بسم الله الرحمن الرحيم

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Red Cell Parameters and its Correlation with Hemoglobin Types in Sickle Cell Trait, Khartoum State, 2017

قياسات كريات الدم الحمراء وعلاقتها بأنواع الهيموقلوبين في خلة الكرية المنجلية، ولاية الخرطوم، 2017

A dissertation submitted in a partial fulfillment of the requirements of the M.Sc. Degree in Hematology and Immunohematology

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قَالَ تَعَالَىٰ:

﴿ وَمَا تَشَآءُونَ إِلَّآ أَن يَشَآءَ ٱللَّهُ رَبُّ ٱلْعَٰلَمِينَ ﴿٢٩﴾ ﴾

صدق الله العظيم

سورة التكوير الايه (29)

Dedications

To my grandfather Hamid Abdulrahman Alameen, and

My grandmother Madeena Hamid Alameen.

To my parents whom are the source of fulfilling,

God showed them good things about us.

To my sisters, my brothers, and my friends

Who are always stand with me.

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Praise and thanks to Allah, Lord of the world, for all countless graces.

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My deepest gratitude to my dear sister who has always helped me Tasnim Mohammed Hamad

Abstract

Red blood cells of individuals with sickle cell traitcontain both Hb A and Hb S, but there is always more Hb A than Hb S. Sickle cell trait is not regarded as disease because complication is mild nevertheless some complication can developed like anemia, joint pain, weakness and abdominal pain.

This study was aimed to study red cell parameters and its correlation with haemoglobin types in Sudanese with sickle cell trait.

A total of 100 subjects were recruited for this study, 50 sickle cell trait individuals and 50 healthy volunteer as a control group. Blood sample were collected from all participants in EDTA containers.

Patients' data was collected by structured interview questionnaire and analysed by statistical package for social sciences (SPSS), version 20

Red cell parameters were measured by automated haematology analyzer. Capillary electrophoresis was used for quantitation of haemoglobin types.

There was statistically significant decrease Hb concentration, PCV and red cell indices in sickle cell trait when compared to healthy controls.

In sickle cell trait individuals mean of HbS percent was 39.1%, HbA was 57.0%, HbA_2 was 2.9% and HbF was 0.7%.

There was statistically significant positive correlation between HbS and Hb concentration and statistically negative correlation between HbA and Hb concentration. The correlation between each of HbF and HbA_2 with Hb concentration was not statistically significant.

There was no statistical difference between HbS and age, also no association between HbS and gender.

In conclusion, red blood cells parameters were significantly lower in individuals with sickle cell trait than healthy controls, and there was statistically significant positive correlation between HbS and red blood cells parameters.

الملخص

تحتوي كرات الدم الحمراء للأشخاص الذين يحملون صفة الكرية المنجلية على هيموقلوبين AوهيموقلوبينS، ولكن دائما هيموقلوبينA أكثر من هيموقلوبينS.

خِلة الكرية المنجلية قد لا تعتبر مرض لأن مضافاتها قليلة و لكن بالرغم من ذلك قد تحدث بعض المضاعفات مثل: فقر الدم, الضعف العام, الام البطن.

هدفت هذه الدراسة لقياس كريات الدم الحمراء وعلاقتها بأنواع الهيموقلوبين في خلة الكرية المنجلية.

مجتمع الدراسة يحتوي علي 50 شخص حامل لصفة الانيميا المنجلية و50 أصحاء, كمجموعة ضابطة.

اخذت عينة الدم في وعاء EDTA .

بيانات المريض اخذت عن طريق استبيان منظم وحللت النتائج عن طريق استخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية.

اظهرت النتائج نقصان ذو دلالة احصائية معنوية في قياسات كريات الدم الحمراءللاشخاص المصابين خلة الكرية المنجلية مقارنة مع الأشخاص الاصحاء.

و اظهرت ايضا ان متوسط نسبة هيموقلوبين S)39.1%), متوسط نسبة هيموقلوبين A) 57%), متوسط نسبة هيموقلوبين F) 0.7%)و متوسط نسبة هيموغلبين A_2)2.7%).

واظهرت علاقة ذو دلالة احصائية معنوية موجبة بين هيموقلوبين Sومستوى الهيموقلوبين , وعلاقة ذو دلالة احصائية سالبة بين هيموقلوبين Aوتركيز الهيموقلوبين, بينما لا توجد علاقة ذات دلالة احصائية معنوية بين هيموغلوبين F, A_2وتركيز الهيموقلوبين.

وايضا لا توجد علاقة ذو دلالة احصائية معنوية بين هيموقلوبين S والعمر وليس هنالك ارتباط بين هيموقلوبين S والنوع.

خلصت هذه الدراسة الى أن قياسات كريات الدم الحمراء للأشخاص الذين يحملون صفة الكرية المنجلية أقل بدرجة مؤثرة من الأشخاص الأصحاء وهنالك علاقة ذات دلالة احصائية موجبة بين هيموقلوبين S و قياسات كريات الدم الحمراء.

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List of Abbreviations

ALA	Amino Laevulinic Acid
ANOVA	Analysis of Variance
ATP	Adenosine Tri Phosphate
CBC	Complete Blood Count
CE	Capillary Electrophoresis
CO_2	Carbon Dioxide
COA	Co enzyme A
DNA	Deoxyribonuclic Acid
EDTA	Ethylenediaminetetra-acetic Acid
EOF	Electric Osmotic Flow
GAG	Guanine Adenine Guanine
GTG	Guanine Thiamine Guanine
Hb	Hemoglobin
HICN	Cyanomethemoglobin
HPLC	High performance Liquid Chromatography
KD_a	Kelo Dalton
LCD	Liquid Crystal Display
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
NO	Nitric Acid
PCV	Packed Cell Volume
RBC_S	Red Blood Cell
SCA	Sickle Cell Anemia

SCD	Sickle Cell Disease
SD	Stander Deviation
SPSS	Statistical Package for Social Sciences
VCAM	Vascular Cell Adhesion Molecule
WBC_S	White Blood Cell

Chapter One

Introduction and Literature Review

1.1Hemoglobin

Haemoglobin (molecular weight 64 500) contains four haemgroups linked to four globin chains, and can bind four moleculesof oxygen. Myoglobin (molecular weight 17 000) accountsfor 4 – 5% of body iron and has a single haem group attached toits one polypeptide chain. It has a higher affinity for oxygenthan haemoglobin and behaves as an oxygen reserve in muscles.The mitochondria contain a series of haem and non - haem ironproteins (including the cytochromes *a* , *b* and *c* , succinate dehydrogenaseand cytochrome oxidase) that form an electron transport pathway responsible for the oxidation of intracellularsubstrates and the simultaneous production of adenosine triphosphate(ATP). Haem is an essential component of microsomaland mitochondrial cytochrome P450, which is concernedwith hydroxylation reactions (including drug detoxification bythe liver), and of cyclooxygenase, involved in prostaglandin synthesis. Ironis also necessary for the function of ribonucleotidereductase, akey enzyme in DNA synthesis (Hoffbrand *et al.*, 2011).

1.1.2 Hemoglobin structure

Normal adult blood contains three types ofhemoglobin .The major component is hemoglobin A with the molecular structureα, βThe minor hemoglobin's contain ɣ (fetal Hb) or δ (Hb A_2) globin chains instead of βchains. Inthe embryo and fetus, Gower 1, Portland, Gower 2and fetal Hb dominate at different stages .The genes for the globin chains occur in two clusters:ε, γ, δ and βon chromosome 11 and ζ, α on chromosome16 (Hoffbrand *et al.*, 2006).

Table 1.1 Hemoglobin structure and types according to developmental stage(Barbara, 2006).

Hemoglobin species	Globin chains	Period when normally present
A	α_2,β_2	Major hemoglobin in adult life
A2	α_2,δ_2	Minor hemoglobin in adult life
F	α_2,γ_2	Minor hemoglobin in adult life; major hemoglobin in fetal life with adeclining percentage through the neonatal period
Gower1	ζ_2,ε_2	Significant hemoglobin during early intrauterine life
Gower2	α_2,ε_2	Significant hemoglobin during early intrauterine life
Portland	ζ_2,γ_2	Significant hemoglobin during early intrauterine life

1.1.3 Hemoglobin function

The hemoglobin molecule contained within redblood cells is essential for human life, being themeans by which oxygen is transported to the tissues.Other functions include the transport of carbon dioxide(CO_2) and a buffering action (reduction of thechanges in pH that would otherwise be expectedwhen an acid or an alkali enters or is generated in ared cell.A normal hemoglobin molecule is composedof two dissimilar pairs of polypeptide chains,each of which encloses an iron-containing porphyrin designated haem. Hemoglobin has amolecular weight of 64–64.5 kDa. Haem is essentialfor oxygen transport while globin serves to protecthaem from oxidation, renders the molecule solubleand permits variation in oxygen affinity (Barbara, 2006).

1.1.4 Hemoglobin synthesis

Hemoglobin synthesis requires the coordinated production of heme and globin. Heme is the prosthetic group that mediates reversible binding of oxygen by hemoglobin. Globin is the protein that surrounds and protects the heme molecule (www.sickle.com)

Heme is synthesized in a complex series of steps involving enzymes in the mitochondrion and in the cytosol of the cell. The first step in heme synthesis takes place in the mitochondrion, with the condensation of succinyl CoA and glycine by ALA synthase to form 5-aminolevulic acid (ALA). This molecule is transported to the cytosol where a series of reactions produce a ring structure called coproporphyrinogen III. This molecule returns to the mitochondrion where an addition reaction produces protoporhyrin IX.The enzyme ferrochelatase inserts iron into the ring structure of protoporphyrin IX to produce heme. Deranged production of heme produces a variety of anemias. Iron deficiency, the world's most common cause of anemia, impairs heme synthesis thereby producing anemia. A number of drugs and toxins directly inhibit heme production by interfering with enzymes involved in heme biosynthesis(www.sickle.com).

Two distinct globin chains (each with its individual heme molecule) combine to form hemoglobin. One of the chains is designated alpha. The second chain is called "non-alpha". With the exception of the very first weeks of embryogenesis, one of the globin chains is always alpha. A number of variables influence the nature of the non-alpha chain in the hemoglobin molecule. The fetus has a distinct non-alpha chain called gamma. After birth, a different non-alpha globin chain, called beta, pairs with the alpha chain. The combination of two alpha chains and two non-alpha chains produces a complete hemoglobin molecule, a total of four chains per molecule (www.sickle.com)

1.1.5 Hemoglobinopatheis

Inherited abnormalities of hemoglobin synthesis may be divided into two groups: thosecharacterized by structurally abnormal hemoglobin variants and those in which one ormore of the normal polypeptide chains of hemoglobin are synthesized at a reduced rate.(John*et al*.,2003).

Table1.2: The clinical syndromes produced by hemoglobin abnormalities (Hoffbrand *et al*., 2006).

Syndrome	Abnormality
Haemolysis	Crystalline haemoglobins(Hb S, C, D, E, etc.) Unstable haemoglobin
Thalassaemia	α or β resulting from reducedglobin chain synthesis
Familial polycythaemia	Altered oxygen affinity
Methaemoglobinaemia	Failure of reduction

1.2Sickle cell disease

Sickle cell disease (SCD) is an inherited chronic haemolyticanaemia whose clinical manifestations arise from the tendencyof the haemoglobin (HbS or sickle haemoglobin) to polymerizeand deform red blood cells into the characteristic sickleshape. This property is due to a single nucleotide change inthe β - globin gene leading to substitution of valine for glutamicacid at position 6 of the β- g lobin chain (β6 glu→ val or β s). Thehomozygous state (HbSS or sickle cell anaemia) is the mostcommon form of sickle cell disease, but interaction of HbS withthalassaemia and certain variant haemoglobins also leads tosickling. The term ‘ sickle cell disease ’ is used to denote all entitiesassociated with sickling of haemoglobin within red cells (Hoffbrand *et al*., 2011).

1.2.1 Sickle cell anemia

Sickle cell anemia is an inherited form of anemia — a condition in which there aren't enough healthy red blood cells to carry adequate oxygen throughout your body.Normally, your red blood cells are flexible and round, moving easily through your blood vessels. In sickle cell anemia, the red blood cells become rigid and sticky and are shaped like sickles or crescent moons. These irregularly shaped cells can get stuck in small blood vessels, which can slow or block blood flow and oxygen to parts of the body (Iughetti, 2016).

1.2.2 History

In 1910, James B. Herrick reported "peculiar, elongated, sickle-shaped red corpuscles" in"a case of severe anemia" in a black student.He described the clinicalmanifestations in his patient in considerable detail. The sickle cells, he thought, werefreakish poikilocytes, and, with considerable prescience, he suggested that they were amanifestation of a peculiar chemical or physical condition, emmel, in 1917, observed thetransformation of the biconcave red cell to the sickle form in vitroHe also noted thatsickling occurred both in persons with severe anemia and in others who were apparentlyhealthy, thus recognizing both sickle cell anemia and sickle cell trait, the comprehensive studies of Hahn and Gillespie in 1927 delineated the conditionsaffecting sickle cell formation in vitro, including pH, temperature, fixatives, tonicity, andothers. They postulated that similar effects of oxygen couldoccur in vivo, with hypoxia leading to cellular distortion and consequent hemolysis. Later,Hahn applied the term sickle cell trait to the asymptomatic condition associated with invitro sickling. He performed studies in families and concluded that the trait was inheritedas a dominant character(Keith, 2017)

1.2.3 Prevalence and geographic distribution

The highest prevalence of Hb S is in tropical Africa and among blacks in countries thatparticipated in the slave trade. It occurs with lower frequency in the Mediterranean basin,Saudi Arabia, and parts of India. Results of studies of DNA polymorphisms linked to the ßS gene suggest that it arose from three independent mutations in tropical Africa.The most common $ß^S$ chromosome is found in Benin (neighboring Nigeria)and central West Africa. A second haplotype is prevalent in Senegal and the African WestCoast, and a third haplotype is seen in the Central African Republic (Bantu-speakingAfrica). The same three haplotypes are associated with the ß S gene in black Americansand Jamaicans (John, *et al*, 2003).

The Hb S gene in the Eastern Province of Saudi Arabia and inCentral India is associated with a different DNA structure not encountered in Africa andprobably represents a fourth independent occurrence of the sickle cell mutation.Only the Benin and Senegal haplotypes are prevalent among North Africans, Greeks, andItalians, suggesting that the $ß^S$ mutation spread to the Mediterranean basin from WestAfrica. In some parts of Africa, as much as 45% of the population has sickle celltrait. In the United States Latin America, and the Caribbean approximately 8% of blacks carry the sickle gene. In the United States, the expectedincidence of sickle cell anemia (Hb SS) at birth is 1 in 625. The validity of this estimate isborne out by prospective studies of the Hb phenotypes of black infants at birth taking into account increased mortality; approximately 70,000 cases of sickle cell diseasewould be expected among black Americans in the United States (John,2003).

1.2.4 Pathophysiology

The sickle mutation substitutes thymine for adenine in the sixth codon of the ß gene (GAG__GTG), thereby encoding valine instead of glutamine in the sixth position of theß-chain. This ostensibly minor change in structure is responsible for profound changes inmolecular stability and solubility. The tendency of deoxygenated Hb S to

undergopolymerization underlies the innumerable expressions of the sickling syndromes (Schechter and Noguchi, 1994).

1.2.4.1 Effect on erythrocytes

Red cells acquire the sickle or elongated shape upon deoxygenationas a result of intracellular polymerization of HbS, a phenomenonthat is reversible on reoxygenation. Even in thenormally shaped red cells, however, the presence of HbSpolymer reduces deformability, with consequent increase inblood viscosity. Repeated or prolonged sickling progressivelydamages the red cell membrane, which is a phenomenon ofprimary importance in the pathophysiology of SCD. Membranedamage causes movement of potassium ions and water out ofthe cell by the Gardos pathway and potassium – chloride co-transport,leading to dehydration of red cells. The intracellular haemoglobinconcentration rises (producing dense cells), whichshortens the delay time to sickle polymer formation. A secondkey consequence of membrane damage is alteration of thechemistry of the red cell membrane. Perturbation of lipidorganization causes negatively charged phosphatidylserine toappearon the red cell surface instead of its normal location inthe inner monolayer. In addition, the red cells become abnormallyadherent to the vascular endothelium through vascularcell adhesion molecule (VCAM) - 1, thrombospondin andfibronectin (Hoffbrand *et al.*,2011).

1.2.4.2Haemolysis

Intravascular hemolysis results from the lysis of complement-sensitive red cells andHb lost during sickling- or shear-induced membrane fragmentation. Extravascular hemolysis may occur by two mechanisms: monocyte and macrophagerecognition and phagocytosis of red cells that have undergone sickling-oroxidation-induced membrane changes and physical entrapment ofrheologically compromised red cells. Sickling- and oxidation-induced membranechanges promote cell dehydration and clustering of membrane protein band 3. This leadsto

accumulation of IgG and complement on the sickle cell surface(Hoffbrand*et al.*, 2011).

1.2.4.3 Vaso-occlusion

Several processes contribute to development of vaso– occlusion in SCD. Slowing of blood flow arises from abnormal regulationof vascular tone as a result of diminished nitric oxide (NO) -induced vasodilatation. This is aggravated by increase in bloodviscosity, resulting from less deformable red cells, a phenomenon called abnormal rheology. Vaso-occlusion is initiated byadhesion of young deformable red cells to the vascular endothelium,and is followed by trapping of rigid irreversibly sickled cells. Adhesion occurs in the post - capillary venules and is promoted by leucocytosis, platelet activation and inflammatory cytokines. Genetic influences independent of the sicklemutation probably modulate the tendency for vaso–occlusionin individuals and account for some phenotypic variation seenin this disease (Hoffbrand*et al.*, 2011).

1.2.5 Clinical feature

Sickle cell disease (SCD) usually manifests early in childhood. For the first 6 months of life, infants are protected largely by elevated levels of Hb F; soon thereafter, the condition becomes evident(Joseph, 2017)

The most common clinical manifestation of SCD is vaso-occlusive crisis. A vaso-occlusive crisis occurs when the microcirculation is obstructed by sickled RBCs, causing ischemic injury to the organ supplied and resultant pain. Pain crises constitute the most distinguishing clinical feature of sickle cell disease and are the leading cause of emergency department visits and hospitalizations for affected patients.Approximately half the individuals with homozygous Hb S disease experience vaso-occlusive crisis. The frequency of crisis is extremely variable. Some have as many as 6 or more episodes annually, whereas others may have episodes only at great intervals or none at all. Each individual typically has a

consistent pattern for crisis frequency.Pain crises begin suddenly. The crisis may last several hours to several days and terminate as abruptly as it began.The pain can affect any body part. It often involves the abdomen, bones, joints, and soft tissue, and it may present as dactylitis (bilateral painful and swollen hands and/or feet in children), acute joint necrosis or avascular necrosis, or acute abdomen. With repeated episodes in the spleen, infarctions and autosplenectomy predisposing to life-threatening infection are usual. The liver also may infarct and progress to failure with time. Papillary necrosis is a common renal manifestation of vaso-occlusion, leading to isosthenuria (ie, inability to concentrate urine).Severe deep pain is present in the extremities, involving long bones. Abdominal pain can be severe, resembling acute abdomen; it may result from referred pain from other sites or intra-abdominal solid organ or soft tissue infarction. Reactive ileus leads to intestinal distention and pain.The face also may be involved. Pain may be accompanied by fever, malaise, and leukocytosis.Bone pain is often due to bone marrow infarction. Certain patterns are predictable, since pain tends to involve bones with the most bone marrow activity and because marrow activity changes with age. During the first 18 months of life, the metatarsals and metacarpals can be involved, presenting as dactylitis or hand-foot syndrome(Joseph, 2017)

1.2.2 Sickle cell trait

Sickle cell trait, the heterozygous state for the Hb S gene, is present in approximately 8%of black Americans and in as many as 30% of some African populations. The red cells ofsuch individuals contain both Hb A and Hb S, but there is always more Hb A than Hb S (Schechter and Noguchi, 1994).

1.2.2.1 Clinical feature

Sickle cell trait usually is not regarded as a disease state because it has complications that are either uncommon or mild. Nevertheless, under unusual circumstances serious morbidity or mortality can result from complications related

to polymerization of deoxy-hemoglobin S. Such problems include increased urinary tract infection in women, gross hematuria, complications of hyphema, splenic infarction with altitude hypoxia or exercise, and life-threatening complications of exercise, exertional heat illness (exertionalrhabdomyolysis, heat stroke, or renal failure) or idiopathic sudden death (1-4). Pathologic processes that cause hypoxia, acidosis, dehydration, hyperosmolality, hypothermia, or elevated erythrocyte 2,3-DPG can transform silent sickle cell trait into a syndrome resembling sickle cell disease with vaso-occlusion due to rigid erythrocytes. Compound heterozygous sickle cell disease can be mistaken as uncomplicated sickle cell trait, particularly when an unusual globin variant is involved (John,2000)

In addition some disease associations have been noted with sickle cell trait which might not result from polymerization of hemoglobin S but from linkage to a different gene mutation. The association of hemoglobin S with cases of renal medullary carcinoma, early end stage renal failure in autosomal dominant polycystic kidney disease, and surrogate end points for pulmonary embolism are not necessarily the result of hemoglobin S polymerization. Complications from sickle cell trait are important because about three million people in the United States have this genotype, about 40 to 50 times the number with sickle cell disease (John, 2000)

1.2.2.2 Laboratory finding

1.2.2.2.1 Blood count

The hemoglobin concentration is normal, except inthose with coexisting a thalassaemia trait who may be slightly anaemic. Similarly, the mean cell volume(MCV) and mean cell hemoglobin (MCH) arereduced in those with coexisting a thalassaemia trait, but otherwise are normal. Athalassaemia trait is somewhat more prevalent inAfricans and Afro-Americans with sickle cell traitthan in those with normal βglobin genes, so thatit is not rare for subjects with sickle cell trait to haveborderline anaemia or reduction of the MCV andMCH(Barbara, 2006).

1.2.2.2.2 Blood film

The blood film may be completely normal or mayshow microcytosis or target cells. If a subjectwith sickle cell trait develops iron deficiency, targetcells are often prominent. Although classical sicklecells are not seen, small numbers of plump cells thatare pointed at both ends have been reported;such cells were described in about 96% of individualswith sickle cell trait in comparison with 4% of normalsubjects.During P. falciparummalaria, subjects with sicklecell trait have a blood film showing a lower percentageof parasitized cells than is seen in subjects without a haemoglobinopathy(Barbara, 2006).

1.2.2.2.3Sickling test

The presence of haemoglobin S can be demonstratedby a sickle test in which sickle cell formation is inducedwhen blood is deoxygenated. Adrop of bloodis placed between a glass slide and a cover slip andis sealed with molten paraffin wax so that the metabolicactivity of white cells leads to deoxygenation.After an appropriate period of time, the preparationis observed with a microscope. In a common modificationof the method, the drop of blood is first mixedwith a drop of 2% sodium metabisulphite and thepreparation is observed at 24 h. These methods arenot very suitable for use in a routine diagnostic laboratorywhere their place has largely been taken by thesickle solubility test (Barbara, 2006).

1.2.2.2.4.Hb S Solubility Test

Sickle cell haemoglobin is insoluble in the deoxygenatedstate in a high molarity phosphate buffer. The crystals thatform refract light and cause the solution to be turbid(Bain *et al*., 2011).

A positive solubility or sickling test indicates the presenceof Hb S and as such is useful in the differential diagnosisof Hbs D and G, which migrate with Hb S on cellulose acetateelectrophoresis at alkaline pH. Positive results are alsoobtained on samples containing the rare haemoglobinsthat have both the Hb S mutation and an

additional mutation in the β chain. A positive solubility test merely indicates the presence of a sickling haemoglobin and does not differentiate between homozygotes, compound heterozygotesand heterozygotes. In an emergency, it may benecessary to decide if an individual suffers from sickle celldisease before the haemoglobin electrophoresis results areavailable. In these circumstances, if the solubility test ispositive, a provisional diagnosis of sickle cell trait can bemade if the red cell morphology is normal on the bloodfilm. If the blood film shows any sickle cells or numeroustarget cells, irrespective of the Hb, a provisional diagnosis of sickle cell disease should be made; many patients withsickle cell/Hb C compound heterozygosity will have a normalHb (Bain *et al*., 2011).

1.2.2.2.5 Hemoglobin electrophoresis

Haemoglobinelectrophoresis is still the mostcommon technique for the initial detection andcharacterization of a variant haemoglobin, although high performance liquid chromatography (HPLC)is increasingly taking its place. Haemoglobin electrophoresisdepends on the principle that, when proteinsapplied to a membrane are exposed to a charge gradient, they separate from each other andcan then be visualized by either a protein stain or ahaem stain. Haemoglobin electrophoresiscan be carried out on filter paper, a cellulose acetatemembrane, a starch gel, a citrate agar gel or anagarose gel. Haemoglobin electrophoresis is bestperformed on lysed packed red cells so that a consistentamount of haemoglobin is applied and there areno bands caused by the presence of plasma proteins.If whole blood is used, the presence of a paraproteinor a very high concentration of polyclonal immunoglobulins may lead to a prominent band whichcan be confused with a variant hemoglobin .If this is suspected in a laboratory usingwhole blood for lysate preparation, plasma shouldbe removed and packed red cells should be washedbefore a new lysate is prepared (Barbara, 2006).

It might be expected that heterozygotes for hemoglobin S would have equal amounts of hemoglobinS and hemoglobin A. In fact, hemoglobin Ais somewhat more than 50% and hemoglobin S issomewhat less, usually around 40%. Hemoglobin S is readily detected byhemoglobin electrophoresis and other techniques. A sickle solubility test should always be performed when the presence of a significant proportion of haemoglobin S is suspected. (Bain and Phelan, 1996)

Haemoglobin S can also be distinguished fromhaemoglobinAandhaemoglobin C by immunological techniques based on monoclonal antibodies to sequencesincluding the amino acids which are substitutedin haemoglobin S and haemoglobin C. It is thuspossible to distinguish sickle cell trait from sicklecell anaemia and sickle cell/haemoglobin C disease.However, sickle cell/b+ thalassaemia may not bedistinguished from sickle cell trait. Such monoclonalantibodies were previously commercially availablein kit form as HemoCard A plus S and HemoCard C. (Bain and Phelan, 1996).

Hemoglobin S maythen be as low as 10%. The proportion of hemoglobinS is also reduced if there is coexisting iron deficiency, It has been observed to fall markedly inmegaloblastic anemia and may be low in lead poisoning. (Steinberg, 2001)

The percentage of hemoglobin A_2 may be slightlyelevated in sickle cell trait. However, this is not aparticularly useful investigation to perform (Almeid*etal*.,2001)

1.2.3 Variant sickle cell syndrome

Several of the doubly heterozygous states for Hb S and a second disorder of Hbsynthesis are characterized by clinical and hematologic aberrations that to some extentmimic the features of sickle cell anemia. The clinically significant disorders resulting fromdouble heterozygosity for Hb S and a second Hb variant are considered forms of sicklecell disease(john,*et al*.2003)

1.2.3.1 Hemoglobin SC Disease

Hemoglobin SC disease is the second most common type of sickle cell disease. It occurs when you inherit the Hb C gene from one parent and the Hb S gene from the other. Individuals with Hb SC have similar symptoms to individuals with Hb SS. However, the anemia is less severe (Graham, 2017)

1.2.3.2 Hemoglobin S–ß-Thalassemia

Hemoglobin SB+ (beta) thalassemia affects beta globin gene production. The size of the red blood cell is reduced because less beta protein is made. If inherited with the Hb S gene, you will have hemoglobin S beta thalassemia. Symptoms are not as severe (Graham, 2017)

1.2.3.3 Hemoglobin S/Hereditary Persistence of Fetal Hemoglobin

The doubly heterozygous condition for Hb S andHereditary Persistence of Fetal Hemoglobin results ina heterogeneous disorder that is generally extremely mild and associated with apancellular distribution of Hb F, normal blood counts, microcytosis, target cells, and 20 to30% Hb F (Kinney and Ware, 1994)

1.2.3.4 Hemoglobin SE Disease

Hb E is characterized by the substitution of lysine for glutamic acid at position 26 of theß-chain and results in a mild ß-thalassemia phenotype. Because of the increase in theAsian population in the United States, the doubly heterozygous condition of Hb SE is nowoccasionally seen. Patients with Hb SE may have mild anemia and microcytosis along withapproximately 30% Hb E, but blood smears look relatively normal (except for target cells), and patients are usually asymptomatic(Kinney and Ware, 1994)

1.2 Previous studies

In India Sawaimal, *et al*(2017) studied the hematological parameter in sickle cell trait compared by healthy control and results showed that there was statistically significant decrease in Hb concentration, but not PCV and red cell indices. (Sawaimul*et al.,* 2017)

Munsoor and Alabid(2011) also studied the hematological parameter in Sudanese with sickle cell trait compared with healthy control, the results showed no statistically significant differences. (Munsoor and Alabid, 2011)

In India Chikhlikar and Wilkinson (2014) conducted hospital based case control study, sample size was 200 sickle cell trait individuals grouped in to 100 symptomatic i.e. patient suffering from severe anemia, joint pain, weakness, etc and 100 Asymptomatic i.e. patient free from any of above symptoms.100 age and sex matched AA were used as a control group. The results showed that, there was no statistically significant difference between healthy and asymptomatic individuals, while there was highly statistically significant different between healthy and symptomatic patients except in MCHC, also there was highly statistically significant differeance between symptomatic and asymptomatic individuals except in MCHC (Chikhlikar and Wilkinson, 2014).

Many previous studies were reported in percent of HbS in patient with sickle cell trait. Al-Awamy, (1999) reported the general level of Hb in sickle cell trait (20-50%).

In Jordan Bilto and Assaf(1988) reported the level of HbS among sickle cell trait was 39±3.8 %(Bilto and Assaf, 1998), and Reid and famodu (1988) reported mean value of HbS in sickle cell trait was 38±5%(Reid and famodu, 1998).

A study done in Iraq by Shakour and Suhail (2000) determine the proportion of HbS in 170 sickle cell trait individuals, they found that (44.1%) had HbS>38%, (31.8%) had HbS between 31%-38% and (24%) HbS<31%, there was positive correlation between HbS and hemoglobin concentration, packed cell volume, mean cell volume and mean cell hemoglobin (Shakour and Suhail, 2000).

1.3 Rationale

Sickle cell trait is not regarded as a disease because complicationsare mild; nevertheless some complications can developed like anemia, joint pain, weakness and abdominal pain(John,2000).

Variable concentrations of HbS have been reported in individuals with sickle cell trait, and they have been reported to affect red cells parameters.

In this study calculate the percent of Hb A, S, F and A_2 in Sudanese with sickle cell trait and to study its effect on red blood cells parameters.

1.4 Objectives

1.4.1 General objective

To study red cell parameters and its correlation with haemoglobin types in Sudanese with sickle cell trait.

1.4.2 Specific objectives

1- To estimate Hb A, S, F, and A2 in sickle cell trait using capillary electrophoresis.

2- To compare red blood cell parameters between sickle cell trait individuals and healthy controls.

3- To correlate between Hb A, S, F, and A2 and red blood cell parameters in individuals with sickle cell trait and age and gender.

Chapter Two

Materials and Methods

2.1 Materials

2.1.1 Study design

This was a hospital based case control study.

2.1.2 Study area and duration

This study was conducted at Aliaa hospital and laboratory diagnosis and consultation center, Khartoum state, in the period from January to May 2017.

2.1.3 Sample size

Fifty sickle cell trait individuals, fifty healthy controls were enrolled.

2.1.4 Inclusion criteria

Sudanese individuals categorized by electrophoresis as sickle cell trait.

2.1.5 Exclusion criteria

Individuals with known cause of anemia were excluded such as chronic blood loss, chronic infection, inflammatory disease and pregnancy.

2.1.6 Ethical considerations

Informed consent was obtained from each participant before sample collection

2.1.7 Sample collection

Blood samples (3ml) were collected from 50 individuals with sickle cell trait and 50 healthy volunteer as control group in EDTA container.

2.1.8 Laboratory methods

Frequency of Hb S, A, F and A_2 were measured by capillary electrophoresis and CBC was performed using hematology analyzer (sysmex KX-2IN).

2.1.9 Principle of capillary electrophoresis

Capillary electrophoresis is an emerging diagnostic tool in many laboratories separate Hb fraction and calculates the percentage of each fraction.

Capillary electrophoresis (CE) is the technique of performing electrophoresis in buffer-filled narrow capillaries, 25-100μm in diameter. The separation relies on differences in the speed of migration (migration velocity) of iron or solution, but the vitally important feature of CE is the bulk flow of liquid through the capillary, which is called Electric Osmotic Flow (EOF).

The inside surface of the capillary has ionisablesilanol groups, which readily dissociate giving a negative charge to the capillary wall. The negative charge attracts the positive charged iron from the buffer, creating an electrical double layer and there with a potential difference close to the capillary wall. When a voltage is applied across the capillary, cations in the diffuse layer are free to migrate towards the cathode, carrying the bulk solution with them. The result is an Electro Osmotic Flow and separation of the differently charged Hb fractions. These fraction are detected directly at an absorbance wavelength of 415nm,which is optimal to hemoglobin's, in the following order from cathode to anode:

Hb C, A2, E, S, D, F, A, Barts, J, and H.

2.1.10 Principle of sysmex KX-2IN

Hemoglobin concentration obtained by aspiration of small volume of (EDTA) by sample probe and mixed with isotonic diluents in nebulizer,and release of hemoglobin which measured in build colorimeter based in cyanomethemoglobin method (HICN). Through three sensing apertures for each cell type ,cell counted and size information generated in triplicate pulses acting to electronic conductively. Hence three values directly measured (RBCs,WBCs,Hb) and displayed on (LCD). The result printed out according to the setting mode (Bain, 2011).

2.1.11 Data collection and analysis

Data was collected using structured interview questionnaire, and then analyzed using statistical package for social sciences (SPSS), version 20. Qualitative data was represented as frequency and percentage. Quantitative data was presented as mean ±SD. Independent T- test was used to compare means of quantitative variable in two groups. Person correlation test was used to correlate between two quantitative variables. ANOVA was used to compare between quantitative variable and qualitative (more than two branches). Chi- square test was used to investigate the association between two qualitative variables.

Chapter Three

Results

A total of 50 individuals with sickle cell trait were enrolled in this study, 23 (46%) were males and 27 (54%) were females (Figure3.1), their age ranged between 1-55 years (mean±SD:16.6+13.8).

Fifty healthy volunteers were enrolled as a control group 23 (46%) were males and 27 (54%) were females; their age ranged between 1-40 years, mean±SD: 17.4+10.0.

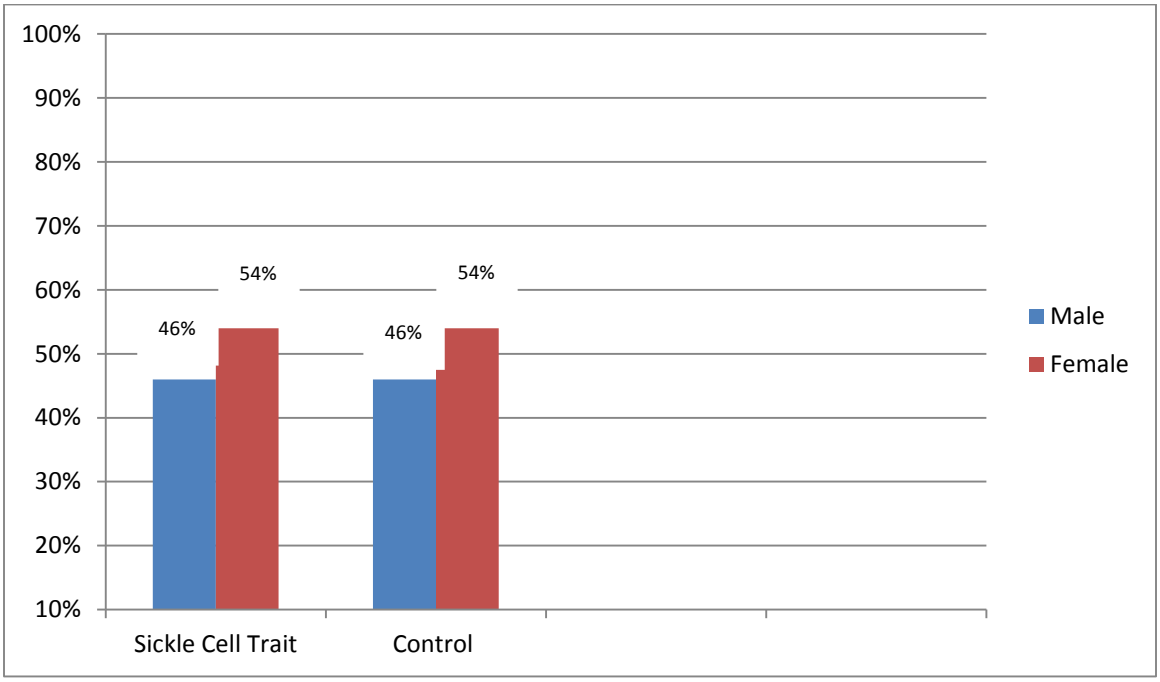

Figure 3.1 Distributions of Gender among study population

There was statistically significant decrease in mean Hb, PCV, MCV, MCH and MCHC in sickle cell trait individuals compared with healthy controls (Table3.1).

Table(3.1) Comparison of red blood cell parameters in sickle cell trait individuals and healthy controls.

Parameter	***Sickle cell trait***	***Normal control***	***P. value***
Hemoglobin (g\dl)	11.4	13.0	0.000
Packed cell volume (%)	35.5	39.8	0.000
Mean cell volume (fl)	78.9	86.1	0.000
Mean cell hemoglobin(pg)	25.3	27.9	0.001
Mean cell hemoglobin concentration(g\dl)	31.3	32.7	0.045

In sickle cell trait individuals HbS was ranged between 28.5-47.9% (Mean±SD:39.1+4.7)HbAwas ranged beween49.4-67.3% (Mean±SD:57.0+4.7), HbA_2 was ranged between 0.2-4.1% (Mean±SD:2.9+0.59) while HbF was ranged between 0.0-10.1% (Mean±SD:0.7+1.5)(Figure3.2).

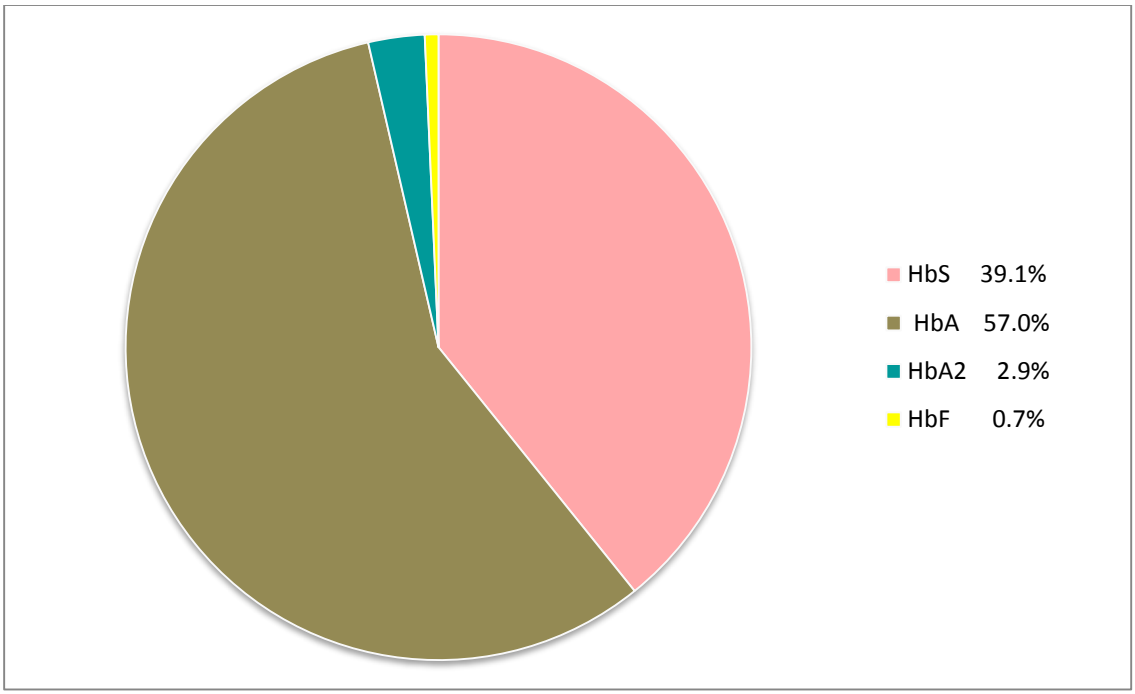

Figure 3.2 Quantitation of hemoglobin A, S, F, and A_2 in sickle cell trait by hemoglobin electrophoresis.

There was statistically significant positive correlation between HbS and Hb concentration and statistically negative correlation between HbA and Hb concentration. The correlation between each of HbF and HbA_2 with Hb concentration was not statistically significant (Table 3.2).

Table 3.2: Correlation between Hb A, S, A_2, F and hemoglobin concentration:

Parameter	***Person correlation(r)***	***P. value***
Hb\ HbA	-0.651	0.000
Hb\HbS	0.682	0.000
Hb\HbA_2	0.003	0.981
Hb\HbF	0.093	0.522

The participants was subdivided a corroding to HbS concentration into three groups group A (6%) with low HbS (<31%), group B (28%) with medium HbS (31-38) and group C (66%) with high HbS (>38%) (Table 3.3).

Table 3.3 Distribution of participant according to HbS concentration.

HbS percentage	**Number**	**Percent%**
Group *A* (HbS% < 31%)	3	6%
Group *B* (HbS%=31%-38%)	14	28%
Group *C* (HbS% > 38%)	33	66%

The red blood cell parameters were lower in group A than group B and group C (Table 3.4).

Table 3.4 Red blood cell parameters in sickle cell trait individuals according to HbS percentage.

Hematological parameter	**Group A**	**Group B**	**Group C**	***P. value***
Hemoglobin g\dl	6.2	9.7	12.5	0.000
Packed cell volume	25.0	34.6	36.9	0.009
Mean cell volume	69.7	75.9	81.0	0.025
Mean cell hemoglobin	18.0	21.5	27.5	0.000
Mean cell Hbconcentaration	25.1	28.3	33.2	0.000

Comparison of mean age in individuals with low, medium, and high HbS showed no statistical significant difference (Table3.5).

Table 3.5 Comparison of mean age in individuals with low, medium and high HbS.

Group	**Age**	***P.value***
Low HbS	8.3	0.865
Medium HbS	12.0	
High HbS	19.3	

There was no statistically significant association between gender and HbS percent (Table 3.6)

Table 3.6 Association between HbS and gender.

Variable	**Male**	**Female**	***p.value***
Low HbS	2	1	0.268
Medium HbS	4	10	
High HbS	17	16	

Chapter four

Discussion, Conclusion, and Recommendations

4.1 Discussion

Our present study showed that was statistically significant decrease in mean of Hb, PCV, MCV, MCH and MCHC in sickle cell trait individuals than healthy controls. This result was agrees to Sawaimal in Hb significant decrease and disagree in red cell indices and PCV statistically insignificant (Sawaimul*etal.*, 2017). Also this result was disagreed with Munsoor and Alabid in Elobied who showed no statistical significant differences between sickle cell trait and healthy controls.(Munsoor and Alabid,2011). The difference may be due study area and sample size.

Chikhlikar and Wilkinson works hospital based case control study, sample size was 200 sickle cell trait individuals grouped in to 100 symptomatic i.e. patient suffering from severe anemia, joint pain, weakness, etc and 100 Asymptomatic i.e. patient free from any of above symptoms.100 age and sex matched AA were used as a control group. The results showed that, there was no statistically significant difference between healthy and asymptomatic individuals, while there was highly statistically significant different between healthy and symptomatic patients except in MCHC, also there was highly statistically significant between symptomatic and asymptomatic individuals except in MCHC(Chikhlikar and Wilkinson, 2014).

The aim of this study was to calculate the percentage of hemoglobin A, S, F and A2 in sickle cell trait. The result show that HbS was ranged between 28.5-47.9% (mean±SD:39.1+4.7), HbA was ranged between 49.4-67.3% (mean±SD:57.0+4.7), HbA2 was ranged between 0.2-4.1% (mean±SD:2.9+0.59), HbF was ranged between 0.0-10.1% (mean±SD:0.7+1.5), this finding agrees with Alawamy who

conducted HbS in SCT ranges from 20-50%(Al-Awamy,1999). Also agrees with Reid and Famodu, Bilto and Assaf, who reported a mean value of HbS 38±5%, 39.8±3.8%, respectively. (Reid andFamodu, 1988),(Bilto andAssaf ,1998).

The result showed there was statistically significant positive correlation between HbS and hemoglobin concentration, this result agrees with Al-Shakour and Al-Suhail who reported same results(Shakour and Suhail,2000).

The participants were subdivided a corroding to HbS concentration in to three groups: group A (6%) with low HbS(<31%), group B (28%) with medium HbS (31-38%), group C (66%) with high HbS (>38%),(Barbara ,2006).

The hematological parameters for three groups were lower in group A than group B and group C. the differences were statistically significant as A < B < C in value of hemoglobin concentration, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration. This result was agrees with Al-Shakour and Al-Suhail,2000. and also agrees with Kennedy who found a significant differences in hemoglobin concentration and mean cell volume found in four groups divided by their HbS(<30%) being associated with the lowest Hb concentration and MCV.. (Kennedy,*etal*,1986).

Warpe*et al (*2016) related the presence of an association between HbS percent and anaemia parameters to co-existence of alpha-thalassemia (Warpe*et al.,* 2016).

4.2 Conclusion

- There was statistically significant decrease in red blood cells parameters in sickle cell trait individuals than healthy controls.
- There was statistically significant positive correlation between HbS and red blood cell parameters.
- There was no statistical significant correlation between HbS concentrations and age and gender.

4.3 Recommendations

- Individuals with sickle cell trait should be followed regularly by CBC, folate and iron profile those with anemia should be further investigated and treated.
- Further study and increase sample size should be conducted in the future to clarify the cause of the presence of positive correlation between HbS percent and red cell parameters.

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