطلعت على المعلومات الحالية والتي تم شرحها لي وأتيح لي طرح الأسئلة عنها كيفما شئت ، وقد تلقيت الإجابات الوافية عن كل الأسئلة ، وأنا أقر بالموافقة على المشاركة متطوع في هذه الدراسة وأعلم بحقي في التوقف عن المشاركة في أي وقت دون أن يؤثر ذلك على حقوقي في تلقي العناية الطبية اللازمة في أي وقت لاحقا

.....: إسم المشارك

.....: توقيع المشارك

من ينوب عن المشارك (في حال عدم قدرة المشارك على قراءة الإقرار ويحتاج الى من يشرح او يترجم له)

.....: إسم من ينوب عن المشارك

.....: توقيع من ينوب عن المشارك

.....: رقم الهاتف

مع خالص الشكر لتعاونكم

.....: توقيع الباحث