

## **Introduction and Literature Review**

### **1.1.0. General introduction of sickle cell anemia:**

Anemia is a condition in which the number of red blood cells or their oxygen carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status (WHO, 2014). Reduction in oxygen carrying capacity is functionally best characterized by hemoglobin concentration below normal level, which is 13.5g/dl in adult males, less than 12 g/dl in adult non pregnant women, and less than 11g/dl in pregnant women and children (Table 1-1), although it can also be described as a reduction in red cell count or reduction in packed cell volume or hematocrit. Blood values always do not accurately reflect alterations in the red cell mass, for example hemoglobin may be falsely low in patients who have an expanded blood volume as in pregnancy or congestive heart failure. Thus one has to be careful in evaluating anemia in these patients. (*Asha shah, 2004*).

If the patient does have symptoms these are usually shortness of breath, particularly on exercise, weakness lethargy, palpitation and headaches. In older subjects' symptoms of cardiac failure, angina pectoris or intermittent claudicating or confusion may be present .The general signs include pallor of mucus membranes, tachycardia feature of congestive heart failure may be present and etc. and specific signs are associated with particular types of anemia e.g. koilonychias (spoon nails) with iron deficiency anemia, jaundice with hemolytic or megaloblastic anemia's, leg ulcers with sickle cell and other hemolytic anemia's and bone deformities with thalassaemia major. The most useful classification of anemia is that based on red cell indices and it divides the anemia into

microcytic, normocytic and macrocytic. (A.V.Hoffbrand & P.A.A.Moss, 2011).

	<i>Hb in g/dL</i>	<i>(%) PCV</i>	<i>RBCs ((10<sup>12</sup>/L</i>	<i>(MCV (fL</i>	<i>(MCH (pg</i>	<i>MCHC ((g/dL</i>
Age	Mean	Mean	Mean	Mean	mean	mean
Birth	4 ± 18	15 ± 60	1.0 ± 6.0	10 ± 110	3 ± 34	3 ± 33
Day 3	3 ± 18	11 ± 56	1.3 ± 5.3	13 ± 105	3 ± 34	4 ± 33
Month 1	2.5 ± 14	10 ± 43	1.2 ± 4.2	12 ± 104	3 ± 33	4 ± 33
2 Months	1.8 ± 11.2	10 ± 35	0.6 ± 3.7	8 ± 95	3 ± 30	3.5 ± 32
6–3 months	1.5 ± 12.6	5 ± 35	0.6 ± 4.7	8 ± 76	3 ± 27	3 ± 33
year 1	1.5 ± 12.6	4 ± 34	0.6 ± 4.5	6 ± 78	2 ± 27	2 ± 34
6–2 years	1.5 ± 12.5	3 ± 37	0.6 ± 4.6	6 ± 81	3 ± 27	3 ± 34
12–6 years	2 ± 13.5	5 ± 40	0.6 ± 4.6	9 ± 86	4 ± 29	3 ± 34
Men	2 ± 15	5 ± 45	0.5 ± 5.0	9 ± 92	2.5 ± 29.5	1.5 ± 33
Women	1.5 ± 13.5	5 ± 41	0.5 ± 4.3	9 ± 92	2.5 ± 29.5	1.5 ± 33

.(Table (1-1): Normal red blood cell values (Ronald Hoffman, 2008

Sickle-cell anaemia also known as sickle cell disorder or sickle cell disease is a common genetic haemoglobin disorder due to inheritance of mutant haemoglobin genes from both parents. Such haemoglobinopathies, mainly thalassaemia and sickle cell anaemia, are globally widespread, about 5% of the world's population carries genes responsible for haemoglobinopathies. (*World Health Organization*, 2006).

Sickle haemoglobin is a mutant haemoglobin in which valine has been substituted for the glutamic acid normally at the sixth amino acid of the  $\beta$ -globin chain. This haemoglobin becomes polymerized and becomes poorly soluble when the oxygen tension is lowered and red cells that contain this haemoglobin become distorted and rigid. Sickle cell disease occurs when an individual is homozygous for the sickle cell mutation or is a compound heterozygote for sickle haemoglobin and  $\beta$ -thalassaemia, haemoglobin C, and some less common  $\beta$ -globin mutations (Marshall, et al. 2005).

Normal hemoglobin is a tetrameric protein complex consisting of two pairs of globin chains with a combined molecular mass of 64,400Da. One type of globin chain is coded at the tip of the short arm of chromosome 16 where  $\alpha$  globin genes are located. The other type is coded on the short arm of chromosome 11 where the  $\beta$  globin genes are located, the globin chains are coded  $\alpha$  and  $\beta$  gene cluster and contain 141 and 146 amino acids respectively. (Robert J Arceci. et al, 2006). The normal hemoglobins are hemoglobin A ( $\alpha_2\beta_2$ ), hemoglobin A2 ( $\alpha_2\delta_2$ ), and hemoglobin F ( $\alpha_2\gamma_2$ ). In the adult, HbA represent more than 95% of the hemoglobin, HbA<sub>2</sub> represents less than 3 to 4%, and Hb F represents more than 1 to 2%. In embryo the Hb is Hb-Portland ( $\zeta_2\epsilon_2$ ), Hb-Gower1 ( $\zeta_2\epsilon_2$ ) and Hb-Gower2 ( $\alpha_2\epsilon_2$ ) (table 1-2). (William kern, 2002). The globin chain synthetic

pattern and the extent of DNA methylation within embryonic, fetal, and adult beta-like globin gene domains were evaluated in greater than or equal to 90% purified human erythroblasts from yolk sacs and fetal livers in the 6 to 12 week of gestational period as well as from adult marrows. The six week erythroblasts produce essentially embryonic epsilon chains, whereas the twelve week erythroblasts synthesize largely fetal gamma globin and the adult marrow erythroblasts synthesize almost exclusively adult beta chains (table 1-2) (figure 1-1). ( Mavilio, etal.,1983).

HEMOGLOBIN SPECIES	Globin chain	Period when normally present
A	$\alpha_2\beta_2$	Major hemoglobin in adult life.
A2	$\alpha_2\delta_2$	Minor hemoglobin in adult life, even more minor in fetal and neonatal life.
F	$\alpha_2\gamma_2$	Minor hemoglobin in adult life; major hemoglobin in fetal life with declining percentage through the neonatal period.
Gower 1	$\zeta_2\epsilon_2$	Significant hemoglobin during early intrauterine life.
Gower 2	$\alpha_2\epsilon_2$	Significant hemoglobin during early intrauterine life
Portland or Portland 1	$\zeta_2\gamma_2$	Significant hemoglobin during early intrauterine life.

Table (1-2): Haemoglobins normally present during adult, fetal and embryonic periods of life (Barbara J. 2006).

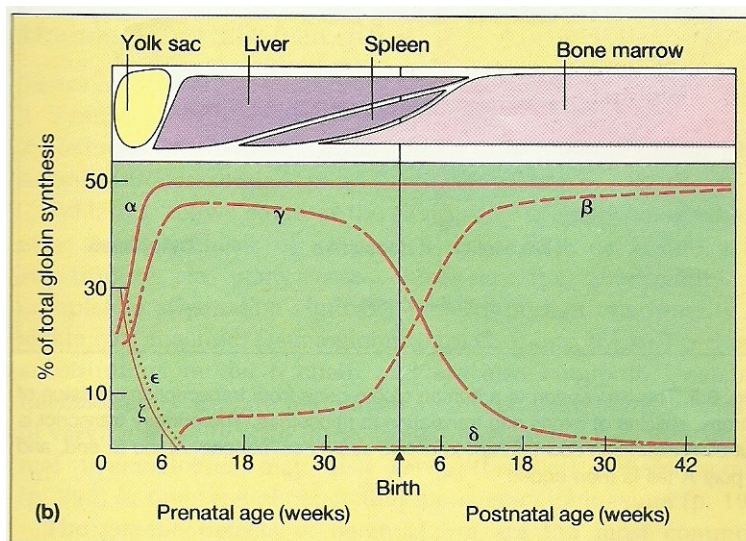


Figure (1-1): Synthesis of individual globin chains in prenatal and post life. (Hoff brand & Pettit, 1993)

Sixty- five percent of hemoglobin is synthesized in the erythroblast and thirty-five percent at reticulocyte stage. Heam synthesis occurs largely in the mitochondria by a series of biochemical reactions commencing with condensation glycine and succinyle coenzyme A under the action of the key rate- limiting enzyme  $\delta$ -aminolaevulinic acid (ALA) synthetase. Pyridoxal phosphate (vitamin B6) is a coenzyme for this reaction which is stimulated by erythropoietin and inhibited by heam. Ultimately, protoporphyrin combines with iron in the ferrous state to form heam, each molecule of which combines with a globin chain made on the polyribosome. A tetramer of four globin chains each with its own heam group in a pocket is then formed to make up haemoglobin molecule. The hemoglobin molecule contained within red blood cells is essential for human life, being the means by which oxygen is transported to the tissues. Other functions include the transport of carbon dioxide (CO<sub>2</sub>) and a buffering action (fig1-2). (Barbara J. 2006).

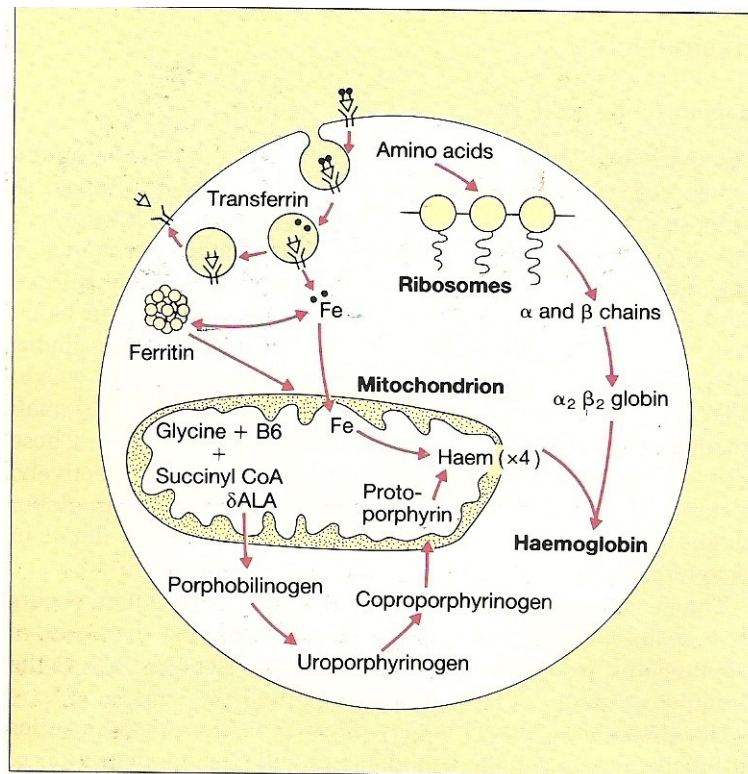


Figure (1-2): Hemoglobin synthesis in the developing red cell. (Hoff brand & Pettit, 1993)

The hemoglobin tetramer consists of two pairs of unlike globin polypeptide chains, each associated with a heam group. Normal hemoglobin has two  $\alpha$  and two  $\beta$  globin chains; the interaction of these chains is responsible for the quaternary structure of the hemoglobin molecule and normal oxygen transport (Fig 1-3). (Ronald Hoffman, 2008).

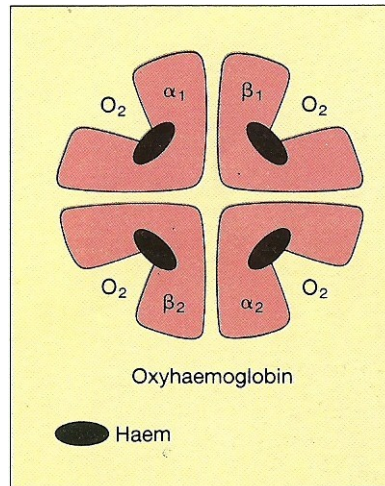


Figure (1-3):  $\alpha$ ,  $\beta$ , globin chains of normal adult hemoglobin attached to the haem molecules (Hoffbrand, et al., 2011).

The globin genes are arranged on chromosomes 11 and 16 in order in which they are expressed, in embryonic, fetal, and adult life different genes are activated or suppressed, the different globin chains are synthesized independently and then combine with each other to produce the different hemoglobins. The  $\gamma$  gene may have two sequences, differing by whether there is a glutamic acid or alanine residue at position 136 ( $G\gamma$  or  $A\gamma$ , respectively) (fig. 1-4). Certain embryonic hemoglobins are usually only expressed in early fetal life, but the main switch to adult Hb occurs 3-6 months after birth when synthesis of the gamma chain is largely replaced by the beta chain. How this switch comes about is largely unknown. (Hoffbrand, et al., 1993).



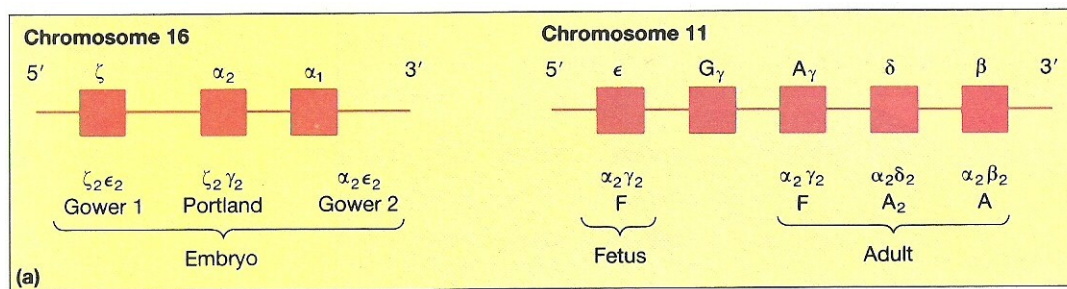


Figure (1-4): The globin gene clusters on chromosome 16 and 11. Hoffbran, et al. (2005).

Sickle cell Hb is designated S because erythrocytes with Hb S can transform into a sickle shape, hemoglobin's that tend to oxidize to metahemoglobin are designated Hb M other Hemoglobin's are named according to their migration on alkaline routine electrophoresis. As additional variants were named based on city, place, or hospital in which they discover e.g. Hb Kansas, sometimes in combination with letter Hb M Boston that discover in Boston. All sickling Hb have the same  $\beta_6$  mutation found in typical HbS, but other Hb variants have addition mutation. Some of these variants have the same mobility as common HbS on alkaline electrophoresis and are thus designated as HbS variant (e.g. S. Travis); however other sickling Hb can have altered mobility on electrophoresis and are given different alphabet destinations corresponding to their migration on alkaline electrophoresis (eg C-Harlen, C-Georgetown) .(William Kern, 2002).

Other structural abnormalities of the Hb molecule cause HbC, HbE disease, and other disorders. Each haemoglobinopathy occurs in homozygous and heterozygous forms. Clinical manifestations are usually only present in the homozygous state. Heterozygotes are referred to as having a trait of the haemoglobinopathy. (Reinhold Munker, et al., 2007)



### **1.1.1. History of sickle cell anemia:**

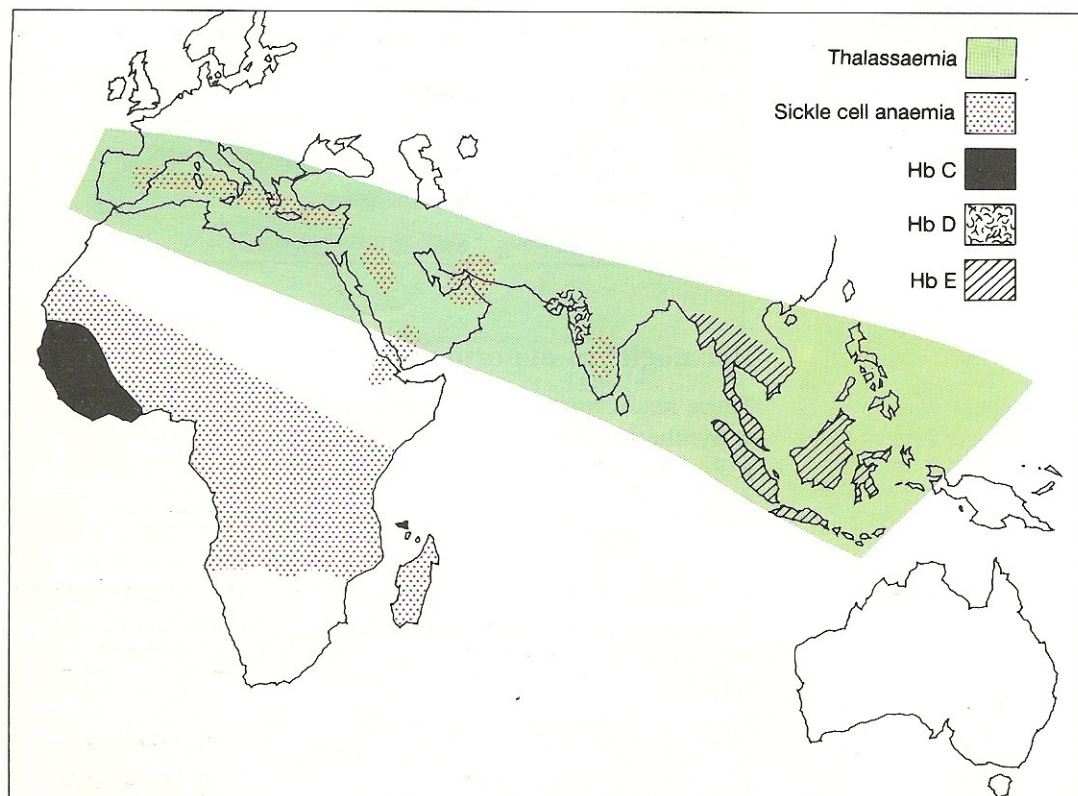
Sickle cell anemia was first observed by Herrick in 1910 (Herrick j.B, 1910) between that time and 1923 only three additional reports appeared those of Washburn (Washburn R., 1911), Cook and Myer (Cook, Myer J., 1915), and Mason (Mason V.R., 1922) each of whom reported one case. In 1949 Pauling and Itano described sickle cell anemia as the first molecular disease when they found that hemoglobin from a patient with sickle cell anemia (HbS) differed in electrophoretic mobility from normal hemoglobin (HbA) and that hemoglobin from a subject with sickle cell trait was a mixture of HbS and HbA (Linus Pauling, Itano S, 1949). Beside sickle cell anemia the finding of hemoglobin S with other  $\beta$  - globin chain variants of adult hemoglobin was first demonstrated by Itano and Neel in 1950 in the well known discovery of sickle cell / hemoglobin C disease. Since then Hemoglobin S has been observed with hemoglobin D, hemoglobin E, G, J, and hemoglobin K (Ibrahim, et al., 1967). Conley has chronicled the fascinating history of sickle cell disease. (Conley, 1980).

### **1.1.2 Geographical distribution of sickle cell anemia:**

Sickle cell anemia (SCA) is common in tropical Africa and the clinical manifestations of the disease were recognized by the native Africans Centuries (Luzzatol, 1981), Since then, several studies have documented the presence of the disease in other parts of the world particularly in the Arabian Peninsula, the Mediterranean, India and the United States (table 1-3) )fig 1-5) below. (Padoms, et al., 1991) (Leikin, 1989).

<i>Geographic region</i>	<i>%Heterozygote rate</i>
<i>Northern Africa</i>	<i>1-2</i>
<i>Western Africa</i>	<i>10-30</i>
<i>Central Africa</i>	<i>7-37</i>
<i>Southern Africa</i>	<i>0-5</i>
<i>Northern Greece Southern Italy</i>	<i>1-27</i>
<i>United State (African ancestry)</i>	<i>8</i>
<i>Caribbean (African ancestry)</i>	<i>1</i>
<i>Brazil (non white)</i>	<i>7</i>
<i>Saudi Arabia (South West)</i>	<i>5</i>
<i>Saudi Arabia ( Eastern province)</i>	<i>25</i>
<i>Central India (tribal population)</i>	<i>20-30</i>

.(Table (1-3) Areas of high prevalence of sickle mutation (Hoffbrand, etal., 2011



**Figure (1-5):** The geographical distribution of the sickle cell anemia and the more common, inherited structural haemoglobin abnormalities. (Barbara, 2006)

### 1.1.3. Classification and inheritance of Sickle cell anemia:

Sickle cell anemia is an inherited disorder due to substitution of thymine by adenine purine base in glutamic acid which in turn results in the substitution of valine to glutamic acid in position six of beta globin chain, this will cause de oxygenated sickle cell hemoglobin to form polymers that ultimately destroy red blood cells (Beutler, 2002).

The inheritance pattern of sickle cell anemia is autosomal recessive; these conditions are passed on to children by parents who carry mutated globin gene. A child who inherits two trait genes, one from each parent who is a carrier, will have the disease. A child of two carriers has a 25% chance of receiving two trait genes and developing the disease, a 50% chance of

being a sickle cell trait, and a 25% chance of being unaffected as shown below ( figure 1-6), in the situation where one parent is a carrier, there is a 50% chance of a child being a carrier and in a situation where one parent is a carrier and one parent is affected, then there is a 50% chance of the individual being a carrier and 50% chance of being affected. In addition, it is possible for co-inheritance of alpha and beta globin gene mutations to occur, in these situations, an individual may inherit a different abnormal mutant allele from each. (Zulema, etal., 2002).

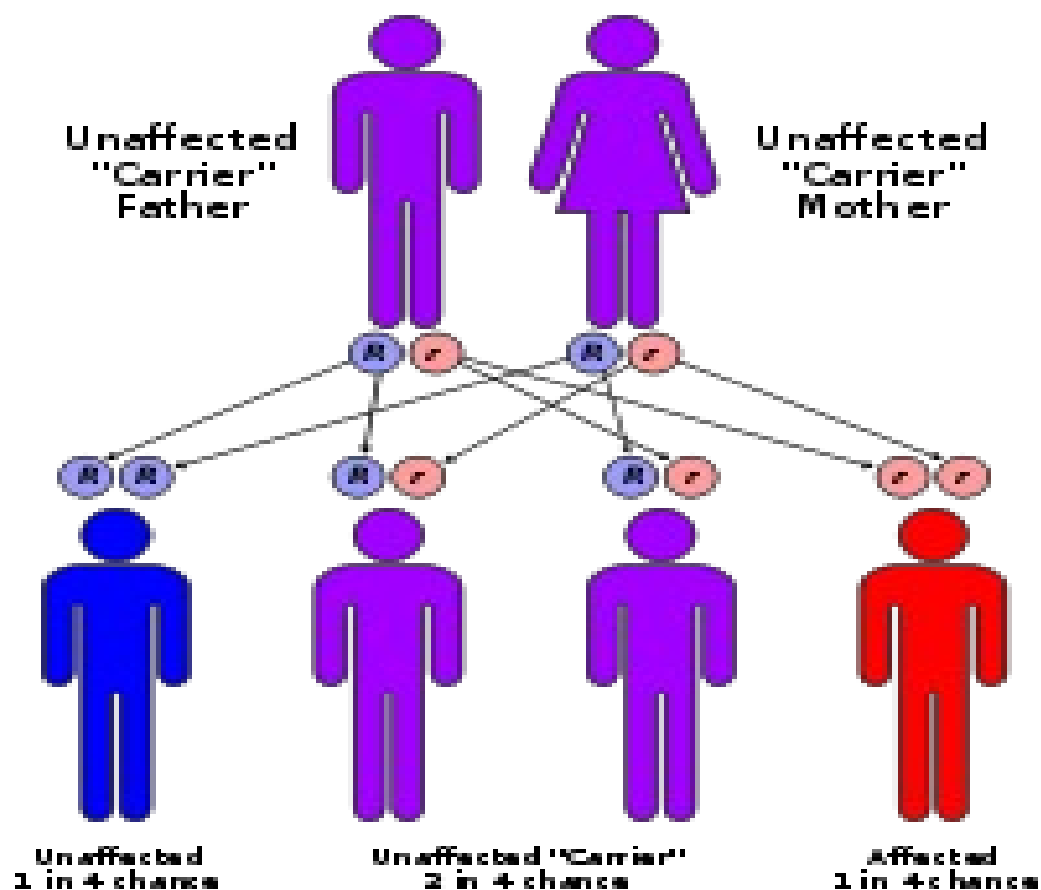


Figure (1-6) expected chances of children from carrier parent (Zulema, etal., 2002).

The common sickling disorders consist of the homozygous state for the sickle cell gene, that is sickle cell anemia (Hb SS), and the compound heterozygous state for the sickle cell gene and for either HbC (another  $\beta$  chain variant) or  $\beta$  thalassaemia (termed HbSC disease or sickle cell  $\beta$  thalassaemia respectively). Heterozygote's have one normal beta chain ( $\beta^A$ ) and one affected beta chain ( $\beta^S$ ) chain gene and produce about 60% of HbA and 40% of HbS, homozygote produce mainly HbS with small amounts of HbF. Compound heterozygote's for HbS and HbC produce almost equal amounts of each variant, whereas those who inherit the sickle cell gene from one parent and  $\beta$  thalassemia from the other make predominantly sickle hemoglobin (table 1-4). (Drew Proven, 2007).

Severe anemia/disease	Mild anemia/disease	Minimal anemia/no disease
Haemoglobin S/S	S/ $\beta^+$ -thalassemia	Haemoglobin S trait
Haemoglobin S/C	Haemoglobin C/C	Haemoglobin C trait
S/ $\beta^0$ -thalassemia	Haemoglobin S/Lepore	Haemoglobin D trait
Haemoglobin S/E	Haemoglobin S/D	
	Haemoglobin S/O <sup>-arab</sup>	

Table (1-4): The common haemoglobinopathies. ( Hillman Robert, etal.,2005)

#### 1.1.4. Pathophysiology of Sickle cell anemia:

The abnormality of HbS is substitution of valine for glutamic acid at position 6 of  $\beta$  globin chain. Deoxygenated Hb S tends to polymerize into long rigid structures which distort the cell into the characteristic sickle shape, anything that causes deoxygenating of Hb predisposes to sickling, including hypoxia, acidosis and increased temperature. The polymerization of Hb is reversible, and cell that have sickled may return to normal shape with reoxygenation, however the repeated cycle of

sickling and unsickling damage the cell and eventually the erythrocytes become irreversibly sickled. The rigid elongated sickle cells obstruct small blood vessels resulting in tissue infarction, Common site of infarction include the spleen, bone and bone marrow, medulla of the kidney, mesenteric vessels and pulmonary vessels .Sickled erythrocytes are also sticky and adhere to endothelial cells predisposing to thrombosis. (William, 2002).

### **1.1.5. Clinical feature of sickle cell anemia:**

Sickle cell disease (SCD) is a major health problem in many countries with wide spectrum of clinical severity. The SCD can cause numerous disorders that vary with respect to degree of anemia, frequency of crises and duration of survival (Luzzattol L, 1981), so the variability in clinical severity from the virtually symptomless sickle cell trait to the potentially lethal state characteristic of sickle cell anemia. Wide variation in the severity of clinical manifestations also occurs among patients with sickle cell anemia, some die within the first few years of life, while others have been discovered late in life as a result of a chance survey. (Ernest Beutler, 2001). Sickle cell anemia survival to adulthood in Africa was reported to be 10-15% in the first decade of life, with the death rate of about 5% during subsequent decades. Large portion that died have shown no overt chronic organ failure but died during acute episodes of pain, infections, acute chest syndrome, stroke and anaemic crises (Ambe, etal, 2012). Various types of crises occur, which include vasoocclusive (painful) crisis, aplastic crisis, sequestration crisis, and hemolytic crisis. (Ernest Beutler, 2001)

#### **1.1.5.1. Vasoocclusive (painful) Crisis:**

The vasoocclusive crisis is the most common and is the hallmark of the patient with sickle cell disease. (Serjeant, et al., 1994), it is result from complex interactions between endothelium, plasma factors, leukocytes, and rigid sickled red cells leading to the obstruction of blood vessels, tissue hypoxia occurs and ultimately leads to tissue death and localized pain. (Antal P, et al., 1998). Vasoocclusive crises may affect any tissue, but the pain occurs especially in bones, chest, and abdomen. Infarctions in the spleen are so common in sickle cell anemia that after age 6 to 8 year the spleen usually becomes very small because of scarring (autosplenectomy). (Powars, 1975). Stroke, is the most serious type of vasoocclusive complication. (Russell, et al., 1984).

#### **1.1.5.2. Aplastic Crisis:**

Aplastic crises in sickle cell disease show the reticulocyte count falls to low levels, indicating that red cell production has decreased dramatically, depression of erythropoiesis is generally associated with infections with the B19 strain of Parvovirus that, appear to be the most important cause of such crises (Rae, 1992), (Serjeant, 1993) and (Pagliuca, et al., 1993), and may be accompanied by extensive marrow necrosis. Because of the short red cell life span in sickle cell disease, even in the steady state, a temporary depression of marrow activity can cause a catastrophic fall in hemoglobin level, manifesting as an aplastic crisis (Godeau, et al., 1991). Marrow output failure may also result from a deficiency of folic acid, (Hoffbrand, 2011).

#### **1.1.5.3. Sequestration Crisis:**



The sequestration crisis occurs particularly in infants and young children (Kinney, et al., 1990), although it may occur in adults with Splenomegaly, particularly those with hemoglobin SC disease or sickle  $\beta$ -thalassemia. (Solanki, et al., 1986),(Bowcock, et al, 1988) It is characterized by sudden massive pooling of red cells, especially in the spleen. Hypovolemic shock and cardiovascular failure may develop rapidly (Kinney, et al., 1990).

#### ***1.1.5.4. Hyper hemolytic crisis:***

Significant change in blood picture characterized by a precipitous fall in the hemoglobin level associated with jaundice, marked reticulocytosis, polychromasia on the blood smear, increased unconjugated hyperbilirubinaemia, and increased urobilinogen content in urine above the steady state level for each patient. (Aljuwan, et al., 2004).

#### **1.1.5.5. Other clinical features:**

Chronic damage to liver may occur through micro-infarcts, and ulcers of lower legs are common, due to vascular stasis and local ischemia, the spleen also is enlarge in infancy and early childhood but later Splenomegaly usually resolves due to repeated infarctions of the spleen and it is unusual to be able to feel the spleen after the first decade of life (autosplenectomy) (Jones, et al., 2003). A proliferative retinopathy, hand-foot syndrome (dactylitis), and priapism are other clinical complication (Alexander, et al., 2004). The kidneys are vulnerable to infarctions of the medulla with papillary necrosis, the failure to concentrate urine aggravates the tendency to dehydration and crises and nocturnal enuresis is an uncommon severe complication (Ataga, 2000).

#### **1.1.6. Laboratory diagnosis of sickle cell anemia:**

The peripheral blood picture in sickle cell anemia depends on the type of sickle cell syndrome. The Hb level is normal in new born but anemia develops and sickle can be observed in the peripheral blood by 3-4 months of age as HbF declines. In HbSS disease the red cell is normocytic, normochromic with polychromasia, fewer target cells, the average reticulocyte count is 10% (4-20%) and normoblast maybe observed. The red cells are microcytic in the presence of coexisting  $\alpha$  thalassemia or iron deficiency. In HbS/ $\beta$  thala. Cigar-shaped, target cells and microcytic are prominent. The red cell morphology in the HbSC disease is characterized by predominant target cells and rare cigar shaped. The occasional Howell-Jolly body indicative of loss of splenic function. In SCD, may be observed the TWBCs are elevated as a result of an increase in mature neutrophils. The platelet count is also elevated as a result of decreased splenic function. Others laboratory features indicates mild activation of coagulation system, low ESR, the serum unconjugated bilirubin and Lactate Dehydrogenase are elevated, haptoglobin is decreased ( Hoffbrand, etal., 2004).

The diagnosis of sickle cell Hb is preferably by electrophoresis (Beutler, etal, 2002). The rapid methods that are less reliable for the detection of sickle cell Hb include the observation of sickling of RBCs, containing sickle cell Hb, microscopically under a cover slip by suspending the cells in a droplet of 2% solution of sodium metabisulfate (Daland, 1948), and the solubility test that utilizes a reducing agent such as sodium dithionite which is added to the hemolysate. Deoxy HbS is insoluble and render the solution turbid ( Mitchell, etal., 2006), both these tests are unable to distinguish sickle cell trait from

sickle cell anemia and cannot be used for primary diagnosis, they are useful aids in the identification of an abnormal electrophoretic band as HbS and for identifying sickle cell trait in units of red cells prior to transfusion. High performance liquid chromatography (HPLC) can be used instead of electrophoresis to identify and qualitative HbS and others hemoglobin ( Hoffbrand, Etal., 2005). Using of the gel diffusion technique for detecting of HbS in Sudanese patient, showed that although this technique is cheap, simple with high sensitivity and specificity, the positive predictive value compared with cellulose acetate electrophoresis in detecting of HbSS could not differentiate HbAS from HbSS in the presence of high level of HbF (Nimir, etal., 2009).

However such tests do not detect HbC or  $\beta$  thalassemia and do not reliably distinguish between SCT and SCD and therefore of limited value. The use of polymerase chain reaction (PCR) to detect sickle cell mutation is the method of choice for prenatal diagnosis (Maryam, etal., 2004). Chorionic villus biopsy has been used extensively to obtain fetal DNA for diagnosis in the first trimester ( Fitches, etal., 1986). Sickle cell anemia can be diagnosed at birth by subjecting cord blood samples to electrophoresis ( Baelen, etal, 1969).

Ideally, all babies of ethnic groups with a high frequency of the sickle cell gene should be screened at birth, because of a demonstrated decrease in mortality of very young children when the diagnosis is made. The cost-

effectiveness of screening depends on the composition of the target population. Screening is particularly desirable if the mother has sickle cell trait. (Fitches, 1987).

### 1.1.7. Sickle cell trait:

Sickle cell trait (SCT) is a benign condition with no anemia and normal appearance of red blood cells on blood film, it is remarkably common in some parts of the world, carriers have a mixture of sickle cell hemoglobin and normal hemoglobin, their erythrocyte do not sickle in vivo so they have no hematological abnormality (Allison, 1954) . Nevertheless, under unusual circumstances, serious morbidity or mortality can result from complications related to polymerization of deoxy-hemoglobin S. (Niton, 2010).

Sickle cell trait affects 8 – 10% of African - Americans and up to 25 – 30% of the population in West Africa (table1-5). ( Hoffbrand, etal., 2011).

<b><i>country</i></b>	<b><i>Carrier frequency %</i></b>
Nigeria	19-27
Mali	7-29
Niger	5-33
Cameroon	8-34
Ghana	3-22
Uganda	4-33
Kenya	2-32
United Republic of	10-38
Tanzania	1-29

Saudi Arabia	0-22
Iraq	20
India (Madras)	30
India (Gujarat)	25
India (Orissa)	

**Table (1-5):** Carrier frequencies for HbS (Drew proven, 2007)

Sickle trait reduces the risk of severe *falciparum* malaria, but not the prevalence of parasitaemia, in carriers the infection continues for the normal length of time but that the presence of sickle-cell hemoglobin limits the multiplication of trophozoites so that parasite counts remain low. Since the mortality from *falciparum* malaria is closely related to the height of the parasite count in peripheral blood, it is inferred that sickle-cell trait carriers will have a greater chance of surviving through this particularly dangerous stage of first exposure to malaria than will those without the trait, the limited available evidence suggests that the protective effect of the sickle-cell trait is less effective against *P. malariae* than against *P. falciparum* (Alliso, 1957), the analysis of people with sickle cell trait and people homozygous for hemoglobin A in the regions with endemic malaria in fact show a lower mean parasite burden in people with sickle cell trait relative to hemoglobin A homozygote's (Fleming, et al., 1979). In contrast, children with sickle cell disease have a high fatality rate, with acute malarial infections being a chief cause of death (Fleming, 1989). While it has been known for some time that sickle cell trait offers protection against malaria, the mechanism has never been clear (Williams, et al., 2005), for example one study suggest that Sickle trait red cells infected with the *P. falciparum* parasite deform, presumably because the parasite reduces the oxygen tension within the

erythrocytes to very low levels as it carries out its metabolism, deformation of sickle trait erythrocytes would mark these cells as abnormal and target them for destruction by phagocytes ( Luzzatto, et al., 1970), Other investigations suggest that oxygen radical formation in sickle trait erythrocytes retards growth and even kills the *P. falciparum* parasite (Anastasi, 1984),and etc. sickle cell trait present in individuals who possess one copy of the normal beta globin gene (HbA) and one copy of the sickle variant (HbS), but these individuals do not express symptoms of sickle cell disease. Hence, sickle cell traits present with varied problems including increased urinary tract infection in women, gross hematuria, complications of hyphema, splenic infarction with altitude hypoxia, idiopathic sudden death or exercise, in which sickling and vaso - occlusion under these extreme circumstances cause rhabdomyolysis, acute renal failure and cardiac arrhythmias. These individuals are advised to gradually increase exercise intensity, avoid dehydration, and to stop physical activity with the onset of muscle cramp or fatigue, a slight risk of sudden death during exercise has been reported predominantly in sickle cell trait and the treatment of sudden collapse consists of rapid intravenous hydration and oxygen supplementation.(Sears, 1978), (Eichner, 2007).

People with uncomplicated sickle cell trait have a normal blood examination as assessed by conventional clinical methods, including normal red cell morphology, indices,

reticulocyte counts, and red blood cell survival by chromium labeling. Conventional methods of detecting hemolysis are negative, such as measurements of serum haptoglobin, bilirubin, and Lactate dehydrogenase. Erythrocyte density distribution is normal, adherence to endothelium is not increased, altered membrane lipids and proteins are not detectable, cytoplasmic inside-out vesicles with high calcium content are absent, and permanently distorted erythrocytes are not observed (Niton, 2010).

#### **:Other sickling syndromes .1.1.8**

##### **:Hb SC disease .1.1.8.1**

Hemoglobin SC disease results from the inheritance of HbS gene from one parent and HbC gene from the other parent. Red cells contain approximately equal amounts of the two hemoglobins. HbA is absent, and Hb F is normal or slightly increased. (Greer, et al., 2003). The mean haemoglobin levels indicated mild anaemia although individual haemoglobin levels were often within the normal range, the clinical features were qualitatively similar to those of homozygous sickle cell disease although they were generally less frequent and of lesser severity (Serjeant, et al., 1973). Parameters of hemolysis and the complications of chronic hemolytic anemia (cholelithiasis, leg ulcers, hepatomegaly, and cardiomegaly) are milder in HbSC disease than in sickle cell anemia and also asplenia and its sequelae (increased platelet count and reduced serum IgM levels) are less frequent in Hb SC disease. Cerebrovascular accidents and the decreased leukocyte alkaline phosphatase scores are similar in both diseases.



Thromboembolic complications, retinopathy, and renal papillary necrosis are more frequent in Hb SC disease. (Samir, et al., 1982).

#### **1.1.8.2. Sick cell/ Beta thalassaemia:**

Sickle cell/  $\beta$  thalassaemia compound heterozygote's account for less than 10% of patients with sickle syndromes. The majority of these patients have the  $\beta^+$  phenotype, with the proportion of HbA ranging from 3 to 25%. The clinical phenotype is mild and disease severity correlates with the amount of HbA present. The clinical manifestations of the less frequent HbS/  $\beta^0$  genotype are similar in severity to those of HbSS. The red cells are microcytic and hypochromic and variable numbers of target cells and sickle cells are observed. Reticulocytosis (10 – 20%) is present and the level of HbA<sub>2</sub> is elevated. ( Hoffbrand, et al., 2011).

#### **:Sickle cell anemia with coexistent $\alpha$ thalassaemia .1.1.8.3**

Patient with homozygous sickle cell disease may be homozygous for alpha thalassaemia ( $\alpha^-/\alpha^-$ ), may be heterozygous for alpha thalassaemia ( $\alpha^-/\alpha\alpha$ ) or may have a normal  $\alpha$  globin gene ( $\alpha\alpha/\alpha\alpha$ ). The patient with homozygous alpha thalassaemia have significantly higher RBCs count and higher level of HbA<sub>2</sub> as well as significantly lower level of HbF, MCV, MCH, MCHC, reticulocyte count, serum bilirubin levels and irreversibly sickle cell counts, than those with normal alpha globin gene, the heterozygous have intermediate values. Alpha thalassaemia inhibit in vivo sickling in homozygous sickle cell disease and may be an important genetic determinant of its hematologic severity. (Douglas, et al., 1982).

#### **Sickle cell / hereditary persistence of fetal hemoglobin .1.1.8.4** **:((HPFH**

A sickle cell homozygote who had apparently inherited the HPFH determinant had 20.3% Hb F. (**Stamatoyannopoulos**, et al., 1975), high HbF is associated with generally milder but not asymptomatic disease. (**Idowu**, et al., 2011), It is associated with a reduced rate of acute painful episodes, fewer leg ulcers, less osteonecrosis, less frequent acute chest syndromes, and reduced disease severity. (Steinberg, et al., 2009).

#### **1.1.8.5. Sickle cell/ Hb Lepore disease:**

Compound heterozygous Hb S–Hb Lepore Boston resembles sickle cell anemia or sickle cell– $\beta^0$  thalassemia electrophoretically but clinically have less severe anemia, resembling that of sickle cell– $\beta^+$  thalassemia. The diagnosis is also suggested by the low to low-normal Hb A<sub>2</sub> levels, HbF levels vary. The peripheral smear shows microcytosis, hypochromia. Vasoocclusive complications occur, and splenomegaly is common. (Ronald Hoffman, et al., 2009).

#### **1.1.8.6. Sickle cell/ Hb D disease:**

Patients with hemoglobin S-D disease present with anemia, circulating sickle cells in the venous blood, and hemoglobin that migrates with hemoglobin S on paper electrophoresis at pH 8.6, they are frequently diagnosed as sickle cell anemia. (Cawein, et al., 1966).

#### **:Sickle cell/ Hb O Arab disease .1.1.8.7**

HbO Arab resembles HbC on alkaline electrophoresis and produces a moderately severe hemolytic anemia in association with HbS. The disease

is more severe than HbSC, and numerous sickled erythrocytes are  
.(observed on the peripheral smear. ( Hoffbrand, etal., 2011

#### **:Sickle cell/ Hb E disease .1.1.8.8**

Hb E is characterized by the substitution of lysine for glutamic acid at position 26 of the  $\beta$ -chain and results in a mild  $\beta$ -thalassemia phenotype. Because of the increase in the Asian population in the United States, the doubly heterozygous condition of Hb SE is now occasionally seen. Patients with Hb SE may have mild anaemia and microcytosis along with approximately 30% Hb E, but blood smears look relatively normal (except for target cells), and patients are usually asymptomatic. (Greer, etal., 2003).

#### **1.1.9. Epidemiology:**

Sickle cell disease affects approximately 50,000 Americans of all racial and ethnic backgrounds (Fletcher, 1992)). Among infants born in the US, sickle cells occur in 1 in every 375 Africans Americans, 1 in 3,000 Native Americans, 1 in 20,000 Hispanics, and 1 in 60,000 Whites (Agency for health care policy and research, 1993). One in every 150 African American couples in the US is at risk of giving birth to a child with sickle cell disease ( Wong, etal., 1992). Sickle cell disease has a prevalence of less than 0.35% among African American children in the United States. Double heterozygotes, including those with HbSC or S- $\beta$ -thalassemia, are even less common. The prevalence of sickling disease is lower among adults because the life span of SS homozygote's and double heterozygotes is decreased. Screening surveys have documented a prevalence of sickle trait of 7.4% among African American veterans and

8.7% in the African American community of San Francisco. Some studies have shown regional differences in prevalence. Prevalence does not decrease with age. Sickle cell trait is present with a low frequency in southern Italy and with a higher frequency in parts of Greece. It remains a rare finding in Americans of Mediterranean extraction (Allan, et al., 2009). In Europe, a high prevalence of the disease has been observed in France (Bardakdjian, et al., 2004).

In recent years, the (SCD) incidence has increased dramatically in Europe and North America because of the high rate of migration of people from endemic areas. From January 2009 to January 2010 the number of foreign residents in the province of Ferrara (Italy) increased by 12.2%: most of the immigrants were from countries at high risk of SCD (Ballardini, et al., 2012).

Sickle cell disease is prevalent in many parts of India, where the prevalence has ranged from 9.4-22.2% in endemic areas (Awasthy, et al., 2008). The sickle cell (HbS) gene occurs at a variable frequency in the Middle Eastern Arab countries, with characteristic distribution patterns and representing an overall picture of blood genetic disorders in the region (Mohsen, 2011), in Saudia Arabia the prevalence of SCD in varies significantly in different parts of the country, with the highest prevalence is in the Eastern province, followed by the southwestern provinces. The reported prevalence for sickle-cell trait ranges from 2% to 27%, and up to 2.6% will have SCD in some areas (Wasil, 2011). Sickle cell disease (SCD) is a more common and severe disease in Africa (Ambe JP. Et al, 2012) and extremely common in West Africa where malaria is also a big factor, contributing to health problem (Roth, et al., 1978). Nigeria the most populous black nation in Africa has the largest number of sickle cell anaemia (SCA) patients in the world. Borno and Yobe State has the

largest number of sickle cell trait in Nigeria with prevalence of 27.9% and 32.6% respectively (Ambe, etal., 2012). South Africa has a low incidence of sickle cell disease (SCD). However, its demographics are changing because of immigration from sub-Saharan African countries where SCD is prevalent (table 1-6) down. (Wonkam A. etal, 2012).

Ethnic groups		Homozygous HbSS %	Heterozygous HbAS %	Double Heterozygous SC/ %
USA	Black Americans	3 - 9	8 - 16	
USA	Whites Americans (Portuguese- Hispanics)	1 - 8	8 - 10	8 – 14
Europe	UK (Pakistanis- Blacks)	3 -7	6 - 15	10 – 12
Other European countries	Mediterranean	2 - 8	1 - 15	8 – 10
Caribbean		1 -3	3 - 8	
Middle East		1 - 2	7 - 8	1 – 3
Africa		1 - 10	15 - 30,5 (40,5)*	< 1
Cameroon		1 - 3	10 - 30	Rare

Table (1-6) Geographic distribution of Sickle Cell disease (frequency among total population): Summary from US Task force Preventive Service and American Academy of Family Physicians, \* West Africa and Nigeria, 1994 (Reprint N° 510)

In Sudan sickle cell anemia is one of the major types of anemia especially Western Sudan where the sickle cell gene is frequent the analysis of the haplotypes associated with the S gene indicated that the most abundant haplotypes are the Cameroon, Benin, Bantu and Senegal haplotypes (

Abdelrahim, etal., 2006). The first report of the presence of HbS gene in the Sudanese appeared in 1950 (Abbott, 1950). Later it was shown that the frequency of the gene varies significantly in different tribes (Foy,

etal., 1954). Omer et al. (1972) studied the incidence of abnormal hemoglobin among five indigenous [Nubians (Northern Sudan), Kalakla (Central), Dinka (Southern), Ingassena (South Eastern), and Beja (Eastern)] and two immigrant groups [Nigerians (Western bank of the Blue Nile) and Tchiendians (Central Sudan)] in Sudan. The highest sickle cell trait incidence was found in the immigrant tribes, the Nigerians (27%) and Tchiendians (20%). Ingassena, Beja and Kalakla tribes showed no evidence of abnormal hemoglobin, while the Dinka tribe showed. Another study showed the Frequency of haemoglobin AS and AC variants was greater in Hausa than Fulani (Fleming, et al., 1979). The presence of Hb S is already well documented among the Albagara, an Afro-Arab constellation of tribes with a predominantly African descent (Taha, et al., 1985). In a sub group of Albagara (Misseria) studies showed the prevalence of sickle cell disease (SCD) to be 30%, 16% among immigrants from the province of Blue Nile (Ahmed, Baker, 1986). The frequency of sickle cell trait has not been studied satisfactorily in Sudan especially Western and Eastern parts where the gene frequency of sickle cell disease is quite prevalent. The problem becomes augmented due to population unawareness, consanguineous marriage which employ widely in that area, lack of health counseling and undertaken serious research (Munsoor, et al., 2011). One previous study suggested that the sickle cell gene may have been preferentially introduced through males of migrating West African tribes to Sudan particularly Hosa, Folani, and Bargo (Bereir, et al., 2007). Another study about haemoglobinopathies in Sudan showed that HBS is the most common abnormal Hb in Western Sudanese ancestry (Abozer, et al., 2008). A recent study in Sudan about the prevalence of sickle cell anemia in the Northern area of Algardaf state showed a high frequency of sickle cell anemia HBSS was found among Masaleet and Maslam. Also this study showed that the prevalence of SCT

is higher than SCD in the study area (Nisreen, et al., 2010). Another recent study about the SCT among relatives of sickler's patients in Western Sudan showed the higher prevalence of HbAS followed by HBAA and a low percentage of HBSS. It also showed the highest distribution of SCT among Bedaria followed by Fulani and Selehab(Munsoor, et al., 2011).



## **1.2. Rationale:**

The frequency of sickle cell trait has not been studied satisfactorily in Sudan especially Western and Eastern parts where the gene frequency of sickle cell disease is quite prevalent.

As Gadaref have different ethnic groups, the previous studies showed the prevalence of sickle cell trait and Sickle cell disease in the Northern area, my goal here is to study the frequency of Sickle cell trait among relatives of sickle cell anemia patients in Al-Gadaref state-Sudan in order to reduce the spreading of the disease in, and hematological parameters of such study will provide valuable informative baseline data.

### **1.3. Objectives:**

#### **General objective**

To estimate frequency of sickle cell Trait among relatives of sickle cell anaemia patients in Al-Gadaref State-Sudan

#### **Specific objectives:**

- 1- To detect pattern of sickle cell on basis of hemoglobin electrophoresis.
- 2- To study the distribution of sickle cell anemia among ethnic and Geographic groups in Al-Gadaref state.
- 3- To compare the results obtained with other ethnic groups inside Sudan and outside.

## **2. Material and methods**

### **2.1. Type of study:**

This was a descriptive, cross-sectional, analytical study carried out during the period of April 2012 - April 2014 to detect sickle cell trait among relatives of sickle cell anemia patients in Al-Gadaref state -Sudan.

### **2.2. Study population:**

Sicklers' relatives in Al- Gadaref state were selected randomly regardless of their age or sex and were interviewed by special questionnaire which include (age, sex, father tribe, mother tribe, their area of origin, and history of sickness).

### **2.3. Sampling:**

In this study sampling method was a non probability sample calculated to achieve 114 specimens of blood. The sample size was determined according to statistical calculation.

#### **2.3.1. Inclusion criteria:**

All families having at least a confirmed patient diagnosed as having sickle cell anemia (HbSS).

#### **2.3.2. Exclusion criteria:**

All families who have no any member diagnosed as having any type of sickle cell anemia.

### **2.3.3. Collection of blood:**

Two and half ml of venous blood was collected in EDTA container from 114 enrolled Sicklers' relatives and 30 healthy individuals as control group.

Blood was collected with care and adequate safety to ensure reliability of the result.

### **2.3.4. Preparation of hemolysate:**

two volumes of washed packed cells were lysed in one volume of distilled water. Then one volume of carbon tetrachloride ( $\text{CCl}_4$ ) was added. the tubes were shook vigorously for 1 min. the tubes was centrifuged at 1200q for 5min. then the supernatant was transferred to a clean sample container and the Hb was adjusted to 9-11 g/dl with water, and stored at  $-20^\circ\text{C}$ ( Lewis, etal.2006)

## **2.4. Ethical consideration:**

Sample and data were obtained from the participators after taken their permission and agreement.

## **2.5. Data analysis:**

Data was analyzed to obtain the mean, standard deviation, frequencies using (SPSS) computer program

## **2.6. Laboratory analysis:**

### **2.6.1. Complete blood count:**

Small volume of well mixed EDTA blood samples was aspirated, measured to a predetermined volume, diluted to specified ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture there are the electrodes between which flows direct current resistance to change between the electrodes. As direct current resistance changes, the blood cells size was detected as electric pulses. Blood cell count was calculated by counting the pulses, and a histogram of blood cell size was plotted by determining the pulse sizes. Also analyzing a histogram made it possible to obtain various analysis data, Hb also was detected by non cyanide Hb analysis method (Kobe, 1999-2006).

### **2.6.2. Thin blood film:**

Small drop of blood was placed in a centre line of slide about 1cm from one end. Then, a spreader was placed in front of the drop at an angle of about 30° to the slide and was moved back to make contact with the drop. After that the drop was spread along the slide to obtain the ideal thickness, and dried film was put in staining rack, and was flooded with commercially Leishman's stain and was leaved for 1min to fix, twice buffered distilled water was added then it was mixed carefully and was leaved to stain for 10min after that the stain was washed up with tap water ( Lewis, etal., 2006). Dried film was checked under microscope with 100X/1.25 oil.

### **2.6.3. Erythrocyte Sedimentation Rate:**

#### **2.6.3.1. Westergren method:**

Diluted blood was sedimented in an open-end straight glass tube 30 cm in length and 2.55mm in diameter mounted vertically on stand. The test was performed on venous blood, diluted accurately in the proportion of one volume of (32g/l) trisodium citrate (anticoagulant diluents solution) to four volumes of blood that was mixed with ethylene diamine tetra acetic acid anticoagulant . the blood sample was mixed thoroughly and then was drew up into the Westergren tube to the 200mm mark, then the tube was placed vertical and undisturbed for 60 min free from vibrations and draughts and not exposed to direct sunlight, then the nearest 1mm the height of the clear plasma above the upper limit of the column of sedimenting cells was read. (Lewis, etal., 2006)

### **2.6.4. Sickling test:**

#### **2.6.4.1. Principles:**

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

#### **2.6.4.2. Reagents:**

A- Disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ). 0.114mol/l (16.2 g/l).

B- Sodium dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ). 0.114mol/l (19.85 g/l).prepared freshly just before use.

Working solution. Mix 3 vol. of A with 2 vol. of B to obtain a pH of 6.8 in resultant solution.

#### 2.6.4.3. Method:

Five drops was added from freshly prepared reagent to one drop of anticoagulated blood on a slide, a nail varnish was used to seal between slide and cover glass. The slide was put in Petri dish with wetted cotton. Then the result was observed after 30min, one hour, two hour and 24hour. A positive control of Hb AS was included. Sickling took place almost immediately in sickle cell anemia, but delayed in sickle cell trait.

### 2.6.5. Hb electrophoresis:

#### 2.6.5.1. Principles:

At alkaline pH, hemoglobin is negatively charged protein and when subjected to electrophoresis will be migrate towards the anode (+). Structural variants which have change in the charge on the surface of the molecule at alkaline pH will separate from Hb A. Hb variants which have an amino acid substitution which is internally sited may not separate and those which have an amino acid substitution which has no effect on overall charge will not separate by electrophoresis (S mitchell Lewis.etal, 2006).

#### 2.6.5.2. Equipment:

Horizontal electrophoresis tank that allow abridge gap of 7cm , direct current power supply capable of delivering 350V at 50 mA ,wicks of

filter or chromatography paper , blotting paper, applicators or fine micro capillaries, cellulose acetate membranes and staining equipment.

#### 2.7.5.3. Reagents:

Electrophoresis buffer. Tris/EDTA/borate (TEB) pH 8.5. Tris-(hydroxymethyl) aminomethane (Tris) 10.2g, EDTA (disodium salt) 0.6g, boric acid 3.2g, water to 1 litre. The buffer should be stored at 4°C and can be used up to 10 times without deterioration.

Wetting agent, such as Zip-prep solution (Helena laboratories). 1 drop Zip –prep in 100ml water.

Fixative/stain solution. Ponceau S, 5g, trichloro-acetic acid, 7.5g, water to 1 litre.

Destaining solution. 3% (v/v) acetic acid, 30ml, water to 1 litre.

#### 2.6.5.4. Method:

Electrophoresis tank was prepared by placing equal amount of Tris/EDTA/borate (TEB) buffer in each of the outer buffer compartments. two chamber wicks was wet in the buffer and place one along each divider/bridge support ensuring that they make good contact with the buffer. Then the cellulose acetate was soaked by lowering it slowly into a reservoir of buffer and left to soak for 5min before use. The sample well plates were filled with 50µl of each diluted sample or control, and then a second sample well plate was loaded with Zip-prep solution. the applicator tips was cleaned immediately prior to use by loaded it with Zip-prep solution and applied them to a blotter, and then the cellulose acetate strip was removed from the buffer and blotted twice between two layers of clean blotting paper without allowing them to dry. the applicator was loaded by depressed the tips into the sample wells twice and applied



the first loading onto some clean blotting paper and then it was reloaded and the samples was applied to the cellulose acetate and placed it across the bridges, with the plastic side upper most and then was electrophoreses at 350V for 25 min. after that the cellulose acetate was transferred to Ponceau S and was fixed and stained for 5min, then excess stain was removed by washing for 5min in the first acetic acid reservoir and for 10 min in each of the remaining two. Then it was blotted once using clean blotting paper and was leaved to dry after that the membranes was labeled and stored in a protective plastic envelope. ( Lewis. etal., 2006).

### 3. Results

The data showed that the gender enrolled in the study as in table (3-1). From the results of Hb electrophoresis its clear, that the highest frequency among the study population occur in HbAS (66.7%), followed by Hb AA (24.6%), HbSS (5.3%), and the lowest frequency among the study population is (1.8%) in HbAC, and HbSC, table (3-3).Table (3-4) also showed the Hb electrophoresis among the different tribes enrolled in the study.

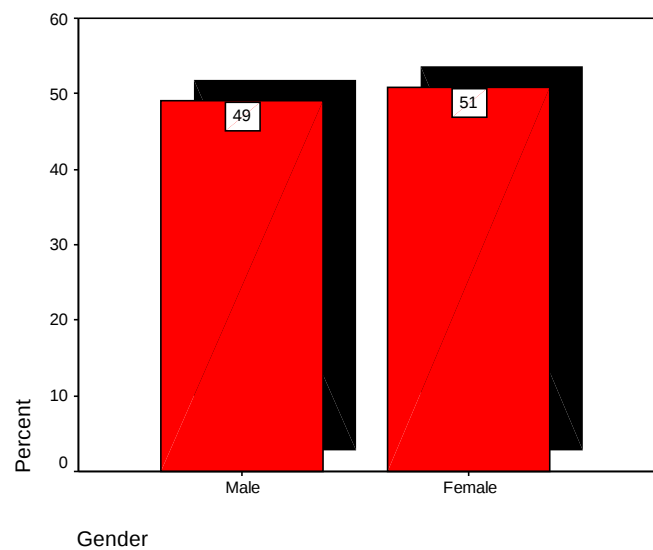


figure (3-1): The gender frequency among the study population.

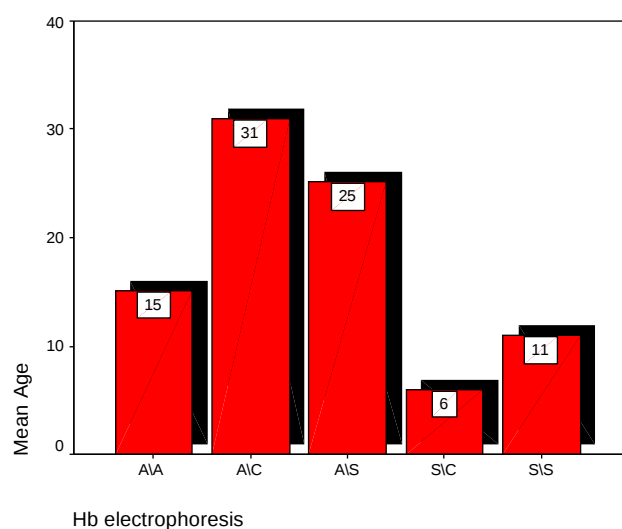


Figure (3-2): The mean age among study population. (The mean age/year).

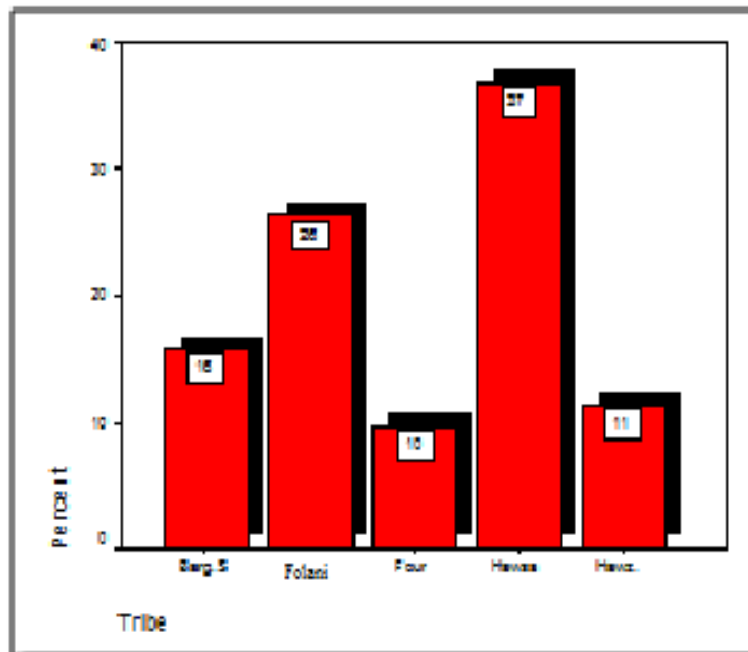


figure (3-3): The tribal frequency among the study population.

### 3.1. Laboratory Tests Results:

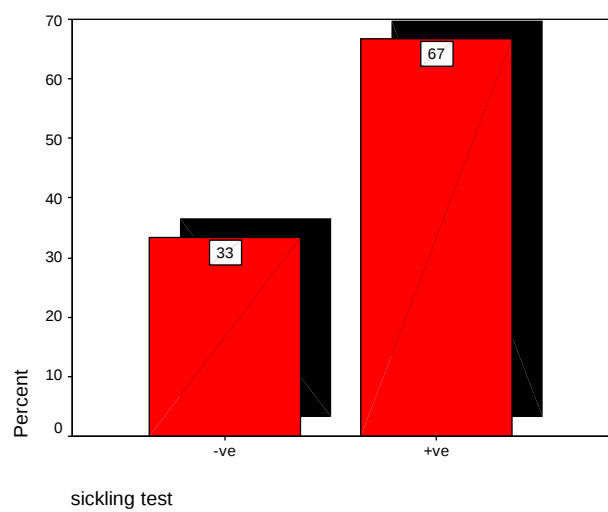


Figure (3-4): The sickling test among study population

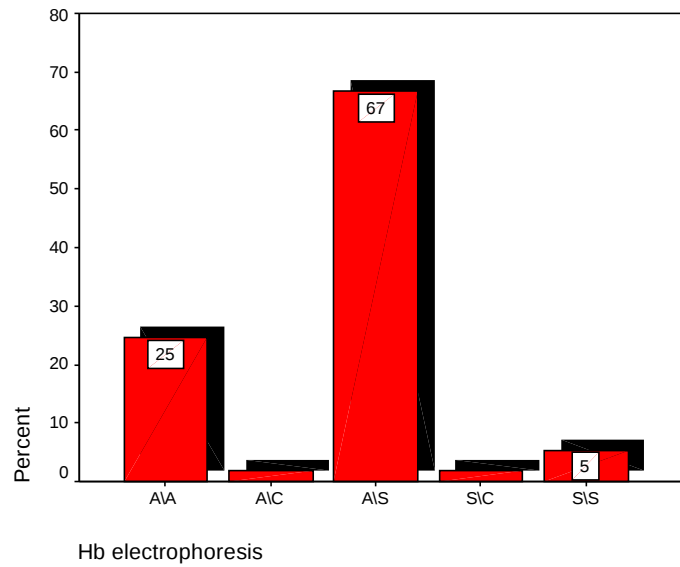


Figure (3-5): The result of hemoglobin electrophoresis among the study population.

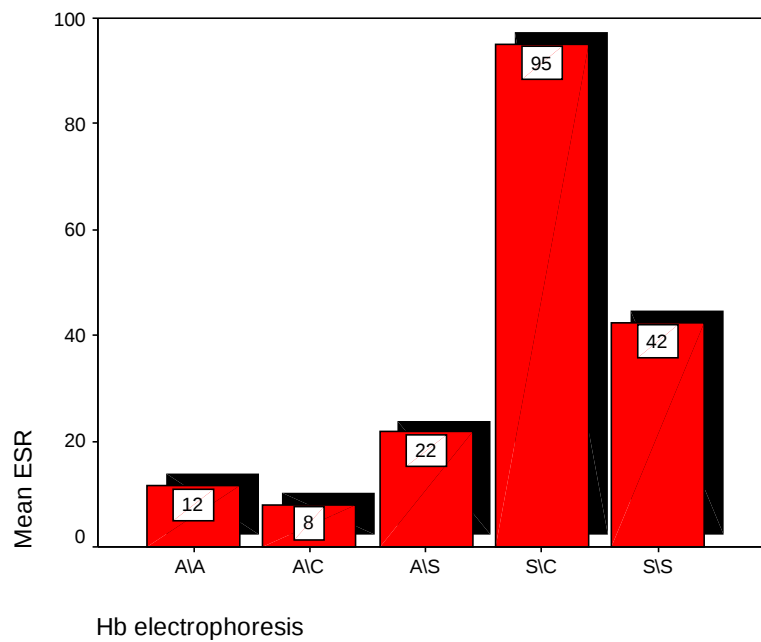


Figure (3-6): The mean of ESR in HbAA, HbAC, HbAS, HbSC, and HbSS groups.

The mean of ESR in mm/h.

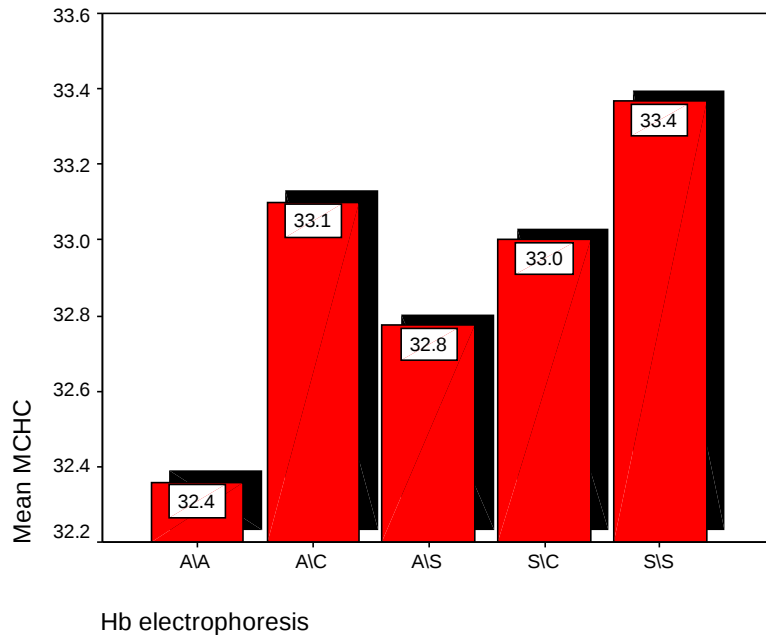


Figure (3-7): The mean MCHC in HbAA, HbAC, HbAS, HbSC, and HbSS groups.

The mean of MCHC in mg/dl.

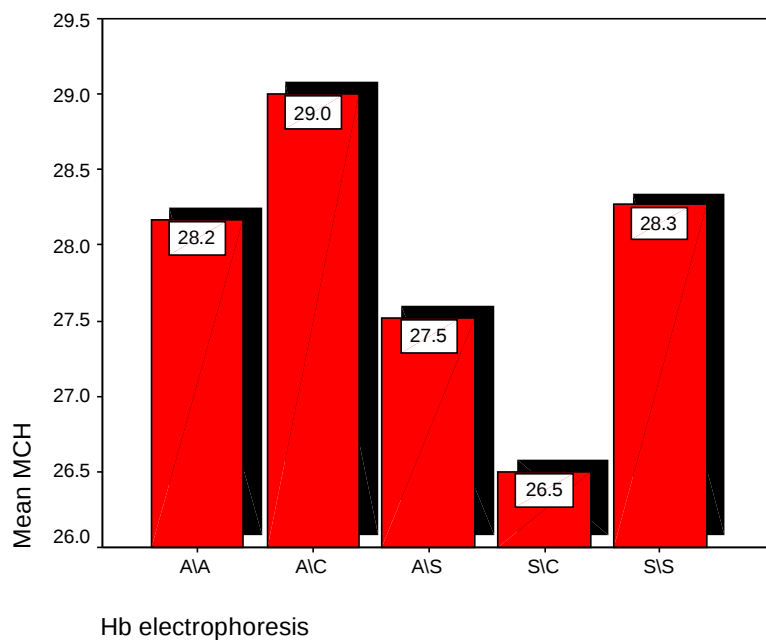


Figure (3-8): The mean MCH in HbAA, HbAC, HbAS, HbSC, and HbSS groups.

The mean of MCH in pg.

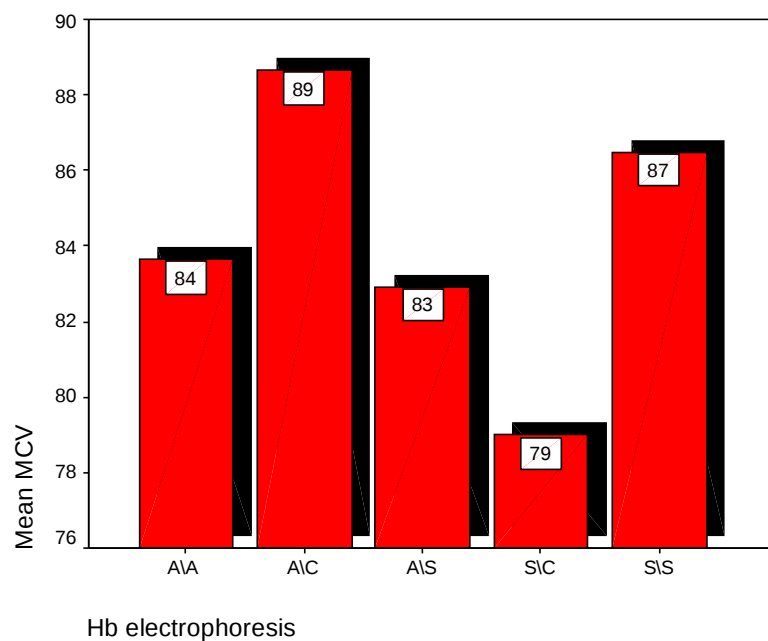


Figure (3-9): The MCV in HbAA, HbAC, HbAS, HbSC, and HbSS groups.

The mean MCV /fl.

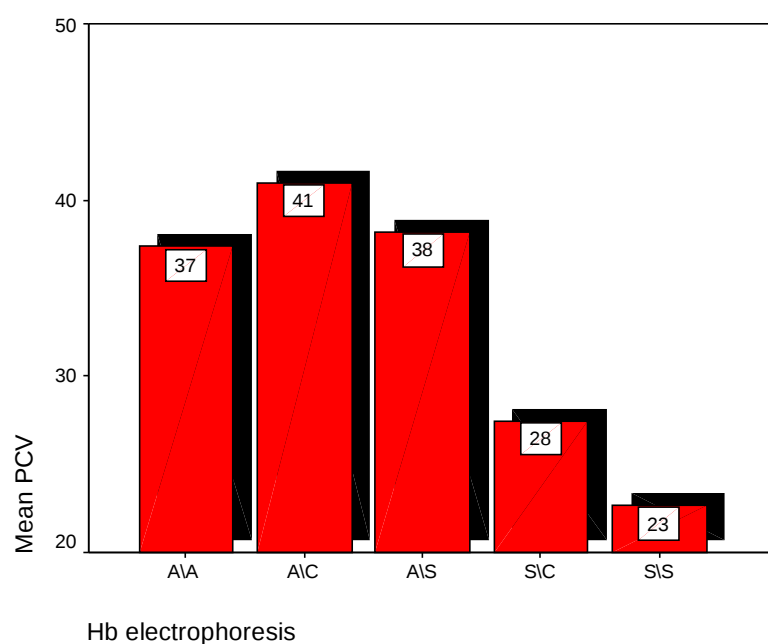


Figure (3-10): The mean PCV in HbAA, HbAC, HbAS, HbSC, and HbSS groups.

The mean of PCV in %.

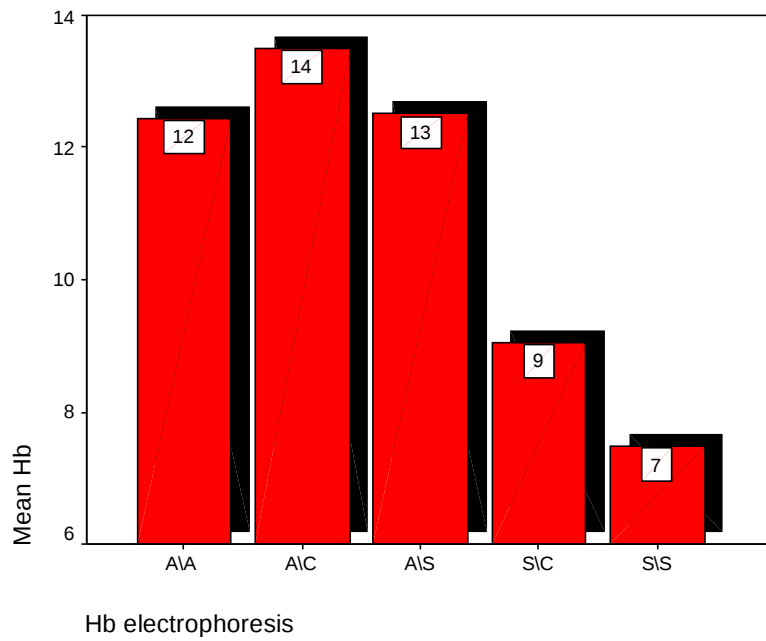


Figure (3-11): The mean Hb in HbAA, HbAC, HbAS, HbSC, and HbSS groups.

The mean Hb g/dl.

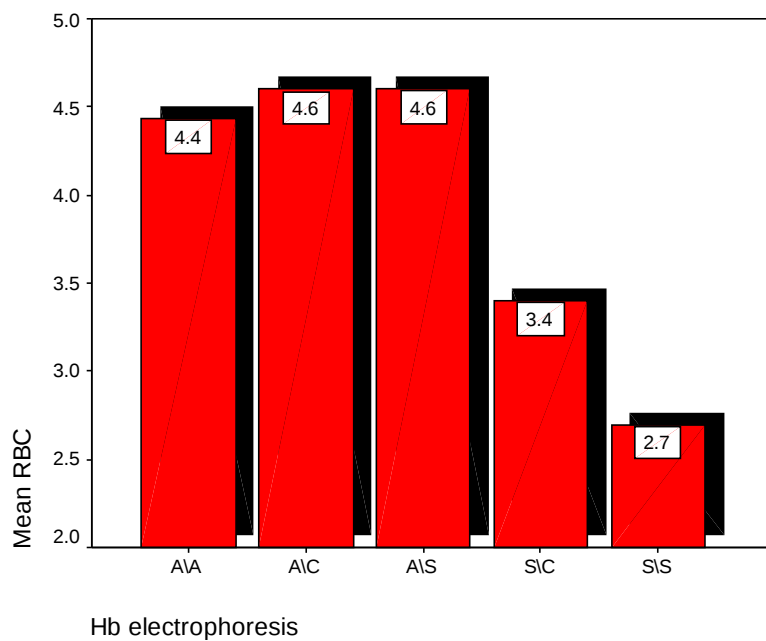


Figure (3-12): The mean RBC in HbAA, HbAC, HbAS, HbSC, and HbSS groups.

The mean RBC $\times 10^6/\mu\text{l}$ .

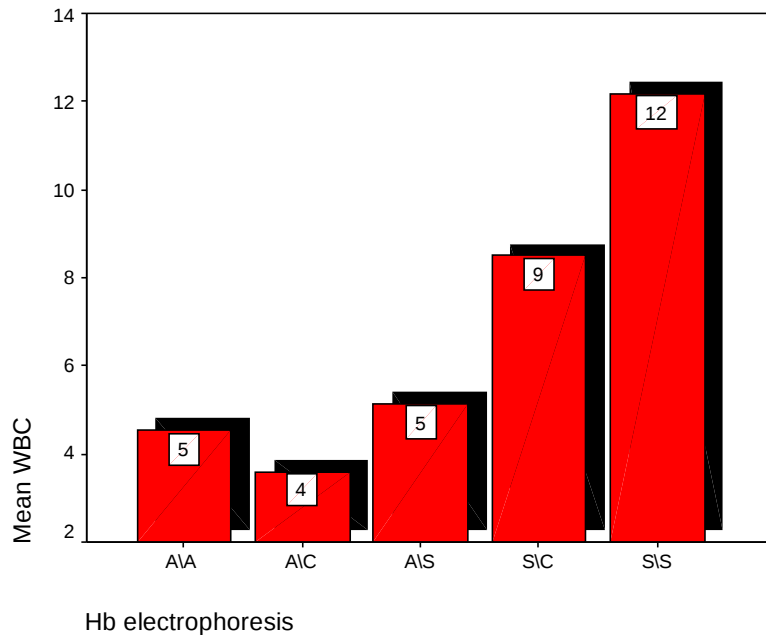


Figure (3-13): The mean of WBC in HbAA, HbAC, HbSC, and HbSS groups.

The mean  $WBC \times 10^3/\mu l$ .

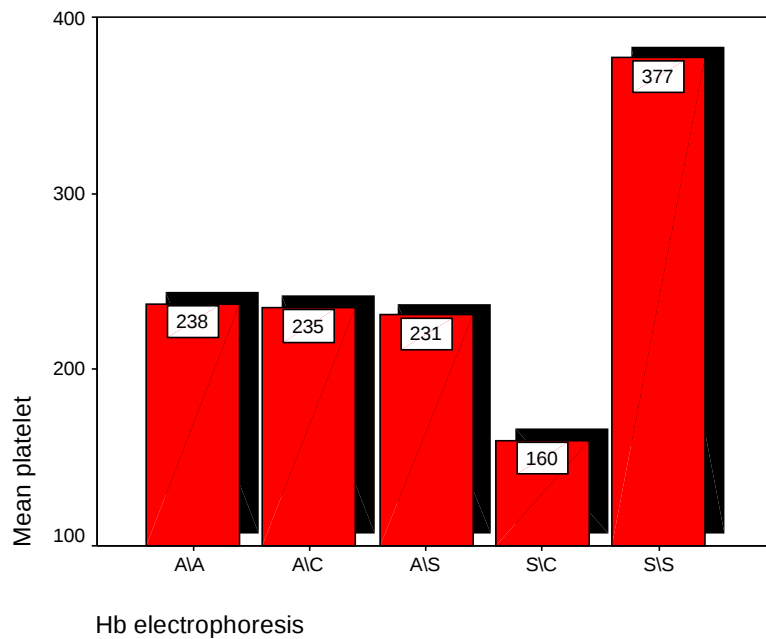


Figure (3-14):The mean of platelets in HbAA, HbAC, HbAS, HbSC, and HbSS groups. (The mean of platelet  $\times 10^3/\mu l$ ).



Group Statistics

	Hb electrophoresis	N	Mean	Std. Deviation	P. value
ESR	A\A	28	11.5000	6.58562	.483
	A\C	2	8.0000	9.89949	
platelet	A\A	28	237.50	42.568	.938
	A\C	2	235.00	63.640	
MCHC	A\A	28	32.3571	2.34006	.665
	A\C	2	33.1000	1.55563	
MCH	A\A	28	28.1679	2.17205	.598
	A\C	2	29.0000	.00000	
MCV	A\A	28	83.6429	7.18132	.342
	A\C	2	88.6500	3.32340	
PCV	A\A	28	37.36	4.399	.267
	A\C	2	41.00	4.243	
Hb	A\A	28	12.439	1.0884	.189
	A\C	2	13.500	.7071	
RBC	A\A	28	4.432	.4691	.625
	A\C	2	4.600	.2828	
WBC	A\A	28	4.536	1.7498	.465
	A\C	2	3.600	.8485	
Age	A\A	28	15.14	12.753	.095
	A\C	2	31.00	1.414	

Table (3-5): Complete blood count and ESR in HbAA, and HbAC groups. (The mean of ESR in mm/h, platelets in  $\times 10^3/\mu\text{l}$ , MCHC in mg/dl, MCH in pg, MCV in fl, PCV in %, Hb in g/dl, RBC in  $\times 10^6/\mu\text{l}$ , WBC in  $\times 10^3/\mu\text{l}$ ).

Group Statistics

	Hb electrophoresis	N	Mean	Std. Deviation	P. value
ESR	A\A	28	11.5000	6.58562	.000
	A\S	76	21.6974	13.53122	
platelet	A\A	28	237.50	42.568	.530
	A\S	76	230.71	50.828	
MCHC	A\A	28	32.3571	2.34006	.292
	A\S	76	32.7737	1.52686	
MCH	A\A	28	28.1679	2.17205	.229
	A\S	76	27.5197	2.50902	
MCV	A\A	28	83.6429	7.18132	.680
	A\S	76	82.9250	8.08594	
PCV	A\A	28	37.36	4.399	.513
	A\S	76	38.20	6.223	
Hb	A\A	28	12.439	1.0884	.860
	A\S	76	12.508	1.9402	
RBC	A\A	28	4.432	.4691	.173
	A\S	76	4.601	.5857	
WBC	A\A	28	4.536	1.7498	.193
	A\S	76	5.136	2.1772	
Age	A\A	28	15.14	12.753	.007
	A\S	76	25.20	17.679	

Table (3-6): Complete blood count and ESR in HbAA, and HbAS groups.( The mean of ESR in mm/h, platelets in  $\times 10^3/\mu\text{l}$ , MCHC in mg/dl, MCH in pg, MCV in fl, PCV in %, Hb in g/dl, RBC in  $\times 10^6/\mu\text{l}$ , WBC in  $\times 10^3/\mu\text{l}$ ).

Group Statistics

	Hb electrophoresis	N	Mean	Std. Deviation	P.value
ESR	A\A	28	11.5000	6.58562	.000
	S\C	2	95.0000	7.07107	
platelet	A\A	28	237.50	42.568	.017
	S\C	2	160.00	14.142	
MCHC	A\A	28	32.3571	2.34006	.705
	S\C	2	33.0000	.00000	
MCH	A\A	28	28.1679	2.17205	.295
	S\C	2	26.5000	.70711	
MCV	A\A	28	83.6429	7.18132	.376
	S\C	2	79.0000	1.41421	
PCV	A\A	28	37.36	4.399	.005
	S\C	2	27.50	3.536	
Hb	A\A	28	12.439	1.0884	.000
	S\C	2	9.050	1.3435	
RBC	A\A	28	4.432	.4691	.006
	S\C	2	3.400	.5657	
WBC	A\A	28	4.536	1.7498	.004
	S\C	2	8.500	.7071	
Age	A\A	28	15.14	12.753	.328
	S\C	2	6.00	4.243	

Table (3-7): Complete blood count and ESR in HbAA, and HbSC groups.( The mean of ESR in mm/h, platelets in  $\times 10^3/\mu\text{l}$ , MCHC in mg/dl, MCH in pg, MCV in fl, PCV in %, Hb in g/dl, RBC in  $\times 10^6/\mu\text{l}$ , WBC in  $\times 10^3/\mu\text{l}$ ).

Group Statistics

	Hb electrophoresis	N	Mean	Std. Deviation	P.value
ESR	A\A	28	11.5000	6.58562	.000
	S\S	6	42.3333	11.84342	
platelet	A\A	28	237.50	42.568	.000
	S\S	6	376.67	13.663	
MCHC	A\A	28	32.3571	2.34006	.308
	S\S	6	33.3667	.66533	
MCH	A\A	28	28.1679	2.17205	.924
	S\S	6	28.2667	2.83314	
MCV	A\A	28	83.6429	7.18132	.395
	S\S	6	86.5000	8.31264	
PCV	A\A	28	37.36	4.399	.000
	S\S	6	22.67	2.875	
Hb	A\A	28	12.439	1.0884	.000
	S\S	6	7.483	.9948	
RBC	A\A	28	4.432	.4691	.000
	S\S	6	2.700	.2280	
WBC	A\A	28	4.536	1.7498	.000
	S\S	6	12.167	.7528	
Age	A\A	28	15.14	12.753	.448
	S\S	6	11.00	6.356	

Table (3-8): Complete blood count and ESR in HbAA, and HbSS groups. (The mean of ESR in mm/h, platelets in  $\times 10^3/\mu\text{l}$ , MCHC in mg/dl, MCH in pg, MCV in fl, PCV in %, Hb in g/dl, RBC in  $\times 10^6/\mu\text{l}$ , WBC in  $\times 10^3/\mu\text{l}$ ).

## **Discussion:**

Sickle cell disease is a collective name for a group of conditions due to abnormal haemoglobins that share the sickling phenomena of red cells when exposed to low oxygen tension. (Serjeant, 1997). It is one of the commonest but preventable inherited diseases. (Joel, et al., 2011). Heterozygote's have one normal ( $\beta^A$ ) and one affected ( $\beta^S$ ) chain gene, homozygote produce mainly Hb S with small amounts of Hb F. Compound heterozygote's for Hb S and Hb C produce almost equal amounts of each variant (Drew, 2007). In Sudan sickle cell anaemia is one of the major types of anaemia especially western Sudan where the sickle cell gene is frequent (Abdelrahim, et al., 2006). The sickle cell gene may have been preferentially introduced through males of migrated West African tribes to Sudan particularly Hosa, Folani, and Bargo (Bereir. Et al., 2007).

This study was done to investigate and detect the frequency of sickle cell trait among the relatives of sickle cell anemia patient in Algedarif state in eastern Sudan. Seventeen families with 114 individuals were investigated for CBC, ESR, Sickling test and Hb electrophoresis.

The sickling test was positive in 67% among study population and negative in 33%. The Hb electrophoresis showed the highest frequency among study population occur in HbAS (66.7%), followed by Hb AA (24.6%), HbSS (5.3%), and the lowest frequency among study population is (1.8%) get by HbAC, and HbSC.

The frequency of sickle cell trait in our study was higher as compared with other results in Nigera and Saudia Arabia that reported by Ambe JP. et.al., 2012, in Borno and Yobe State that has the largest number of sickle cell trait in Nigeria with prevalence of 27.9% and 32.6% respectively

(Ambe, Etal., 2012), and that register by Wasil Jastaniah who reported the prevalence for sickle-cell trait ranges from 2% to 27%, in some areas of Saudi Arabia (Wasil, 2011). Our study was within range of the study had done in western area of Sudan that showed the frequency of sickle cell trait in the study area was 54 which means that 54% of the studied population were carrier of sickle cell gene (Munsoor, Afaf , 2011).

The high frequency of sickle cell disease is among Hawsa (36.8%), followed by Fulani (26.3%), Bargo Selehab (15.8%), Hawzma (11.8%), and lastly Four (9.6%). (79%) of study population their tribes origin came from west Africa and (21%) their origin from western area of the Sudan, no any case of sickle cell anemia was detected from eastern, northern or southern tribe of the Sudan. this result was match with study done by Bakhita Attalah and Abderahim O that show SCA was found to be predominant among the Afro-Asiatic-speaking groups (68.4%) including nomadic groups of Arab and non- Arab descent that migrated to the Sudan in various historical epochs. It is also similar to study done by Omer, etal 1972 about the abnormal haemoglobins in the indigenous and immigrant tribes of the Sudan that showed the highest sickle cell trait incidence was found in the immigrant tribes. The majority of the study population (98.24%) belonged to families of single ethnic descent, which reflect the high degree of within -group marriage, thus in a high risk of augmenting the sickle cell gene.

Erythrocyte sedimentation rate in the study population is significantly higher in HbSS and HbSC people .This may be due to any type of infection, or other cause because the erythrocyte sedimentation rate of asymptomatic patients with sickle cell anemia is abnormally low and in patients with sickle crisis and medical complications, the sedimentation rates were even higher (Lawrence, 1986). Also the higher ESR was found

in HbAS than HbAA this may be related to their older age or having any other cause that increases their ESR.

The mean of Hb level, TRBCs, and PCV in patient with sickle cell anemia and HbSC are lower than in sickle cell trait, and HbAC which are not significantly differ than normal person HbAA. These results were match with (Akinsegun, 2012) and (Hoffbrand, 1993), who showed a reduction of the above values in HbSC, HbSS, with no significant change between HbAA, HbAS, and HbAC. The result of MCV, MCHC, and MCH, showed no significant difference between all electrophoresis groups, these result were in agreeing with the study of Hoffbrand, et al., In 2005, that showed sickle cell anemia was belonged to normocytic normochromic anemia ,and carrier were asymptomatic which had normal red blood cell indices. Similar Study of Serjeant GR and Serjeant BE. In 1972, also showed that compound heterozygote ( HbSC), individuals their MCV and MCH were lower as compared to sickle cell anaemia individuals with a mean level around the lower limit of the normal range, whereas the MCHC is more often elevated.

The leukocytes were elevated in HbSS and HbSC probably due to infection in most of them and normal in HbAC, HbAS and HbAA, similar results were obtained from study Adedoyin D, in 2012 that showed the higher values of white blood cells and platelets in sickle cell anemia compared to haemoglobin phenotype AA controls. The study of Wong W-Y, Zhou Y, et al, in 1996 showed that The WBC, neutrophils count and monocytes count were elevated in sickle cell/haemoglobin C disease, but less so than in sickle cell anaemia. Similar results were published in the study of Malik H. I. M, et al, in 2013 for frequency of sickle cell disease in Heglig area in Sudan.

The platelet count was higher in HbSS that agree with Akinsegun A and Adedoyin D and lower in HbSC as a result of Splenic sequestration that associated not only with a fall in the haemoglobin concentration, but also with a fall in the platelet count (Zimmerman, 2000), no significant change between platelets values in HbAA, HbAS, and HbAC.

## **Conclusion:**

From the present study it concluded that sickle cell trait is highly frequent among the relatives of sickle cell anaemia patients in the studied area and could be capable of spreading the disease further due to high degree of consanguineous marriage, population unawareness, closure societies, and lack of medical counseling. Also provide an insight into the distribution of the sickle cell gene in that area that showed sickle cell aneamia was found to be predominant among afro – African immigrant groups. In addition to that all sickle cell trait people were had normal life with normal hematological parameters.

## **Recommendations:**

- 1- Raising awareness of sickle cell disease with local, national and international health agencies.
- 2- Initial research efforts should probably focus on newborn screening to study the true prevalence and impact of SCD in Sudan and also screening of newborn infants permits appropriate prophylaxis.
- 3- Government should establish laws to prevent any marriages without laboratories investigations, and marriage among same families should be minimize to control and reduce the spreading of the disease through carriers.
- 4- Screening for sickle trait should be offered to all Sudanese adult in reproductive age groups, and for high-risk pregnant women at their first prenatal visit to facilitate genetic counseling
- 5- Research collaborations between developing countries and well resourced countries to advance knowledge and improve access to optimal care worldwide.



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## **Appendix 1:**

Sudan University of Science and Technology  
College of tgraduate Studies and scientific reseach

### **Questionnaire**

The frequency of sickle cell trait among relatives of sickle cell patients in  
Al-Gadareif state

..... :No  
.....:Age  
.....:Gender  
.....:Father tribe  
.....:Mother tribe  
.....:Area of Origin  
..... :Residence

.....:Phone/Mobile number

.....Date of diagnosis

( ) Family history of disease: Yes ( ) No

( ) Treatment: Folic acid ( ) Hydroxyurea

( ) Transfusion therapy

.....:Relationship to sickler

.....:Associated diseases

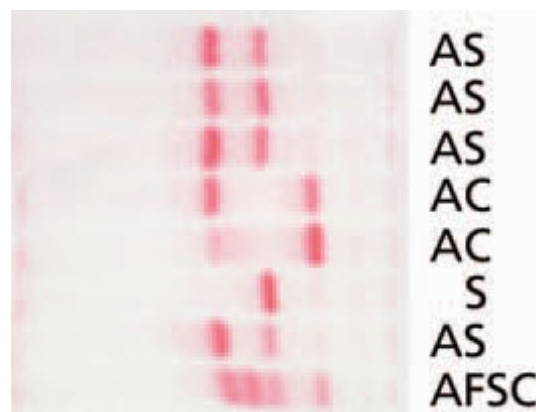
.....:Numbers of sickler in his-her family

.....:Clinical features if present

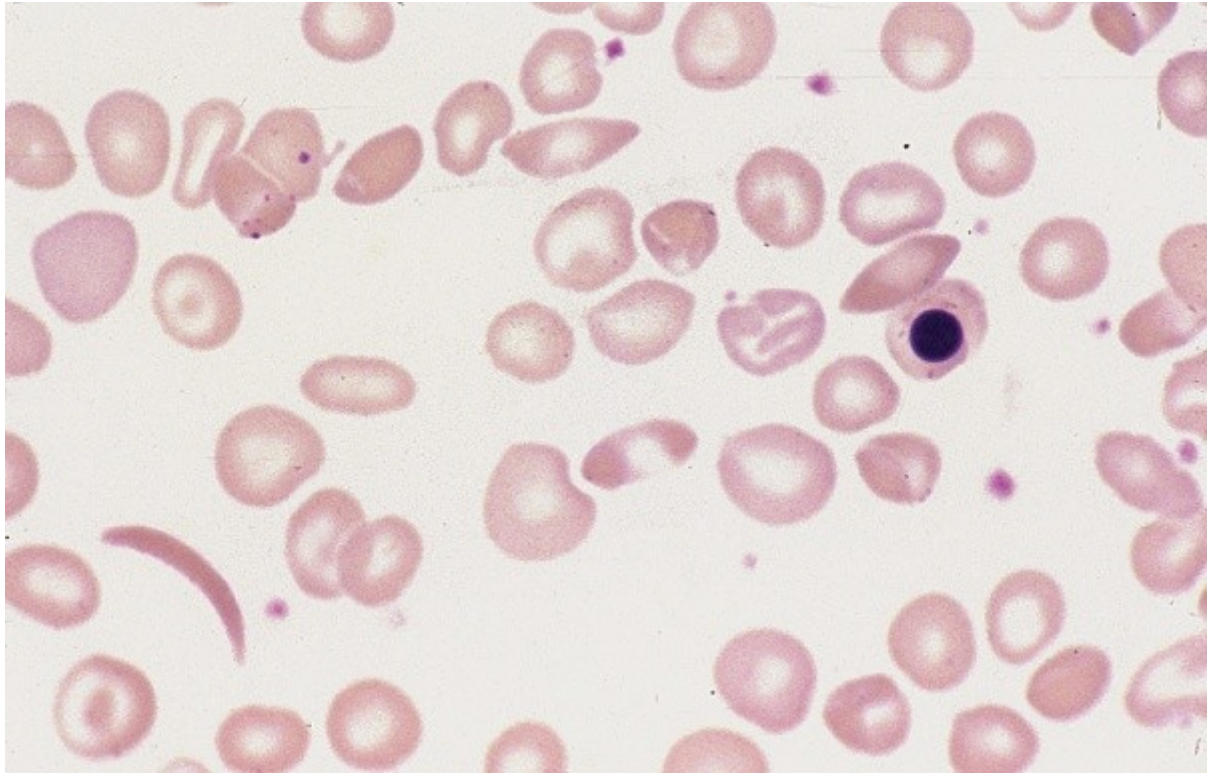
.....

.....Date.....Sig

## Appendix 2:



Haemoglobin electrophoresis on cellulose acetate at alkaline pH showing a patient with sickle cell anaemia (third lane from bottom) with almost all the haemoglobin being haemoglobin S; AFSC, control sample containing haemoglobins A, F, S and C; AS, sickle cell trait; AC, haemoglobin C trait.



Homozygote's sickle cell anemia patient with sickle cell and nucleated RBCs.

### **Appendix 3:**



Electrophoresis tank















.



































