CHAPTER 1

INTRODUCTION AND LITERATURE REVIW

1.1 Introduction

Haemoglobinopathies (HBP) are the commonest recessive diseases in human populations. The WHO estimated that about 5% of the world's populations is carrier of HBP (Mattevi *et al*, 2000) with more than 1% of Africans suffering from sickle cell disease (SCD) (Modell and Darlison, 2008).

Internationally, haemoglobin (Hb)S ($\alpha 2/\beta 2^{6Val}$) is the most common haemoglobin variants and is found most frequently in equatorial Africa and Middle East. (Makani,2007). Globally, approximately 4.5% of the world population carry abnormal Hb (Angastiniotis, 1995), from whom most were detected in parts of Africa; particularly HbS. Sckle cell anemia(SCA) is associated with high morbidity and mortality among sickle cell suffers in developing countries, many children are born with SCA and Approximately 5% of these children are believed to die before they reach five years of age.

HbS was suggested to be more common in Sudan particularly in people from western Sudan (Mohammed, 2006). In the Western regions HbS is very well documented among the Albagara, an Afro-Arab constellation of tribes with a predominant African descent (Bayoumi, 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 Blue Nile province (Ahmed, 1986) and 18% among Nilotes in the south of Sudan(Foy, 1954).

Furthermore, HbS is also known to be prevalent in the White Nile (Ahmed, 1986) and Khartoum states(Omer, 1972).

Electrophoresis, Cation Exchange-High Performance Liquid Chromatography (CE-HPLC), Iso-Electric Focusing (IEF) and DNA analysis are the main methods in detecting Sickle Cell Anaemia (SCA).

Obtaining family history and Full Blood Count are also an important factor in gaining a fuller picture of the case. Complete Blood Count (CBC) is the first investigation for patients with abnormal Hb. It may help in diagnosing patients with HbS, where sickle cells are detected in their blood morphology (Trent, 2006, El-Hazmi, 2001).

This study determines the frequency Of haemoglobinopathies (HBP) and discusses alterations in β–S haplotypes within those patients with HbS in correlation with HbF (Foetal Hb) level, among the population of the Northern Darfur area.

Medical history within the family is particularly important in communities where consanguineous marriage is preferred (Kutlar, 2007, Maroof, 2002). Such awareness within communities will help to prevent carrier parents having children with the disease. According to the available literature on haemoglobinopathies in Northern Darfur area, no population based studies have been carried out on knowledge gaps, attitudes and beliefs of the communities and health workers about haemoglobinopathies. Availability and accessibility to diagnostic services for haemoglobinopathies in the rural settings is lacking.

1.2 Literature Review

1.2.1 Erythropoiesis

Production of red cells -erythropoiesis- is a tightly regulated process. When one of the progeny of the multipotential hematopoietic stem becomes committed to the erythroid lineage, the early erythroid progenitor undergoes a series of divisions that eventually result in morphologically recognizable erythroblasts .The mature erythrocyte is biconcave discs having a mean diameter of about 7.8 micrometers and a thickness of 2.5 micrometers at the thickest point and 1 micrometer or less in the center. In normal men, the average number of red blood cells per cubic millimeter is 5,200,000 (±300,000); in normal women, it is 4,700,000 (±300,000), its life spans is120-days. The main disorders of red cells are: Anaemia, haemoglobinopathies and Polycythaemia (Lichtman *et al*, 2007).

1.2.2 The red cell structure and function:

Mature erythrocytes are unique among the cells of human tissues in that they normally lack nuclei and cytoplasmic structures such as lysosomes, endoplasmic reticulum. They exist in large blood vessels as biconcave discs but their shape changes to parachute-like conformation in the capillaries, which have a diameter less than that of erythrocyte in the biconcave disc form after the red cell re-enter large vessels, loss of flexibility or elasticity leads to membrane damage and shape change which is accompanied by diminished life span of the red cell in many different forms of anaemia , (De Gruchys, 1958). The main function of red cells is to carry oxygen to the tissues and to return

carbon dioxide from the tissues to the lungs. In order to achieve this gaseous exchange, they contain specialized protein, haemoglobin. Each red cell contains approximately 640 million haemoglobin molecules (Hofbrand, 1993).

The membrane is normally a highly deformable, but non-expansible or structure. It is fragile but it is integrity is firmly maintained by the attachment of it is inner surface to lattice –like structure of specialized cytoskeleton proteins which support the membrane and dictates the shape of the erythrocyte. The major protein component of the membrane is designated band 3 protein .Its understood to encase the channels through which the facilitated transport of anions and glucose takes place (De Gruchys, 1958).

Another important trans membrane protein is glycophorin, The suction protruding into the exterior is so heavily substituted with sugars that they comprise nearly two-thirds of the mass of molecule and contain most of sialic acid which contributes to the negative charge of the outer surface of the red cell at physiological pH.Glycolipids in the outer leaflet of the membrane contain specific oligosaccride sequence which constitute to ABO blood group substances. Phospholipids and to a lesser extent, cholestrol are the dominant lipid components of the matrix of membrane (De Gruchys, 1958).

The most important constituent of the cytoskeleton is the protein spectrin .Intertwined spectrin molecules are linked together by a specific protein and actin to form a lattice-like network which is attached to internal surface of the membrane.

This network is a resilient structure which normally causes red cell to resume the biconcave disc form after forces causing distortion have been removed. Specific sites on spectrin serve as points of attachment to molecules that protrude from the membrane (band 4.1 protein), and to the protein ankyrin which links spectrin to the internal pole of band 3 protein fig 1.1 (De Gruchys, 1958).

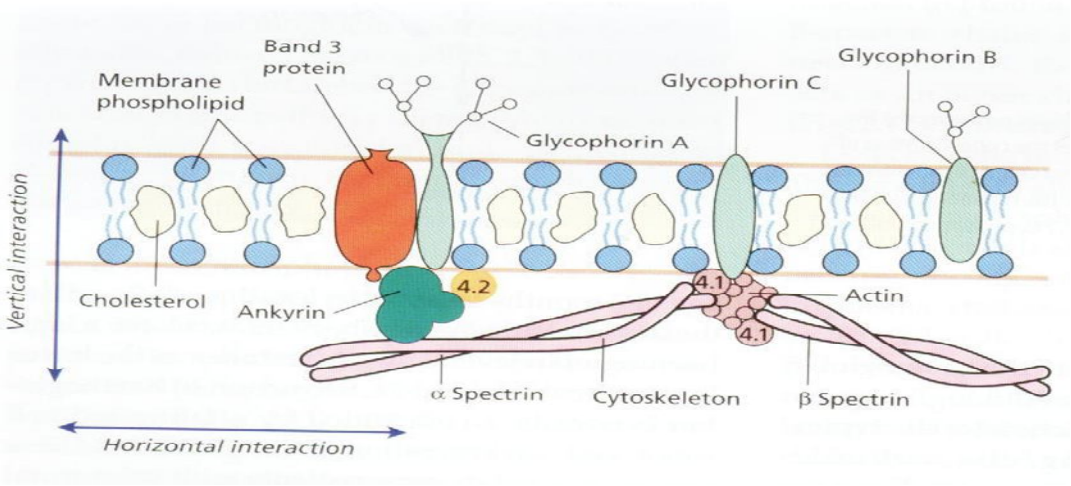

Fig1.1: Red Blood Cell Membrane (De Gruchys, 1958).

1.2.3 Haemoglobin:

Haemoglobin (Hb) is the substance in the blood which gives it its red colour. It carries oxygen around the body and, therefore, it is vital for maintaining life,

Worldwide the usual and most common adult haemoglobin type is known by the letter A. Where a person has inherited haemoglobin A, from his parents they will have the usual

haemoglobin combination AA (Hb AA). In this instance, the red blood cells are round, able to carry oxygen well, and they flow easily around the body (Hofbrand, 1993).

Each molecule of normal adult haemoglobin Hb A (The dominant haemoglobin in blood after the age of (3-6 months) consist of four polypeptide chains two α and two β globin polypeptide chains (α2β2) which have 141 and 146 amino acids respectively. HbA comprises about 97% of the Hb in adults. Two other types, Hb A2 (α2δ2) and Hb F (α2γ2), are found in adults in small amounts (1.5-3.2% and < 1%, respectively).

The molecular weight (MW) of Hb A is 68000 D. Each with it is own haem group contains a ferrous ion (Fe2+) to which oxygen reversibly binds. When oxygen is bound, the beta chains 'swing' closer together; they move apart as oxygen is lost. In the 'open' deoxygenated state, 2,3 diphosphoglycerate (DPG), a product of red cell metabolism, binds to the haemoglobin molecule and lowers its oxygen affinity (Hofbrand, 1993).

The - chains is directed by two α genes, α1 and α2, on chromosome 16, and the beta and gamma chains by single genes on chromosome11. The delta chain is directed by two genes also on chromosome 11 (Dacie and Lewis, l995).

1.2.3.1 Haemoglobin Synthesis:

The synthesis of haemoglobins in mitochondria with formation of delta-aminolevulinic acid from glycine and succinyl-coenzyme A in the presence of pyridoxal phosphate (vitamin B6)and delta aminolaevulinic acid synthetase the process then continues in the cytoplasm of cell .Where two molecules of delta-

aminolaevulinic acid condense in presence of delta-aminolaevulinic acid dehydrase to form prophobilinogin. Four molecules of prophobilinogin combine to form urophyrinogen 111 in the presence of uroporphyrinogen1 synthase and uroporphyrinogen 111cosynthnthetase.Coproporphyrinogen 111 is then formed by the action of uroporphrinogen decarboxylase, which removes four carboxyl groups from the acetic acid side chains (Hofbrand, 1993).

Heme is then produced in the mitochondria where protooporphrinogen 1X is formed by the action of coproporophyrinogen oxidase .Protoporphyrin 1X is formed in the presence of protoporphyrinogen oxidase and ferrous iron is incorporated into the molecule in the presence of the enzyme ferrochelatas (heme synthesis) to form the heme molecule, figure 1.2 (Hofbrand, 1993).

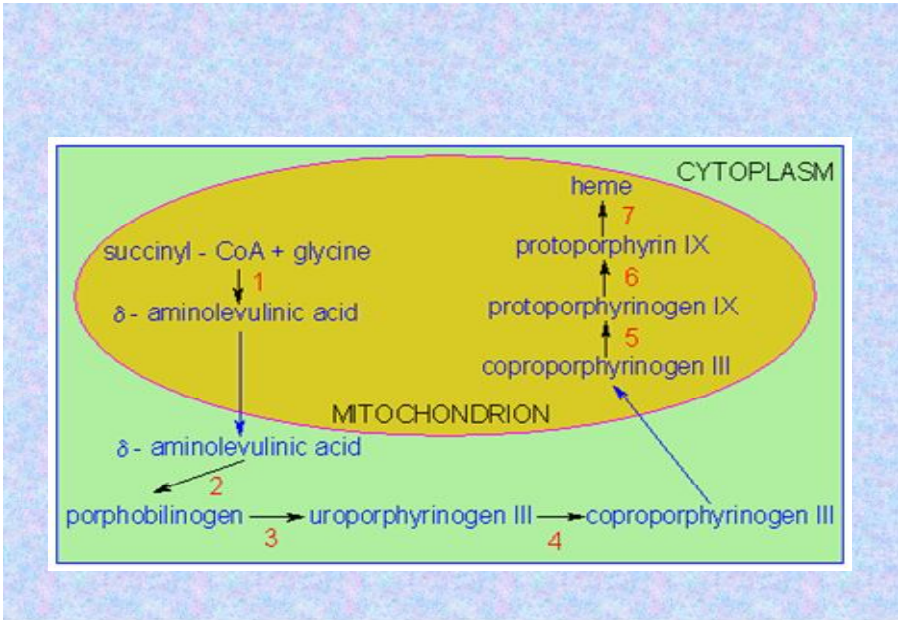

Figure 1.2: Haem syntheses (Hofbrand, 1993).

The atom of the iron is located in the centre of the structure and in the ferrous state binds oxygen. Iron is delivered to the maturing red cell by a specific transport protein siderophilin. Trasferrin carries two atoms of iron in the ferric state the transferrin attaches to receptor on red cell membrane .This causes the membrane to invaginate, forming an intercellular vacuole the iron is then released into the cytoplasm (Barbara *et al,* 2012).

1.2.3.2 Primary structure of globin chains:

The non – α (β, γ, δ or ε) chains are all 146 amino acid in length; the β chains begin with valine and histidine. The C-terminal residues are try β145 and his β146.The δ chains (of haemoglobin A2) differ from the β chain (of haemoglobin A) in only 10 residues. The first eight residues and the C- terminal residues (127 to 146) are the same in δ and β chains, tetramers of β chain (haemoglobin H) may be found in α thalasseamia. The γ chain of fetal haemoglobin (haemoglobin F) differs from β chain by 39 residues. The N-terminal residues of the γ Chain and β chain are glycine and valine respectively, while the C-terminal residues, tyr 145 and his 146, are the same as in γ and in β chains (Hofbrand, 1993).

Appreciable quantities of free γ chains, found in the red cell of some infants with α thalassemia: free γ chains, like β chains, can form homotetramers known as haemoglobin Bart's (Gribben *et al,* 2009).

In addition to the different N-terminal residues, several other differences in primary structure between the γ and β chains are noteworthy the γ chain contains isoleuocine, while the β chains do

not. The increased alkaliuted resistance of haemoglobin F and haemoglobin Bart's (γ4) has been attributed to the different amino acids at residues at 112 and 130 (βCys and try by thr and trp, respectively). The γ genes are duplicated: one codes for glycine (y G) and the other for alnine (γA) resdues 176, giving rise to two Kinds of y chains. In addition, a common polymorphism, the substitution of threonine for isoleucine, is frequently found at residue 75 of the γA chain (Gribben *et al*, 2009).

1.2.3.3 Secondary structure of globin chains:-

About 75 percent of the amino acids α or β chain in helical arrangement, all studied haemoglobins have similar helical content. Eight helical areas, lettered A to H, occur in the β chains. Haemoglobin nomenclature specifies that amino acids within helices are designated by the amino acid number and the helix letter, while Amino acids between helices bear the number of the amino acid And the letters of the two helices. Thus residue EF3 is the third residue of the segment connecting the E and F helices, while residue F8 is the eighth residue of the segment connecting the E and F helices, while residue F8 is the eight residue of the F helix (Makani *et al*, 2007).

1.2.4 Abnormal haemoglobin:

Some people inherit unusual haemoglobins: for example, sickle haemoglobin (S), haemoglobin (C), or any other unusual haemoglobin. There are approximately 400 unusual haemoglobins known in humans (Hofbrand, 1993).

They named haemoglobinopathies is a kind of genetic defect that results in abnormalities of the globin chains of the hemoglobin

molecule, result in life-threatening illnesses, is responsible for significant morbidity and mortality all over the world . Due to spontaneous mutation Hb gene variants are present at low prevalence (carrier 1-1.5/1000) in all sizeable populations (Ashley and Koch, 2000). Most Hb gene variants are rare and many are harmless, but some are common because carriers are less likely than others to die from Falciparum malaria (Modell, 2008). In populations in which malaria is (or was) endemic 3-40% of individuals carry one of these significant variants, and the prevalence of Hb disorders ranges from 0.3 -25 per 1000 live births (Modell, 2008).

These fall into two broad categories- structural variants that change the amino acid and produce an unusual Hb, and thalassaemias that lower or abolish production of glob in chains (Huisman and Marianne, 1996).

There are more than 400 different types of abnormal haemoglobin have been identified, but the most common include(S, C, D, and E) (Hoffbrand *et al*, 2005).

1.2.4.1 Quantitative Disorders:

Thalassaemia is an inherited autosomal recessive blood disease the genetic defect, which could be either point mutation or deletion, results in reduced rate of synthesis or no synthesis of one of the globin chains that make up hemoglobin . Thalassemia is a quantitative problem of too few globins synthesized are classified according to which chain of the haemoglobin molecule is affected in α thalassemias, production of the α globin chain is affected, while In β thalassemia production of the β globin chain is affected (Hoffbrand *et al*, 2005).

1.2.4.2 Qualitative Disorders:

Abnormal haemoglobins are formed when the sequence of globin chain amino acids is altered. There is usually only a single amino acid substitution in one of the globin (polypeptide) chains (Hoffbrand *et al*, 2005).

Haemoglobin C differs from normal Hb A by the single amino acid substitution of lysine for glutamic acid in the sixth position from the NH_2 terminal end of the β chain ($\alpha_2\beta_2^{6Glu\text{-}Lys}$). Homozygous Hb C occurs in West Africa (Northern Ghana and Nigeria) and is most common in Mali (Gribben *et al*, 2009).

Clinical manifestations are mild chronic haemolytic anaemia with associated splenomegaly and abdominal discomfort. Hb is usually >100 g/L. Red cell morphology is typically normocytic, normochromic, with numerous target cells (50 to 90%) and occasionally microspherocytes and fragmented cells. Haemoglobin C crystals (bar of gold crystals) occur particularly in splenectomized patients. The retic count is slightly increased (Gribben *et al*, 2009).

Haemoglobin D Prevalent in parts of India, particularly the Punjab. Glutamine substituted for glutamic acid at position 121.Both heterozygous ($\alpha_2\beta_1\beta_1^{121Glu\text{-}Gln}$) and homozygous ($\alpha_2\beta_2^{121Glu\text{-}Gln}$) states are asymptomatic. The PBF is unremarkable, except for a few target cells (Gribben *et al*, 2009).

Haemoglobin E is Prevalent in Burma, Thailand, Cambodia, Laos, Malaysia and Indonesia. Lysine is substituted for glutamic acid at position 26.The homozygous state ($\alpha_2\beta_2^{26Glu\text{-}Lys}$)

presents with mild anaemia, target cells, microcytosis and hypochromasia. Hb E trait ($\alpha_2\beta_1\beta_1^{26Glu\text{-}Lys}$) is asymptomatic. There are microcytosis, hypochromasia and target cells without anaemia (Gribben *et al*, 2009).

Haemoglobin HbS ($\alpha 2/\beta 2^{6Val}$) is the most common haemoglobin variants and is found most frequently in equatorial Africa and Middle East. (Makani 2007) globally, approximately 4.5% of the world population carry abnormal Hb, from whom most were detected in parts of Africa; particularly HbS (Angastiniotis *et al*, 1995).

Homozygous for the abnormal gene that codes for Hb S. Results from a substitution of valine for glutamic acid at the sixth position from the NH_2 terminal end of the β chain. The structural formula for sickle disease (Hb SS) is ($\alpha_2\beta_2^{6Glu\text{-}Val}$). Hb S polymerises when deoxygenated and forms tactoid polymers. The polymers cause the red cell to deform into the sickle shape and reduce the cells ability to circulate. This leads to hypoxia, pain and infarction of organs. The term sickle cell disease applies not only to the homozygous (SS) but to mixed syndromes (compound heterozygote) in which sickling occurs, e.g. SC, SD, SE, SO (Taliaferro and Huck, 1923).

The disease is prevalent in West and Central Africa, where the gene frequency may reach one in three. Hb SS is usually diagnosed early in life, when the level of Hb F declines. Patients have severe chronic haemolytic anaemia with many complications. Sickle cell trait without significant: clinical symptoms, while homozygosity resulted in sickle cell anaemia (Taliaferro and Huck, 1923).

In1949 Pauling and this colleague found that all the haemoglobin in patients with sickle cell aneamia showed an abnormally slow rate of migration on electrophoresis, while the parents of these patients had normal as well as abnormal haemoglobin. Soon after, other abnormal haemoglobins were discovered by subjecting haemoglobin to electrophoresis .The biochemical nature of the defect in the sickle cell aneamia was elucidated by Ingram, who digested haemoglobin with trypsin and seperated the resulting peptide *(* Emmel, 1917).

On paper by electrophoresis in one direction and chromatography in the other this technique (finger printing) demonstrated that one of the digestion products of the sickle cell haemoglobin migrated differently from that of normal haemoglobin. Determination of the amino acid composition of this peptide indicated that sickle cell anaemia was result of the replacement of glutamic acid residue by valine. This discovery established that the substitution of a single amino acid in a poly peptide chain can alter the function of the gene sufficiently to produce widespread clinical effects .Conley has chronicled the fascinating history of sickle cell disease(Emmel, 1917).

1.2.4.3 Nomenclature of abnormal haemoglobins :

After the discovery that sickle cell haemoglobin, or haemoglobin S (Hb S), was electropholically altered, additional variants were assigned letters of alphabet A,B,C,D,E, etc. The letters of the alphabet were rapidly exhausted, however, and the subsequent abnormal haemoglobins were named after the geographic location in which they were found (eg. Haemoglobin

Memphis, haemoglobin Mexico).If the haemoglobin had the electrophoretic characteristics of one previously described by a letter the geographic designation was added as a subscript (eg.haemoglobin M sakation). In this case M indicates an amino acid substitution resulting in a methomeglobin (Makani *et al*, 2007).

The term sickle cell disorder refers to states in which the red cell undergoes sickling when it is deoxygenated .The sickle cell diseases are those disorders in which sickling produces prominent clinical manifestations. Included are sickle cell–haemoglobin C disease (haemoglobin SC disease), sickle cell–haemoglobin D disease (haemoglobin SD disease) sickle cell beta thalassemia, and sickle cell aneamia. The latter term is reserved for the homozygous state for sickle cell gene (Makani *et al*, 2007).

1.2.5 Single Nucleotide Polymorphism (SNP):

Single nucleotide polymorphisms, frequently called SNPs (pronounced “snips”), are the most common type of genetic variation among people. Each SNP represents a difference in a single DNA building block, called a nucleotide. For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA. SNPs occur normally throughout a person’s DNA. They occur once in every 300 nucleotides on average, which means there are roughly 10 million SNPs in the human genome (Maroof, 2002).

1.2.6 Haplotypes:

Globin haplotypes of the gene are based on a series of restriction-endonuclease defined polymorphisms in the globin-gene cluster

on chromosome 11(Huisman and Marianne, 1996). To date, the existence of five different geographic centers has been postulated on the predominance of major haplotypes associated with the β^s mutation. Four of the centers (associated with haplotypes Bantu, Senegal, Benin and Cameroon) have been found in Sub-Saharan Africa (Pagnier, 1984), whereas the remaining one (Arab Indian haplotype) has been found in India, Saudi Arabia and other places in the Middle East(Pagnier, 1984). Classical β^s haplotypes are named according to their putative geographical origins and seem to have originated independently in at least five times and sites - Benin haplotype (Central West Africa), Bantu (Central African Republic-CAR), Cameroon (Eton people in Cameroon), Senegal (Atlantic West Africa), and Arab-Indian (found among the Adavasi of India and among Arabs in the eastern oasis of Saudi Arabia). These haplotypes have migrated to neighboring ethnic groups and increase in frequency, most likely under the selective pressure of P. falciparum malaria (Nagel, 2005).

The majority of the chromosomes with the β^s gene have one of the five common haplotypes, although in every large series of sickle cell patients there is a minority of chromosomes (5–10%) associated with less common haplotypes, usually referred to as “atypical” haplotypes (Jago, 2000).

The Atlantic slave trade and the Arab slave trade dispersed these genes to the Americas and the Middle East. While the Benin haplotype linked to the sickle gene migrated to Sicily, Portugal, Greece and Turkey several hundreds of years ago, more recently, a more genetically diverse sickle gene has migrated (with the

immigrant flow of ‘guest workers’) to several countries of Europe, including France, Germany, Spain and Italy (Nagell, 2005).

In general within ethnic groups in which the β^s allele has a high frequency, one particular β^s haplotype usually predominates. For instance the Senegal β^s haplotype is the most commonly observed haplotype in Senegal, although the Benin haplotype is also present (Webster, 2003). The Arab- Indian haplotype is very common (~51%) among the population of Southwest Iran, although other haplotypes are also present (Rahimi, 2003). Correlation of β-globin gene cluster haplotype with severity of disease expression has revealed that those with the haplotype designated Senegal have decreased severity, those with the Benin haplotype have intermediate severity, and those with the Central African Republic (CAR) haplotype have the most severe clinical expression (Powers, 1993).

The African haplotypes are generally associated with a more severe clinical course as they are associated with lower fetal Hb levels; except for the Senegal haplotype which is associated with a more benign clinical course and higher levels of Hb F. The Arab-Indian haplotype is also associated with a benign clinical course and higher levels of Hb F. As discussed above the distribution of the sickle gene can be seen and with Sudan being located in the middle of Africa and the population being made up of a mixture of tribes of Arab and African descent (Powersl, 1993).

It would be feasible to expect any of the defined haplotypes to be present in the study population. While β-globin gene cluster haplotypes are convenient markers for genetic regulators of β-

globin gene expression, most of the polymorphic restriction sites used to assign a haplotype have no known role in the differential transcription and temporal regulation of these genes. An exception is the XmnI site that is 5' to the γG-globin gene which is strongly associated with high expression of the γG-gene compared with the γA-gene (Nagell, 2001).

1.2.7.1 The sickle cell disease:

Sickle cell anaemia (SS disease) may be considered the prototype of the sickle cell disease, and in general the clinical features and treatment of all these disorders are the same .The homozygous state, sickle cell aneamia, is the most severe of these disorders. with haemoglobin SC disease and sickle cell β thalassemia tending to be somewhat milder ,and haemoglobin SD disease being the mildest of the group .However, there is a great deal of overlap in the severity of the clinical manifestations of these disorders ,some patients with sickle cell thalasseamia or haemoglobin SC disease may be more anaemic and have severe and frequent crises than some mildly affected patients with sickle cell aneamia .A major difference among these disease is in their laboratory diagnosis(Cao *et al*, 1997).

1.2.7.2 Biochemical Basis of sickling:

There are few diseases of man whose etiology can be traced to as basic a level as sickle cell disease .Sickle cell anaemia is due to substitution of thymine for adenine in the glutamic acid DNA codon (GAG-GTG), which results in turn in substitution of β6 valine for glutamic acid .As haemoglobin exists in two conformation, designated the oxy (relaxed R) and deoxy (tense, T)

states. Deoxygenation of haemoglobin shifts this equilibrium toward the T conformation. Molecules of deoxyhaemoglobin S have a strong tendency to aggregate, and such aggregation requires the substitution of valine for glutamic acid in the β6 position, since only those haemoglobin variants with this substitution (E, G, S and Harlem) undergo sickling .Certain other structural features of the molecule are also of importance (Mirchev and Ferrone *et al*, 1989). The deoxygenated haemoglobin solution turns into a firm gel. The distorted sickled red cell is the visible end result of this molecular aggregation .The process is dependent (Eaton and Hofrichtor , 1976).

Initially there is a rate limiting nucleation process, a few molecules of sickle cell haemoglobin must aggregate, forming a "seed" on which aggregation of further molecules occurs rapidly .Thus the sickling process is characterized by a long delay that is strongly dependent on temperature and Hb concentration. The delay is inversely proportional to approximately the thirtieth power of the haemoglobin concentration (Samuel *et al*, 1990). This delay is quite important in protecting the patient from even more dire consequences than might otherwise be anticipated. Even though the oxygen concentration of venous blood is sufficiently low so that at equilibrium about 85% of the red cells would contain sickle haemoglobin polymer. Kinetic data suggest that about 80% of the cells are prevented from sickling during their round trip through the circulation because they reach the lungs and become reoxygenated before significant polymerization has occurred (Mozzarelli and Laton , 1987).

When a cell sickles and unsickles repeatedly, the membrane is affected and the cell becomes irreversibly sickled, it remains so even when the oxygen pressure is increased, there are the sickled forms seen on air-dried films. An irreversibly sickled cell has a high haemoglobin concentration and a high calcium and low potassium content, and it may be ATP-depleted (Eaton *et al*, 1979). These cells appear to be derived directly from reticulocytes. (Serjeant and Milner,1969). But have a short intravascular life span and the severity of the hemolytic process is directly related to the number of these cells in a patient's circulation (Eaton *et al*, 1979).

1.2.7.3 Nature of problem:

Sickle cell disease (SCD)results from the substitution of a valine residue for glutomic acid at position 6 in the beta- subunit of hemoglobin (Ingram *et al*,1956).With a few minor exceptions , people with only one gene for hemoglobin S (HbAS) are phenotypically normal (sickle trait). People who inherit two HbS genes from their parents have sickle cell disease. Deoxygenated HbS tend to polymerize non-covalently into long strands that deform the erythrocyte, giving the characteristic "sickle cell" morphology Hbs with bound oxygen (e.g, in the arterial circulation) does not polymerize. The term sickle cell disease (SCD) describes of complex, chronic disorders characterized by hemolysis, unpredictable acute complications that can rapidly become life-threatening, and the variable development of chronic organ damage. Expert, comprehensive medical care decreases morbidity and prolongs life expectancy for individuals with SCD (Vichinsky , 1991, Nagel Flemming , 1992).

1.2.7.4 Genetics of sickle cell anemia:

The genetic basis of sickle cell anemia is central to the history of medical genetics. The disease was first described by Herrich (1910) a cardiologist, who observed sickle shaped red cells in the blood of a medical student from Grenada., who suffered from chronic hemolytic anemia .James V. Neel was the first to suggest that sickle cell anaemia was a homozygous state and sickle trait (the asymptomatic carrier state) was a heterozygous state of a not yet defined genetic character (Drew and Gribben, 2000).

Sickle cell disease is a generic term for a group of genetic disorders characterized by the predominance of hemoglobin S (HbS).These disorders include sickle cell anemia, the sickle beta thalassemia syndromes, and hemoglobinopathies in which Hb S is in association with abnormal hemoglobin that not only can participate in the formation of hemoglobin polymers but also is present in sufficient Concentration to enable red cells to sickle. Examples of the latter disorders include hemoglobin SD disease, and hemoglobin SO Arab disease. The sickle cell disorders are found in people of African, Mediterranean, Indian, and middle Eastern heritage. In the United States, these disorders are most commonly observed in Africans Americans and Hispanics from the Caribbean, Central America (Rees *et al*, 2010).

Sickle cell disease is caused by a hereditary defect in the hemoglobin molecule. The two β chains in normal hemoglobin (Hb-A) contain a glutamic acid residue at position 6.In people with sickle cell disease, a valine residue occurs in this position due

to an A to T transversion mutation in glutamic codon GAG to give the valine codon GTG. This residue is on the outer surface of the molecule and this single difference in the sequence of the 146 amino acids of the β chain is enough to produce a "sticky" hydrophobic spot on the surface that results in the abnormal quaternary association of the α and β chains of the abnormal haemoglobin (Hb-S) (Rees *et al*, 2010).

Both deoxygenated Hb-A and Hb-S have a normal, "complementary" sticky patch on the surface of the chains to which the abnormal hydrophobic spot on deoxygenated Hb-S will bind. This normal patch is masked when the hemoglobin is oxygenated. Thus, when oxygen concentrations fall below a critical level, the Hb-S polymerises into linear, insoluble arrays of fibers within the erythrocyte, which become deformed (sickled) and function abnormally .This only happens in sickle cell homozygotes, since the presence of deoxy Hb-A produced by the normal allele in heterozygotes will terminate the polymerization(Rees *et al*, 2010).

Heterozygotes are phenotypically normal but are said to carry the "sickle cell trait. The Glu to Val substitution also causes a charge difference between Hb –A and Hb-S that affects the mobility of the molecule in an electric field. Thus, electrophoresis of haemoglobin (or a red cell lysate – haemolysate, which is predominantly haemoglobin) can be used as diagnostic aid and can readily distinguish between normal, sickle cell homozygote and sickle cell heterozygote individuals (Rees *et al*, 2010).

1.2.7.5 Anaemia in Sickle Cell:

Anemia is not a simple product of haemolysis in sickle cell anemia as demonstrated by the marrow expansion of only five times normal, compared to ten times normal seen in thalassaemia major. One reason for this is the right shift of the oxygen equilibrium curve; this type of O2 binding curve results in increased oxygen delivery to tissues. This explains why erythropoietin increases only below 9 g of Hb. In addition, the response to erythropoietin is blunted, that is inappropriately low for the anaemia, below the threshold of 9 g of hemoglobin. The reason for this is not understood, but the fact that age increases the deficiency of erythropoietin suggest that 'silent' renal damage might be involved in a reduction of erythropoietin response. Another contributor to the anemia (a reduction of erythropoietin response) is the increase of 2, 3-DPG found in sickle red cells (Drew and Gribben, 2000).

The haemolysis is also complex actually there are three red cell population in sickle anemia, the very dense red cells that live only 4-5 days, the F cells (these are sickle cells containing Hb F) that live close to normal life spans and the rest of the cells that fall in between. Co-existence of α thalassaemia and high F reduces haemolysis considerably. Interestingly, the level of Hb F also defines. The properties of progenitors are as well as the circulating haempoietic cytokines. Low Hb F sickle cell anaemia patients have increased number of circulating BFU-e, which is in active cycle. In addition; these patients have con-stitutively circulating GM-CSF and steel factor, unlike normal individuals and high Hb F sicklers. In contrast the low HbF patients have IL-3 circulating

in addition to a haemopiotic inhibitory factor, TNF α (Drew and Gribben, 2000).

Sickle cell anaemia patients are susceptible to an acute anaemia superimposed on their chronic anemia, due to infection with parvovirus B19; witch can produce a life- threatening drop in haemoglobin which is generally self –limited (7-15 days). This result from its ability to infect BFU-E through the blood group p system that serves as receptor. Treatment is by transfusion of red cells in most cases (Drew and Gribben, 2000).

Red cells from patients with sickle cell anaemia, haemoglobin S denatures and forms binding of Heinz bodies to the inner surface of the sickle cell membrane promotes colocalization of the membrane proteins 3, outer-bound autologous IgG an extent, the membrane proteins glycophorin and ankyrin .Loss of transbilayer lipid as found in certain populations of sickle red cells (Drew and Gribben, 2000).

1.2.7.6 Sickle cell Trait:

Sickle cell trait is not a disease. It is not a cause for abnormalities in the blood count and does not produce vaso-occlusive symptoms under physiologic conditions. Sickle cell trait does not adversely affect the individual's life expectancy (Eichner, 1986). Sickle cell trait (AS) is a heterozygous condition in which the individual has one beta S gene and one beta A globin gene, resulting in the production of both Hb S and Hb A with a predominance of Hb A. Sickle cell trait is found in 8 percent of the American black population. The diagnosis should be established by hemoglobin electrophoresis. Persons who have

more Hb S than Hb A on electrophoresis (and have not been transfused) have Hb S beta+thal and not sickle cell trait (Kark *et al*, 1987). Hemoglobin electrophoresis is used to screen persons for sickle cell trait and will also detect persons with sickle cell disease and heterozygotes (carriers) for other hemoglobin disorders such as Hb C .Like sickle cell trait; hemoglobin C trait produces no hematologic abnormalities aside from a few target cells on blood smears. Haemoglobin C trait is not detected by solubility testing or by a sickle cell preparation (Lane, 1985).

Sickle cell trait has been associated with several clinical conditions, including hypothermia, painless haematuria, and an increased risk of urinary tract infection during pregnancy .Haemturia in persons with sickle cell trait must be evaluated thoroughly to exclude other causes, as in other patients. Traumatic hyphenate in individuals with Sickle cell trait requires special management (Mclnnes, 1980): following extreme exertion during basic training in soldiers with sickle when individuals with sickle cell trait become hypoxic, they rarely develop symptoms related to vaso-oclusion. A recent retrospective study of military recruits showed an increased risk of sudden death cell trait compared with individuals with only Hb A. The magnitude of the increased risk was very small (32 per 100,000 compared with 1 per 100,000 for recruits with black parents who had normal hemoglobin) and appeared to be related to the recruit's age (Monplaisir *et al*, 1986).

Individuals with sickle cell trait should receive informative and nondirective genetic counseling. Before counseling couples, it's important to perform hemoglobin electrophoresis and Hb A2 and Hb F measurement on both prospective parents. In parents

who are not carriers of abnormal hemoglobin, measurements of Hb A2 and Hb F are needed to identify thalasseamic conditions. When both parents have sickle cell trait, they have 25 percent chance with each pregnancy of having a child with SS disease (Pritchard *et al*, 1973) .If one parent has sickle cell trait and the other has a beta thalassaemic disorder, they are at the same risk for having a child with a sickle beta thalasseamia syndrome. In couples where one individual has sickle cell trait and one has hemoglobin C trait, the chance of having a child with Hb SC disease is also 25 percent with each pregnancy. If one parent has SS disease and other has sickle cell trait, the risk of having a child with SS disease is 50 percent with each pregnancy (Eichner, 1986).

If both copies of the hemoglobin gene carry instructions to make sickle hemoglobin then this will be the only type of hemoglobin they can make and sickled cells can occur. These people have Sickle Cell Anemia and can suffer from anaemia and sever pain. These sever attacks are known as Crises .Over time Sickle cell sufferers can experience damage to organs such as liver, kidney ,lungs, heart and spleen .Death can be a result(Witkowska *et al*, 1991).

Another problem is that red blood cells containing sickle hemoglobin do not live as long as normal 120 days and this results in a chronic state of anemia. In spite of this, a person with sickle cell disorder can attend school, college and work. People with sickle cell disorder need regular medical attention particularly before and after operations, dental extraction and during pregnancy (Evans, 1903).

Sickle cell trait is benign genetic abnormality which has been wrongly projected as a health hazard in aviation. Conflicting reports on the relationship between this trait and flying exist in the literature. Limitations placed on sickle cell trait individuals unfairly stigmatize large numbers of people, both socially and economically .The imprecise identification of the sickle cell hemoglobinopathies and unrecognized interactions of other abnormal haemoglobins with HbS have perpetuated the controversy. There is tendency to use isolated anecdotal incidents as evidence of increased morbidity in sickle cell trait: however, it has not been documented that hypoxic conditions cause in vivo sickling in pure trait carriers .An analysis of the definitions of the haemoglobinopathies, The molecular basis of haemoglobin S, the interactions of abnormal hemoglobins, and the sickling phenomenon shows both that there is no evidence that the sickle cell trait is a health hazard and that most of the literature contrary to this finding is invalid (Long, 1982).

1.2.7.7 Inheritance of sickle cell anaemia:

Most of our physical characteristics are inherited through the genes we take from our parents; for example, the shape of our nose, the colour of our skin and eyes. We also inherit our haemoglobin (Hb) type from our parents, through the genes. Everyone has two copies of the haemoglobin gene in every cell in their body (apart from eggs and sperm).They gets one from their mother and one from their father. When eggs and sperm are made, only one of the two genes goes into each egg or sperm cell. This is so that when the egg and sperm come together to make a new baby this new person has two genes in every cell in their body as

well. The genes the baby gets will therefore depend on the genes carried in its parents. Sickle cell anaemia is called a recessive condition because you must have two copies of the sickle haemoglobin gene to have the disorder. Sickle haemoglobin is often shortened to S or HbS. If you have only one copy of the sickle haemoglobin along with one copy of the more usual haemoglobin (A or Hb A) you are said to have sickle cell trait. This is not an illness but means that you "carry" the gene and can pass it on to your children. If your partner also has sickle cell trait or sickle cell anemia your children could get sickle cell anaemia (Gribben *et al*, 2009).

If one parent has sickle cell trait (HbAS) and the other does not carry the sickle haemoglobin at all (Hb AA) then none of the children will have sickle cell anaemia. There is a one in two (50%) chance that any given child will get one copy of the Hb S gene and therefore have the sickle cell trait .It is equally likely that any given child will get two HbA genes and be completely unaffected .If both parents have sickle cell trait (HbAS) there is a one in four chance that any given child could be completely unaffected. There is a one in two (50%) chance that any given child will get the sickle cell triat.If one parent has sickle cell trait (HbAS) and the other has sickle cell anaemia (HbSS) there is a one in two (50%) chance that any given child will get sickle cell trait and a one in two chance that any given child will get sickle cell anaemia .No children will be completely unaffected. If one parent has sickle cell anaemia (HbSS) and the other is completely unaffected(HbAA) then all the children will have sickle cell trait .None will have sickle cell anaemia .The parent who has sickle

cell anaemia (HbSS) can only pass the sickle haemoglobin gene to each of their children(Gribben *et al*, 2009).

There are also other different types of haemoglobin such as HbC and beta thalassaemia that can combine with sickle hemoglobin to cause sickling disorders. When someone carries the gene for beta –thalasseamia they cannot make as much amount of haemoglobin is HbS and they can suffer from what is usually a milder form of sickle cell disorder than sickle cell anemia (Gribben *et al*, 2009).

The different Kinds of SCD and the different traits are found mainly in people whose families come from Africa, the Caribbean the Eastern Mediterranean, Middle East and Asia .In Britain SCD is most common in people of African and Caribbean descent (Gribben *et al*, 2009).

Sickle cell syndromes, sometimes abbreviated as (SCS), are commonly called sickle cell "diseases" Sickle cell syndromes are genetically inherited conditions which affect the red blood cells 'haemoglobin. If a person inherits two sickle haemoglobin one from each parent, they will have a condition called sickle cell anaemia (HbSS). Where the sickle haemoglobin is inherited with another unusual haemoglobin, for example haemoglobin C,the individual will have sickle haemoglobin C syndrome (HbSC) If sickle is inherited with beta thalassemia,the individual will have sickle beta thalassemia syndrome (Hb S.Thal). These are just three types of the condition Known collectively as sickle cell syndromes. Sickle cell anaemia is the most and often the most severe of the three (Gribben *et al*, 2009).

In these conditions, the red blood cells lose their ability to carry oxygen properly, the cells become hard, brittle, fragile, break easily, and only live half as long as healthy red blood cells. Therefore, these individuals become anaemic. Sometimes, the red blood cells clog together and block the narrow blood vessels, this give rise to a severe pain known as sickle cell crisis. People with sickle cell syndromes may experience tiredness, feel lethargic, lose their appetite, are prone to getting infections, and will often become breathless during strenuous exercise. During a crisis, a person may need to be admitted to hospital for treatment of infection or for relief of pain. Although these episodes are not constant, they are unpredictable in onset and severity (Gribben *et al*, 2009).

1.2.7.8 Difference between sickle cell trait and a sickle cell syndrome

Sickle cell trait (Hb AS) is not a disease or an illness. Because the individual has one usual haemoglobin A, their red blood cells remain round and healthy. They just carry the unusual sickle haemoglobin gene some people inherit one usual adult haemoglobin from one parent and one sickle haemoglobin from the other and are said to have sickle cell trait (Hb AS).These individuals have healthy red blood cells, they are not ill, they are well and do not have any symptoms. It cannot change into any other form of haemoglobin later in life. Having sickle cell trait they are perfectly healthy and do not have a disease or illness. In fact many athletic people have sickle cell trait and it does not affect their physical performance in any way. The red blood cells of people with sickle cell trait (Hb AS) remain round and carry

oxygen normally. Because there are no symptoms associated with sickle cell trait, these individuals will not know that they carry this unusual haemoglobin unless they have a special blood test, or they have a child with someone who also has unusual haemoglobin and their child is born with a sickle cell syndrome. For a couple who both have sickle cell trait (Hb AS), each time they expect a child there is a 1 in 4 chance that their child could inherit sickle cell anaemia(HbSS),which is one of the sickle cell syndromes(Gribben *et al*, 2009).

Sickle cell syndrome on the other hand, can be a serious condition, even can affect individuals differently. While some people remain fairly well, others may experience many episodes of pain and discomfort which require hospitalization (Overturf *et al*, 1977).

For beta thalassemia if no beta globin is produced by the beta thalassemia gene, the individual has S beta o thalassemia (S beta o thal). If some normal beta globin is produced by the thalassemia gene, the individual has S beta + thalassemia (S beta + thal) (Overturf *et al*, 1977).

In the case of hemoglobin (SC disease), the individual has two abnormal beta globin genes, beta S and beta C, and makes two abnormal hemoglobins, Hb S and Hb C. Because the alpha globin genes are located on a different chromosome from the beta genes, a patient with sickle cell anemia can independently inherit an alpha globin gene abnormality, a common condition in people of African descent that has clinical significance for patients with a sickle cell resulting in alpha thalassemia trait, disorder is the

deletion of two of the four alpha globin genes.In contrast to these disease is sickle cell trait. Individuals with sickle cell trait (Hb AS) have a normal beta globin gene (beta A) and a beta S globin gene, resulting in the production of both normal haemoglobin A and haemoglobin S, with a predominance of Hb A. Their red blood cells sickle only under unusual circumstances such as marked hypoxia and the hyperosmolar environment of the renal medulla resulting in hyposthenuria (Overturf *et al*, 1977).

There are two cardinal pathophysiologic features of sickle cell disorders: chronic haemolytic anaemia and vaso-occlusion (which results in ischemic tissue injury). Haemolytic anaemia may be related cycles of sickling and unsickling, which interact to produce irreversible red cell membrane changes, red cell dehydration, and erythrocyte destruction. Tissue injury is usually produced by hypoxia secondary to the obstruction of blood vessels by an accumulation of sickled erythrocytes. The organs at greatest risk are those with venous sinuses which where blood flow is slow and oxygen tension and pH are low (spleen and bone marrow) or those with a limited terminal arterial blood supply (eye. head of the femur and hummers).The lungs as the recipient of deoxygenated sickle cells that escape from the spleen or bone marrow may be at special risk for vaso-occlusion and infarction. No tissue or organ is spared from this injury. Symptoms of the hypoxic injury may be either acute e.g. Painful events, acute chest syndrome) or insidious in onset (e.g. aseptic necrosis of the hips. sickle cell retinopathy). The effects of acute and chronic tissue injury may ultimately result in failure of organs like the kidney, particularly as the patient ages (Overturf *et al*, 1977).

Appropriate counseling and management requires definitive diagnosis. The diagnosis of sickle cell disease is primarily based on hemoglobin electrophoresis. In those instances where Hb S is found in association with abnormal hemoglobin such as Hb C. the diagnosis can he make by electrophoresis alone, in those instances, however, when the electrophoresis reveals only Hb S. Hb F (fetal hemoglobin), and Hb A_2. there can be difficulty in distinguishing between sickle cell anemia and some of the sickle beta thalassemia syndromes, including S beta o thal and sickle cell trait, in association with hereditary persistence of fetal hemoglobin (S HPFH). It is important that distinctions be made because these disorders differ markedly in their clinical expression. Patients with SS or S beta o thal disease or S HPFI1 all have similar elcctrophoretic patterns. Mean corpuscular volume (MCV) is definitely decreased in thalassemia syndromes and is somewhat decreased in S HPFH. Measurement of Hb A and Hb F may help in distinguishing between these conditions. In general Hb F levels are elevated above 3.5 percent in S beta o thal and are low in patients with S HPFH. Hb F levels are generally higher in the sickle beta thalassemic disorders than in SS disease, although there is considerable overlap between these diagnostic groups, in those instances where S HPFH is suspected measurement of Hb F in the parents and/or siblings can be valuable(Overturf *et al*, 1977).

The diagnosis of sickle cell disease cannot and must not be made from either a sickle cell preparation or solubility test because neither of these tests will reliably distinguish sickle cell trait from sickle cell disease. The diagnosis of a specific sickle cell disorder

can be readily established through an analysis of the alpha and beta globin gene complex by using techniques of molecular biology: however, these are not usually required. The clinician should rely on the clinical history, blood counts, peripheral blood smear, hemoglobin electrophoresis with measurement of the minor hemoglobins A2 and F, and, when available, family studies that include hemoglobin electrophoresis and measurement of Hb A2 and Hb F(Overturf *et al*, 1977).

1.2.7.9 Detection and identification of haemoglohinopathies:

The detection and identification of variant haemoglobins has become complex because of the large number of abnormal haemoglobins that have become known as new and more sophisticated methods of treatment become available it is essential that accurate diagnosis of haemoglobinopathies be made. In the presence of abnormal haemoglobin a review of the patients CBC and red blood cell indices is necessary the blood smear should be reviewed for abnormal characteristics such as target cells and sickle cells. Then cellulose acetate or a citrate ager gel electrophoresis should be performed for the confirmation of the variant haemoglobin. The diagnosis of sickle cell disease cannot and must not be made from either a sickle cell preparation or solubility test because neither of these tests will reliably distinguish sickle cell trait from sickle cell disease. The diagnosis of a specific sickle cell disorder can be readily established through an analysis of the alpha and beta globin gene complex by using techniques of molecular biology (Polymerase chain Reaction): however, these are not usually required. The clinician should rely on the clinical history, blood counts. Peripheral blood smear,

hemoglobin electrophoresis with measurement of the minor hemoglobin A2 and F, and, when available, family studies that include hemoglobin electrophoresis and measurement of Hb A2 and Hb F (Hofbrand, 1993).

1.2.7.10 Fetal Tissue sampling (prenatal Diagnosis):

Analyses are performed on DNA from fetal cells obtained by chorionic villus sampling (CVS) in the first trimester, perhaps as early as the 10th gestational week. As an alternative approach, amniocentesis can be performed safely in the 16th gestational week, a time when there is sufficient amniotic fluid. These sampling methods result in a far lower rate of fetal demise than the fetal blood sampling method practiced previously, and their use now is nearly universal. It is common practice to initiate tissue culture as a backup source of fetal DNA in case insufficient DNA for analysis is obtained initially (Lane and Githens, 1985).

The emphasis on methods of early sampling to safeguard against unacceptable delays in diagnostic testing have encouraged the use of CVS as the method of choice for fetal sampling. However, the use of CVS before 9 weeks' gestation is associated with increased rules of limb reduction anomalies. Moreover, when CVS is used for sampling, confined placental mosaicism may result in mistaken diagnoses of heterozygosity in homozvgous fetuses. CVS is not recommended until after 10 weeks' gestation, and diagnoses of heterozygosity must be confirmed by amniocentesis later in pregnancy. The use of preimplantation test and assay fetal cells in the maternal circulation offer hope for safer sampling in the future (Powledge and Fetcher, 1979).

The Hb S and Hb C genes can be detected directly in fetal DNA samples, if the specific mutation responsible for thalassemia in a parent is known, that gene can also be detected. In some laboratories, several relatively common mutations are sought, but a negative result is not helpful. The use of genetic linkage analysis for indirect identification of' these genes is no longer necessary. Fetal blood sampling is used only in centers where DNA-based testing is unavailable (Evans *et al*, 1993).

1.2.7.11 Newborn screening:

Newborn screening for sickle cells disease is an effective first step to reduce morbidity and mortality in individuals with the disease. Sickle cell disease screening should be coupled with other newborn screening tests performed to detect hypothyroidism and in born errors of metabolism (Grover ***et*** *al* 1983). Red cells of' normal newborn contains hemoglobin F and A. "FA," the hemoglobin in highest concentration being listed first. The hemoglobin pattern or phenotype is due to predominance of Hb F at birth. Newborns with sickle cell trait have an "FAS" phenotype, with more Hb A than Hb S (Pearson *et al*, I 974).

Newborn screening may identify variant hemoglobins other than S or C. When these are co-inherited with Hb S. a definitive diagnosis must be made. Hemoglobin So Arab disease for instance, is as serious a condition as SS disease, while the combination of Hb S and Baltimore is as benign as sickle cell trait (Stern and Davis, 1984).

Most infants with SCD are healthy at birth and become symptomatic later in infancy or childhood after fetal hemoglobin

(Hb F) levels decrease (Vichinsky, 1991). Confirmatory testing of infants with positive neonatal screening results and diagnosis of older patients who present with symptoms require hemoglobin separation by electrophoresis (cellulose acetate and citrate agar). Isoelectric focusing, and/or high-performance liquid chromatography (I IPLC) (Vichinsky *et al*, 1988).

When a newborn's screening test indicates 'sickle cell disease." it is the primary physician's responsibility to either establish a definitive diagnosis or to refer the patient to a pediatric hematologist for this purpose establishing a definitive diagnosis requires accurate characterization of the hemoglobin phenotype and correlation of the phenotype with the clinical history, blood counts, and red blood cell morphology. Recent advances in molecular biology techniques have identified many variations in the beta globin gene area (beta globin gene haplotype) that may modify the clinical expression of sickle cell disease (Powledge andFletcher,1979).

1.2.7.12 Pathophysology of the sickle cell disease:

The primary cause clinical manifestations are the intracellular polymerization of Hb S that occurs when sickle erythrocytes are partially deoxygenated under hypoxia. The intracellular polymerization of Hb S causes the red cell to lose its flexibility. Repeated cycles of oxygenation deoxygenation result in irreversible membrane damage and to the formation of irreversibly sickled cells. More subtle membrane damage leads to intracellular water and potassium loss, and the generation of progressively dehydrated cells in the form of dense erythrocytes. The sickle red cells are less deformable, thus resulting in

microvascular occlusion and hemolytic anemia, which are typical of the disease. Although the exact mechanism of vasoocclusion has not been fully elucidated, many factors play a role in physiopathology the sickle cell vasoocclusion process: adhesion molecules, endothelial cell abnormalities, cytokines and coagulation factors (Pra *et al,* 1988). The most common clinical manifestations in SCD are anemia, jaundice, recurrent vasoocclusive crises, and bacterial infection -mostly by **S** pneumonia. The increased susceptibility to bacterial infection is primarily due to organic or functional asplenia (Farber *et al*, 1985).

It believed that sickle cell provides resistance, to individuals living in malaria endemic regions; the various explanations given are as follows: Malaria is caused by the plasmodium parasite that completes a part of its life cycle in the red blood cells of human beings. It enters the human blood stream when a female Anopheles mosquito, carrying the parasite, bites a healthy individual; it has been observed that the red blood cells of individuals with sickle cell trait break down when infected with the malaria parasite. Since the parasite needs to complete a part of its life cycle inside the red blood cells, destruction of the cells does not allow the disease to get established in the individual, also malaria parasite thrives on hemoglobin in order to grow, the mutated Hb S leads to the polymerization of hemoglobin that does not allow the malaria parasite to ingest hemoglobin The red blood cells of people with sickle cell trait, tend to sickle under very low oxygen tension , malaria parasite reduces the oxygen tension in the blood cells that they infect, because they use up the oxygen

carried by the hemoglobin for their own metabolism this sickles the red blood cells that is destroyed by phagocytes. In case of low oxygen concentration, the potassium in the red blood cells leak out of the cells that contain the abnormal hemoglobin, the parasite needs high levels of potassium to develop and due to its leakage the parasite fails to grow in a blood cell, that have the abnormal hemoglobin(Gribben *et al* 2009).

1 .2.7.13.1 Sickle cell complications:

Sickle cell disease (SCD) is a complex disorder with multisystem manifestations result in anaemia and crisis that requires specialized comprehensive care to achieve an optimal outcome. Appropriate treatment requires usually a pediatric hematologist working in conjunction with a multidisciplinary team (Eaton *et al*, 1995).

The term "sickle cell crisis" is used to describe several independent acute conditions occurring in patients with sickle cell disease that could be of many types including: vasoocclusive crisis, a plastic crisis, sequestration crisis and haemolytic crisis caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ, resulting in ischaemia, pain, necrosis and often organ damage. (Johnson and Giorgio, 1981)The reduced blood flow causes regional hypoxia and acidosis, which further increases the sickling process and may increase the ischemic injury (Mozzarelli *et al*, 1987). Painful crises vary in intensity and duration, usually lasting 4 to 6 days but sometimes persisting for weeks. They may vary in intensity from time to time during the course of a single episode (Brown *et al*,1994).

Hypoxia, infection, fever, acidosis, Dehydration, menstruation, sleep apnea, obstructive snoring, and exposure to cold may precipitate such events; In addition, patients often cite anxiety, depression, and physical exhaustion as precipitators. In many instances, no precipitating event can be identified (Mozzarelli *et al*, 1987).

The frequency and severity of repeated painful crises vary widely among patients. A few patients state that they are always in pain" and repeatedly request hospitalization. Conversely, approximately one-third of patients rarely seek hospital- based treatment. Many patients can manage painful events at home (Mozzarelli *et al*, 1987).

In general, painful crises are a measure of disease severity and have been reported to correlate with early death in adult patients, but we are unable to predict which individuals will be so adversely affected.

High hemoglobin levels, low fetal hemoglobin, and a baseline white blood cell (WBC) count of 15,000 are factors that place individuals at greatest risk. A painful crisis is one of the most common settings in which death occurs in adult patients .Related symptoms are dactylitis, priapism, abdominal pain acute chest syndrome, central nervous system events, bone marrow embolism, and acute multiorgan failure syndrome may suddenly occur during severe painful crises (Lane, 1996).

1.2.7.13.2 Splenic sequestration crisis:

Because of its narrow vessels and function in clearing defective red blood cells, the spleen is frequently affected; it is usually

infracted before the end of childhood in individuals suffering from sickle cell anaemia.

The sinusoids and gates would open at the same time resulting in sudden pooling of the blood into the spleen and circulatory defect leading to sudden fall in hemoglobin levels hypovolaemic shock. Initially the spleen is enlarged, and then progressively becomes smaller. The stasis of flow in the spleen causes hypoxic damage, thrombosis, infarction and fibrosis. In the end the spleen "disappears" completely (termed 'autosplenectomy') (Anson *et al*, 1991).

1.2.7.13.3 Stroke:

Clinically evident stroke is a devastating complication of SS disease that affects from 6 to 12 percent of patients. Strokes arc very rare in persons with Hb SC disease. In children under age 10 years, the most common cause of stroke is cerebral infarction. Ischemic stroke typically presents with signs and symptoms of hemiparesis or monoparesis, hemianesthesia, visual field deficits, aphasia, cranial nerve palsies, or acute change in behavior. Although recovery occasionally is complete, intellectual, motor, and sensory impairments arc typical sequelae. Intracranial hemorrhage becomes increasingly more common with advancing age. In hemorrhagic stroke, more generalized phenomena such as coma, headache, and seizures occur. Recurrent stroke causes progressively greater impairment and increased likelihood of mortality. A "completed stroke" signifies a fixed neurologic deficit, whereas "stroke in progression" implies worsening of the neurologic deficit or appearance of new focal abnormalities while the patient is under observation. Nonfocal complaints such as

dizziness, headache, or fainting are not in themselves presentative of cerebral vascular disease but should be investigated carefully (Vichinsky and Lubin, 1994).

A transient ischemic attack (TIA) is a focal neurologic deficit persisting for less than 48 hours (24 hours for internal carotid, anterior, or middle cerebral arteries and 48 hours for vertebral or basilar arteries that follows a vascular distribution. Typically, there is no clinically apparent residual deficit from a TIA, though newer imaging modalities such as MRI and positron emission tomography (PET) have identified ischemic brain lesions in both the gray and white matter in some patients. TIA often is a harbinger of subsequent Stroke (Vichinsky and Lubin, 1994).

1.2.7.13.4 Infection:

Serious bacterial infections are a major cause of morbidity and mortality in patients with sickle cell disease, Severe, overwhelming septicemia meningitis due to S. pneumoniae is the most common cause of death during early childhood, but enteric organisms emerge as important pathogens in older patients. Because of the patient's asplenic condition, and because of disordered humoral immunity, infections in patients with SS disease are more likely to cause morbidity, disseminate, and resist eradication than in individuals unaffected by the disease (Githens, 1985). Infections also may enhance susceptibility toward vaso-occlusive complications. Prevention and early aggressive treatment of infection are critical in the management of patients with sickle cell disease. Children with SS disease have a normal antibody response to vaccines and should receive all immunizations recommended (Overturf *et al*, 1977).

1.2.7.13.5 Aplastic crisis:

Aplastic crisis is acute worsenings of the patient's baseline anaemia, producing pallor, tachycardia, and fatigue with a substantially decreased reticulocyte count; typically less than 1% is triggered by parvovirus B-19, which directly affects erythropoiesis (production of red blood cells) by invading the red cell precursors and multiplying in them and destroying them. Parvovirus infection nearly completely prevents red blood cell production for two to three days. In normal individuals, this is of little consequence, but the shortened red cell life of sickle-cell patient's results in an abrupt, life-threatening situation (Lehmann and Cutbush, 1952).

Haemolytic crises are acute accelerated drops in haemoglobin level. The red blood cells break down at a faster rate lead to anaemia and jaundice, cholelithiasis (gallstones) and cholecystitis, which may result from excessive bilirubin production and precipitation due to prolonged haemolysis(Gribben *et al*, 2009).

1.2.7.13.6 A vascular necrosis:

Is aseptic bone necrosis of the hip and other major joints, which may occur as a result of ischaemia (Lehmann and Cutbush, 1952).

Sickle cell haemoglobinopathies have the potential to cause ophthalmic complications that affect vision (*Cohen et al, 1986*). It is recommended that patients with sickle haemoglobinopathies have early eye examinations, including indirect ophthalmoscopy.

Retinal neovascularization is most common in Hb SC disease but may also be seen in SS disease and S beta thal (Codon and Serjent, I980).

1.2.7.13.7 Chronic Renal Failure:

Chronic renal failure in sickle cell disease is associated with end-stage renal disease due to one or a combination of the sickle cell nephropathies. Chronic renal failure occurs in a small percentage of patients with sickling disorders, and its prevalence in sickle cell disease appears to be greater than among the general African—American population (Powers et al, 1991).

Surgery and anesthesia, Patients with sickle cell disease who undergo surgery have an increased risk of preoperative complications. Careful preoperative preparation of the patient by a team consisting of a surgeon, hematologist, and anesthesiologist will minimize or eliminate these complications (Bischoff *et al*, 1988).

1.2.8. Previous studies

1.2.8.1 Global prevalence of sickle cell gene and other Variant Hb:

The WHO estimated about 5.2% of the world's populations are carriers for haemoglobinopathies (*Mattevi et al, 2000*). As a result of increasing migration, haemoglobinopathies have become more widespread. The sickle β globin gene is widely spread through Africa, the Middle East, the Mediterranean countries and India. Due to slave trade, it has also been carried to North America, the Caribbean, Central America and a few countries of South America. In some of these regions, SCD has been recognized as a public health problem, since it is the most frequent type of hemolytic anemia.Figure 1.3 (Makani *et al*, 2007).

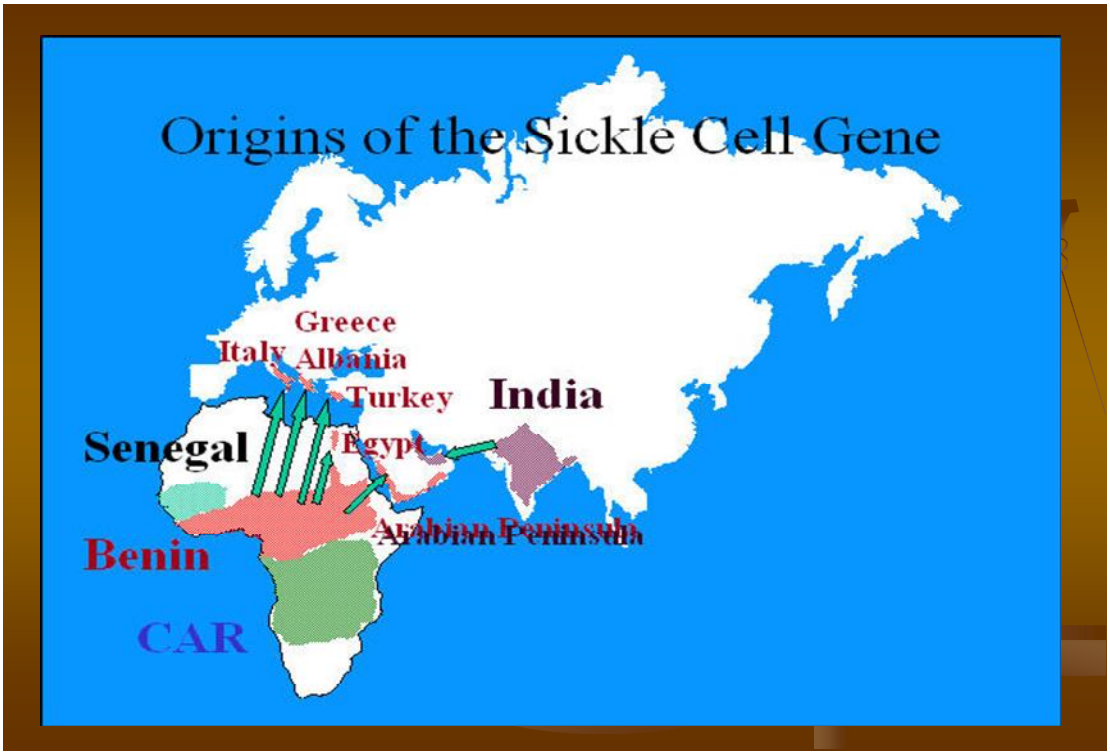

Figure 1.3: Global prevalence of sickle cell gene (Rees *et al*, 2010).

Sickle cell is found in areas of the world where malaria is, or was, common. It has been shown that sickle cell trait protects very young children from the harmful effects of a serious type of malaria; therefore, sickle cell trait is very useful in those parts of the world. Sickle cell trait is found in people whose ancestors originate from Asia, Africa, the Mediterranean islands, the Middle and far East, and South America .Because of migration, it's also found in Caribbean, Black British ,Black Americans, and other whose ancestors originally came from parts of the world where sickle Haemoglobin is common(Rees *et al*, 2010).

For example, it is estimated that approximately: 1 in every 10 Afro- Caribbean, 1 in every 4 West Africans, 1 in every 50 Asians and 1 in every 100 Northern Greeks (Rees *et al*, 2010).

Hb D (α2β2121 glu gln) is designed as Hb D-Punjab and Hb D-Los Angeles in the UK and USA respectively; it is very common in individuals who originate from Northern India. This disorder was the third abnormal Hb to be reported in 1951 in a multiethnic family, of half British and half American-Indian origin (Itano , 1951). Hb D has been detected in several ethnicities, in Europeans as found in English, Portuguese, Italian, German and Greek (Agrawal *et al*, 2007, Modell *et al*, 2007). It was also found in Indian, Persian, Indian American Negro, West Africa and Arab individuals in north Africa particularly Arab-Berbers of Algeria (Vella *et al*,1974, Agarwal *et al*, 1989).

1.2.8.2 Hemoglobinopathies among ethnic groups in Karachi Pakistan:

A brief survey of abnormal hemoglobin variants among the major ethnic groups of Karachi was conducted: 202,600 subjects were studied Patients with low haemoglobin (Hb), low mean cell volume (MCV) and mean cell heamoglobin (MCH) including anemia Microcytosis, hypochromic hemolysis and target cells, were referred for the identification of henioglohinopathy by molecular methods .Population screening showed that 60% had iron deficiency anemia and 40% hemolytic anemia of which 20.6% was due to beta-thalassemia major 13.6% beta — thalassernia trait . 5.1% sickle cell disease 0.76% hemoglobin D Punjab 0.32% hemglobin C, and 0.22% hereditary persistence of fetal hemglobin (HPFH) (ghani, *et al*, 2002).

1.2.8.3 Hemoglobinopathies in Guadeloupe (INDIA):

Survey carried on 8.961 healthy Guadeloupian blood donors, looked for hemoglobinopathies. The results are expressed in regard of the race and site of living (urban or rural) of the subjects, aged 18 to 60. 7.75% were sickle cell trait carriers. 2.36% were heterozygous for Hb C. And 0.2% had a significant elevation of Hb F. Were also report some less frequent phenotypes: three Hb AD, five Hb SC, Hb CC, two Hb SF, one Hb CF and one case of isolated Hb A2 elevation. Two rare hemoglobinopathies are reported: a case of Hb Korle Bu associated with Hb S and a case of Hb N-Baltimore (Fabritius *et al*, 1978)

1.2.8.4 Sickle cell anemia and S-thalassemia in Sicilian children

Thousands of years the Mediterranean basin has been the crossroad for trade, races, ideas, and art. The geographical position of Sicily at the center of the Mediterranean made it a natural stopover on these journeys. Phoenicians, Greeks, Carthaginians, Byzantines, Saracens, Normans, Spaniards, Arabs, Jews, and mercenaries from all over the world came to Sicily in large numbers to settle. In contrast with the past, there has been almost no immigration during the last few centuries. The genetic structure of the Sicilians is clearly not due to recent additions. The consensus is that the gene was introduced into Sicily and Southern Italy from Northern Africa through the trans-Saharan trade routes, or alternatively by means of the Greek colonization, although the introduction of the gene into Sicily during the Muslim invasion cannot be excluded. The overall Hb S gene frequency in Sicily is

2%: it is concentrated on the eastern and southern coasts, reaching frequencies as high as 13% in certain villages. The incidence of these genes is roughly over 5%. Therefore, the most common conditions observed among the patients are S thal. Whereas sickle cell anemia (SS) is less frequent as a result of the domestic migration from Sicily and Southern Italy towards Northern Italy. Sickle cell disease has spread over all of Italy .Moreover recent immigration from foreign countries, in particular from Africa, has contributed to the further diffusion of the disease in Italy. In a recent survey of SCD in Italy, they found that sickle cell disease is widely distributed in Italy. S thal was the most frequent form (Russo and Schiliro , 2014).

1.2.9 Hemoglobinopathies in Africa:

More than 1.07% of Africans suffer from sickle cell anaemia (SCA). In some parts of the African continent, sickle cell anemia has been found to affect 1 in 60 newborn infants, giving a prevalence of (2%), whilst the sickle cell trait range between 10% to 40% across wet equatorial Africa and decreases to less than 2% in the dry parts of Northern and Southern Africa., the African continent has been regarded as the epicenter of sickle cell disease with an annual estimated number of 200,000 new born affected by sickle anemia (Diallo *et al*, 2002)

1.2.9.1 Hemoglobinopathies in Nigeria:

In University of Uyo Teaching hospital (UUTH), Uyo,Akwa Ibom state, Nigeria, eight thousand and ninety seven Haemoglobin genotype tests carried out over a five year period were analysed:

6376 (78.7%) of these were HbAA, 1580 (19.6%) HbAS, 121 (1.5%) HbSS,while HbAC and SC accounted for 16 (0.2%) and 4 (0.04%) respectively. The ratios of Hb AA to Hb AS, HbAA to HbSS and HbAA to HbAC were 4:1, 52:1 and 400:1 respectively. Of the 8097 subjects (Abah *et al*, 2010)

1.2.9.2 Status of sickle cell disease in Uganda:

Sickle cell anemia was first described in Uganda in 1945 by Trowel. The studies undertaken in the late 1940s indicated that the prevalence of sickle cell trait varied among Ugandan tribes, ranging from 1-5% among Karimojong, Bakiga, Banyankole and Bahima, 16-20% among Baganda, Iteso, Acholi and Banyoro, 20-28% among Basoga, Bagisu and Lugbara and 45% among Baamba which is believed to be the highest in the whole world. Of the 900,000 thousand children born annually in Uganda8 approximately 2.8% have sickle cell aneamia (Lehmann and Raper, 1949).

1.2.10 Sickle cell disease in Sudan:

Hb variants are known to be prevalent in Sudan and it is very well documented among people from Khartoum (Elderdery *et al*, 2008), Kordofan (*Mohammed et al, 2006*), Blue Nile (Ahmed *et* al, 1986*)* and eastern states(Gibreel *et al*, 2014). In the Kordofan regions Hb S (α2 /β2 6val) has also reported among the Albagara, an Afro-Arab constellation of tribes with a predominant African descent (Bayoumi *et al*, 1985). In a sub group of Albagara, Misseria, studies showed the prevalence of SCD to be 30%, 16% among immigrants from the Blue Nile province (Ahmed *et al*, 1986), and 18% among Nilotes in the south of Sudan (Foy *et al*,

1954).Furthermore, Hb disorders are also known to be prevalent in the Khartoum area The supposition is that this variety in the population will be accompanied by variation in Hb types as different types of Hb are specifically prevalent in certain tribes as mentioned above(Omer *et al*, 1972).

A survey study was carried out recently among the different ethnicities in Eastern Sudan, Beja Tribes and found that about 0.6% babies were HbAD (Gibreel *et al*, 2014).

1.3 Rationale:

There are differences in the incidence of haemoglobinopathesis genes between people of different ethnic origins. Determination of these genes in North Darfur State is useful because data may be base for future studies, there is no previous study, also due to the medical importance of the haemoglobinopathesis and its consequences of morbidity and mortality which companied by several crisis. Hb abnormality needed diagnostic challenge particularly in multiethnic communities and it would be extremely beneficial to predict its genetic behavior.

North Darfur state has been chosen as the study area, because of its position and its multi ethnicity with a blend of almost all the Sudanese tribes. Additionally, there are immigrants from the West Africa and other region, tribes who moved and settled there. The supposition is that this variety in the population will be accompanied by variation in Hb variant as different Haplotypes of Hb S are specifically prevalent in certain ethnicities.

A new atypical β^S haplotype (Sudan haplotype) was discovered recently in Sudanese patients with sickle cell anaemia. Therefore, further researches in S gene in Sudan are crucial to know more about this haplotype in terms of its clinical symptoms and crisis (Elderdery *et al*, 2012).

1.4.1 General Objective:

Determine the distribution of haemoglobinopathies among ethnic groups (tribes) of Northern Darfur state.

1.4.2 Specific Objective:

1- To determine haematological parameters (CBC) white blood cell count, red blood cell count, Hgb, hematocrit, mean corpuscular volume, mean corpuscular Hgb, mean corpuscular Hgb content and platelet count in North Darfur state.

2- To determine common β globin haplotypes in North Darfur state.

3- To determine the effect of haemoglobinopathies on Hgb concentration level.

4- To find new variant Hb in North Darfur state.

5- To detect the frequency of haemoglobinopathies in every tribe and locality in North Darfur.

CHAPTER 2

2.1 Subject and Methods

2.1.1 Study design:

This is cross section, prospective study; it was a community based study which was conducted in Northern Darfur state from July 2012 to August 2015, to determine the prevalence of haemoglobinopathies among population of Northern Darfur State.

2.1.2 Study populations:

Following written informed consent 666 Individuals were recruited randomly from all localities of the Northern Darfur. All the main Northern Darfur tribal groups are represented (all Northern Darfur population included).

To satisfy the main objective of the study, the total number of subjects required is calculated using the formula:

$$n = 4[\underline{\pi plan(1-\pi plan)}]z^{2}1-\alpha/2$$
$$\text{Ш}^2$$

Where

n the required sample size.

πplan the anticipated population proportion.

α the significant level.

Z 1-α/2 the value from the normal distribution related to and representing the confident interval.

Ш the width of the confidence interval.

we assume the anticipated population proportion π plan is equal to 524.

2.2 Methods:

2.2.1 Collection of data and blood samples:

Data were collected using structural interviewing and questionnaire to collect demographic information age, sex, ethnicity (tribe) and family history. Approval from ministry of health was obtained. Five ml venous blood samples (with K_2-EDTA) were collected from each participant, then complete blood count (CBC) and haemoglobin electrophoresis, by Capillary electrophoresis (CE) was determined and then the samples were stored at -20 C for the analysis of haplotypes.

2.2.2 Procedure of complete blood count:

1- The reagents for operation were checked.

2- Then the power was switched on.

3- Auto rinse and background check were automatically performed.

4- Then three level of control (low count, normal and high) were applied.

5- Whole blood mode of analysis was selected.

6- Sample numbers were introduced by pressing sample number keys.

7- Then enter key was pressed.

8- After that sample was mixed carefully.

9- The sample was bringing in close contact with sample probe.

10- Then start key was pressed and the required volume of blood was aspirated.

11- The sample was removed.

12- Result was displayed in the screen and was printed out.

2.2.3.1 Hemoglobin Electrophoresis:

Capillary zone electrophoresis Minicap Flex Piercing (CZE) was used in this study to separate the Hb variants in whole blood (Joutovsky and Nardi, 2004, Kutlar, 2007). CZE is an alternative to High performance liquid chromatography (HPLC) for first intention haemoglobinopathies screening In addition to the potential gains in efficiency (34 samples/hour on CZE versus 6 samples / hour on HPLC), CZE offers improved peak resolution of hemoglobin A2, especially in the presence of hemoglobin S, and is equivalent to or better than HPLC in diagnosing hemoglobinopathies and thalassemia. CZE analysis is rapid and reproducible, and capable of identifying certain Hb variants such as HbS and HbC with similar migratory paths in Cellulose acetate electrophoresis (CAE). CZE has ability to separate hemoglobin C and E from the A_2 hemoglobin. Also there is Slight difference of migration between S and D hemoglobin allowing an easy identification by overlaying with a memorized reference curve. Perfect individualization and focalization of F hemoglobin between A and S allow a precise quantification (Joutovsky and Nardi, 2004, Ridley *et al*, 2005).

2.2.3.2 Procedure of hemoglobin electrophoresis:

1- The blood cells were let for several hours to form sediment at 2—8 *°C* or centrifuge the blood sample at 5000 rpm for 5 minutes.

2- The maximum volume of plasma was Discarded carefully (less than 3ml of plasma).

3- Then the blood was vortex for 5 seconds.

4- Distilled or deionized water in the water vial was changed and inserted in it is position.

5- The level of MINICAP wash solution vial was verified and inserted in it is position.

6- Sufficient number of reagent cups for the analyses in the reagent cup holder was inserted.

7- Bottle for biological wastes was emptied

8- The level of reagents (Hemoglobin buffers) was verified.

9- Both Minicap and computer was switched on

10- Hemoglobin program was selected.

11- Normal Hb A2 control was placed into a new hemolysing tube and labeled with the specific barcode for normal Hb A2 control, in position No 28 of carrousel.

12- 5 mL of MINICAP hemoglobin hemolysing solution in a tube labeled with the specific barcode for hemolysing solution was placed in position No 27 of the carrousel sample rack (5ml of hemolysing solution is sufficient to analyze 8 samples/controls).

13- The door was closed for analysis normal HbA2 control.

14- The normal HbA2 control was removed at the end of analysis and curve verification.

15- Blood sample tubes were placed (after plasma removal) in positions 1- 26 on the carrousel.

16- The door was closed to launch the analyses.

17- Result was displayed in the screen and was printed out.

2.2.4.1 DNA extraction procedure:

1- A total of 200 µL of a blood was placed into 1.5 ml appendorf tube that contains 1 ml red blood cells lysis buffer, and was vortex

occasionally for few minutes then incubated at room temperature for 30 minutes.

2- Then centrifuged at 3500 rpm for 5 minutes.

3- The supernatant was discarded and the process was repeated till white pellet was formed.

4- Then 1 ml of white blood cell lysis buffer was added, vortex and incubated at room temperature for 15 minutes.

5- Then was centrifuged at 3500 rpm for 5 minutes. And the supernatant was removed.

6- 200 µL of Insta Gene matrix (Chelex) was added to the remaining white pellet and was vortex.

7- The mixture was incubated at 56°C for 15 minutes, then at 100 °C for 8 minutes.

8- Then was centrifuged at 10.000 rpm for 3 minutes and the supernatant was placed in Eppendorf tube.

9- DNA concentration and purity was measured by Gene Quant machine and was stored at-20°C for PCR analysis.

2.2.4.2 DNA quantitation procédure :

1- In a 1.5 ml separate Microfuge tube 10µl DNA to 90µl H_20 was mixed and vortex.

2- The solution was left to stand for 10 minutes at room temperature to insure complete diffusion of DNA throughout the solution, this represented 1/10 dilution , Ideally the reading should be between 0.2 and 1.

3- Equal dilution of EB Buffer was prepared as a blank.

4- The machine was switch on.

5- The DNA dilatation was placed in clean cuvette into the cuvette holder.

6- Set-up key was pressed, 320 nm and dsDNA was selected.

7- Cuvette was inserted and removed rapidly.

8- The DNA concentration and purity was read.

2.2.4.3 DNA and reagents storage conditions:

Extracted Genomic DNA was stored at -20°C, for processing thawed and centrifuged again to pellet down any reaming Chelex and kept on ice cryo-rack, at the same time stock primers and ready master mix were kept on ice cryo-rack for thawing and sterile PCR water was brought out form refrigerator and aliquote on 1.5 ml tube and kept as above

2.2.5 Primer preparation:

Each of the upstream and downstream primers were prepared by adding 10µl of each stock primer (100 µM) to 90µl PCR water and aliquoted in 0.5 ml PCR polypropylene tube to yield a concentration of 10µM, and the solution was mixed carefully using sterile tips to ensure the homogeneity, table 2.1 showed the primers and digestion of β globin gene by endonucleus enzymes.

Ta ble 2.1: The primers and digestion of β globin gene by endonucleus enzymes (Rahimi *et al*, 2003)

	Region name	Primers sequences	Annealing	PCR products size	Digested fragments
1	HindII 5' to €"	TCTCTGTTTGATGACAAATTC AGTCATTGGTCAAGGCTGACC	55	760	314/446
2	XmnI 5' to Gy	AACTGTTGCTTTATAGGATTTT AGGAGCTTATTGATAACCTCAGA C	55	650	450/200
3	HindIII within IVS II Gy	AGTGCTGCAAGAAGAACAACTA CC CTCTGCATCATGGGCAGTGAGCT C	65	323	225/98
4	HindIII within IVS II Ay	ATGCTGCTAATGCTTCATTAC TCATGTGTGATCTCTCAGCAG	55	635	327/308
5	HindII 3' to β	GTACTCATACTTTAAGTCCTAAC T TAAGCAAGATTATTTCTGGTCTC T	55	914	480/434
6	AvaII within IVS II β	GTGGTCTACCCTTGGACCCAGAG G TTCGTCTGTTTCCCATTCTAAACT	65	328	220/108

2.2.6 PCR procedure:

1- Samples and reagents were brought out from the freezer and kept on ice in a frozen cryo-rack during assembly procedure.

2- A4 worksheet with PCR samples data was recorded for each sample to be tested.

3- The amount of each reagent was calculated according to the number of samples to be processed with an extra one more samples than actually being tested to compensate for retention of solution in pipette tips and tube for example if total number of samples is 10, make enough mix for 12 (table 2.2) showed that.

4- PCR reagents, except for samples DNA, were added in the order listed on the worksheet, adding water first and the primers last.

5- All reagents were kept in a frozen-cryo-rack during mixing and returned to the freezer immediately after use, caps were closed tightly.

6- Specified volume of sample was loaded into an appropriately labeled ready mix tube (Maxime PCR premix Kit) table 2.3 showed the component of ready master mix.

7- The tips were always changed and was avoided touching the side tube and capped to avoid contamination.

8- 5 µl of genomic DNA was added into ready prepared master mix for amplification of β globin gene.

9- Positive and negative control was included.

10- Samples was sealed carefully and placed onto PCR machine immediately.

11- Program was started with accurate Temperatures profile, showed in table 2.4

12- After PCR was finished, tubes were spine briefly and stored at -20°C.

Table 2.2: Calculation the amount of the PCR component

Reagents	Volume (µl)	Sample s (X) µl
H_2O	13.0	13X
Forward primer	1.0	1X
Reverse primers	1.0	1X
Master mix	5.0	5X

Table 2.3: Ready Master Mix components

Components
i-TaqTM DNA polymearase 5U/µl
dNTPs
Reaction buffer (10x)
Gel loading

Table 2.4: The Temperatures profile

Profile	Temperature	Cycles
Initial denaturation	94°C/ 6 min	1 cycle
Denaturation	94°C/1 min	35 cycles
Annealing	55°C or 65 °C/ 1 min	
Extension	72°C/2 min	
Final extension	72°C/ 8 min	1 cycle
Hold at	4°C/ ∞	

2.2.7 Genotyping of β globin haplotypes variants alleles by restriction fragment length polymorphisms (RFLP):

Ten microliter of the amplified products of β globin gene was digested with 5Units of special enzyme in a total volume of twenty microliter mixture, incubated at 37°C over night, The beta haplotypes were constructed from the absence (-) and presence (+) of each of the six restriction enzyme sites in individuals with HbSS of the sample studied, table 2.1.

2.2.8 Visualization of the β haplotypes fragments:

15 µl of the digested products were run on 2% agarose gel dissolved in 1X TBE buffer, eletropherosed for 1 hour at 80v, and the fragments were visualized on UV trans-illimantor and photographed, table 2.5 and table 2.6 showed the interpretation and identification of haplotypes.

Table 2.5: interpretation of the fragments in the agarose gel

-ve	+ve	+ve/-ve
Uncut	Cut	Uncut/cut
328	220/108	328/220/108

Table 2.6: Identification of haplotypes

	HindII	XmnI Gy	HindIII Gy	HindIII Ay	HindII	AvaII
Benin	_	_	_	_	+	+
Arab-Indian	+	+	+	_	+	+
Cameron	-	-	+	+	+	+
Senegal	_	+	+	_	+	+
Bantu	_	_	+	_	_	+
Bantu A1	_	_	_	_	_	+
Bantu A2	+	_	_	_	_	+
Bantu A4	_	+	+	_	+	_
Bantu A6	_	_	+	+	_	+

2.2.9 Ethical considerations:

A Study was approved from ethical committee of the Sudan University of Science and technology, consents were taken from the participations after they had been informed about the procedure of blood collection and aim of study, they were used that data will not used for any other purpose than this study.

2.2.10 Data analysis

The data was entered and analysed using soft-ware package for social science 15.0 (SPSS 15.0). The open source epidemiologic statistic programme for public health version 2.2.1 (Openepi) was used for the comparison of these study populations and 95% confidence interval was used and p value of 0.05 was considered statistically significant.

CHAPTER 3

3.1 RESULT

Out of hundred and sixty six individuals enrolled in the study, with mean age 21.7 ± 18.3 years for female and 20.9 ± 15.4 years for males, the age ranged between 15 months to 35 years for AS individuals. It was 11 months to12 years for the SS patients. There were 369 (55%) females and 297 (45%) males. table 3.1 and 3.2 showed the mean ages and gender distribution.

Samples were collected from fifty nine tribes which represented most of Northern Darfur tribes, from all localities of the State figure 3.1 and table 3.3 showed the frequencies of the tribes, table 3.4 showed the frequencies of the localities.

Four variants of Hb were detected. These were Hb AA (86.94%), Hb AS (10.51%), Hb SS (1. 95%) and Hb AD (0.6%), Hb AA (adult Hb or normal Hb) was predominant. Hb SS and Hb AS had a higher frequency than other abnormal Hb, table 3.2 showed the percentage of the haemoglobin variants, (table 3.5 and 3.6) showed the distribution of hemoglobin variant among the study tribes and the localities.

The homozygous form of HbSS was found in four tribes, stratified as follows: Housa tribe 10.26 % followed by Fur 3.1%, Bartey 2.7% and Zagawa 1.74%, (table 3.7 , Figure 3. 2) showed that.

The heterozygous form (HbAS) was found in tribes of Northern Darfur state, as follow: 30.7% Keneen, 23.1% Barno, 14.3% Rezegat, 13.2% Fur, 12.8% Housa, 10.5% Ziadya, 9.6% Zagawa,

9.5% Bartey, 9.1% Tama, 7.4% Fallata and 5.4% Tongour,and other ten tribes 36.1%, table 3.5 and table 3.8 showed that.

Hb AD another Hb phenotype This type was found only in four ethnic groups of Northern Darfur, Gemer, Ziadya, Bartey and Zagawa tribes at 33.3, 5.3%, 1.4% and 0.87% respectively, table 3.5 and table 3.9.

In contrast, HbS was not found in four endogenous tribes of Northern Darfur, Medob, Awlad Mana, Gawama and Shrafa.

The result of complete blood count showed that the mean haemoglobin concentration was 12.6±2.19 g/dl and there about ninety four (14.1%) of the participants are considered as anaemic with Hb concentration lower than 10 g/dl. Mean PCV 38.5%, RBCs count 4.7±0.8× 10^{12}/L. Mean MCV 84.9 fl, one hundred forty seven of the participants (22.1%) with MCV less than 80 fl (microcytic cells) and eleven (1.65%) with MCV more than 100 fl (macrocytic cells). Mean MCH 27.9 pg , MCHC 32.8 g .Mean TWBCs 6.9±2.2×10^3/µL ,there are one hundred and forty of the participants (21%) was leucopenia with TWBCs less than 4 ×10^3/µL and fifty eight (8.7%) was leucocytosis with TWBCs more than 11×10^3/µL. And the mean Plts count was 265±84×10^3/µL, eighty seven of the participants (13.1%) was thrombocytopenia with Plts count below 150×10^3/µL and forty eight (7.2%) was thrombocytosis with Plts count more than 450 ×10^3/µL, figure 3.3, table 3.10, table 3.11, table 3.12, table 3.13 and table 3.14 showed the haematological parameters. HbSS had consistently low blood Hb concentration, ranging from 4.7 to 8.2 g/dl with a mean of Hb 6.95±1.04, mean MCV 92.19±7.44 and high mean TWBCs 18.92±14.94. The Hb F level ranged from

0.4% to 4.8% .The means Hb in Hb AS individuals was 11.8 g/dl, and in Hb AD individuals was 12.4 g/dl, in table 3.15 and table 3.16.

Haplotypes was assigned unequivocally to thirteen chromosomes; four of the five typical β^s globin haplotypes were identified. The most frequent was the Cameroon (84.6%) followed by Benin (53.8%), Bantu (46.2%) and Senegal (154%). The Indian – Arab haplotypes was not observed Figure 3.6 show the frequency of the haplotypes.

Table 3.1: Mean age of study group

Gender	Frequency	Mean Age (years)
Male	297 (45%)	20.9 ± 15.4
Female	369 (55%)	21.7 ±18.3

Table 3.2: gender distribution M: F of the sample size as well as Hb variant

Hb_Electro		Gender			%
		Female	Male	Total	
N	Frequency	315	264	579	
	percent	54.40%	45.60%	100%	86.94%
S	Frequency	44	26	70	
	percent	62.86%	37.14%	100%	10.51%
SS	Frequency	9	4	13	
	percent	69.23%	30.77%	100%	1.95%
D	Frequency	1	3	4	
	percent	25%	75%	100%	0.6%
Total		369 (55%)	297 (45%)	666	

Table 3.3: Frequency of study group according to tribes

Tribe	Frequency	%
ZAGAWA	115	17.27%
TAAYSHA	5	0.75%
FUR	129	19.37%
TONGOUR	56	8.41%
HAWARA	10	1.50%
HOUSA	39	5.86%
DAJO	2	0.30%
MOSABAAT	5	0.75%
MEDOB	15	2.25%
KENANA	2	0.30%
MASALEET	9	1.35%
AWLAD MANA	7	1.05%
BARTEY	74	11.11%
NUBA	1	0.15%
ZIADYA	19	2.85%
FLLATA	27	4.05%
MEMA	8	1.20%
HABANEYA	5	0.75%
SHOYHAT	2	0.30%
KAWAHLA	1	0.15%
KENEEN	13	1.95%
ETAFAAT	1	0.15%
BARNO	13	1.95%
TAMA	11	1.65%
BRAGO	8	1.20%
BNI HESAIN	2	0.30%
MARARET	4	0.60%
GEMER	3	0.45%
REZEGAT	7	1.05%
BNI HLBA	1	0.15%
SLEHAB	1	0.15%
KROBAT	4	0.60%
MESERIA	10	1.50%
AWLD RASHED	2	0.30%
ARAB BASHER	3	0.45%
HEMEDIA	1	0.15%
BNI OMRAN	2	0.30%
GALEIA	1	0.15%

DADENGA	1	0.15%
GAWAMA	7	1.05%
SLAMAAT	1	0.15%
SHYGIA	1	0.15%
DANGLA	3	0.45%
MAALYA	1	0.15%
SHRAFA	6	0.90%
HAMR	1	0.15%
TKAREER	1	0.15%
ASERAA	1	0.15%
AWLAD ALREEF	2	0.30%
KHOZAM	2	0.30%
QURAN	4	0.60%
BNI MANSOOR	4	0.60%
BAZAA	5	0.75%
BAGRMA	1	0.15%
DAR HAMID	1	0.15%
WAHEIA	1	0.15%
MANASEER	1	0.15%
KALEMBO	1	0.15%
KTENGA	3	0.45%
Total	666	100%

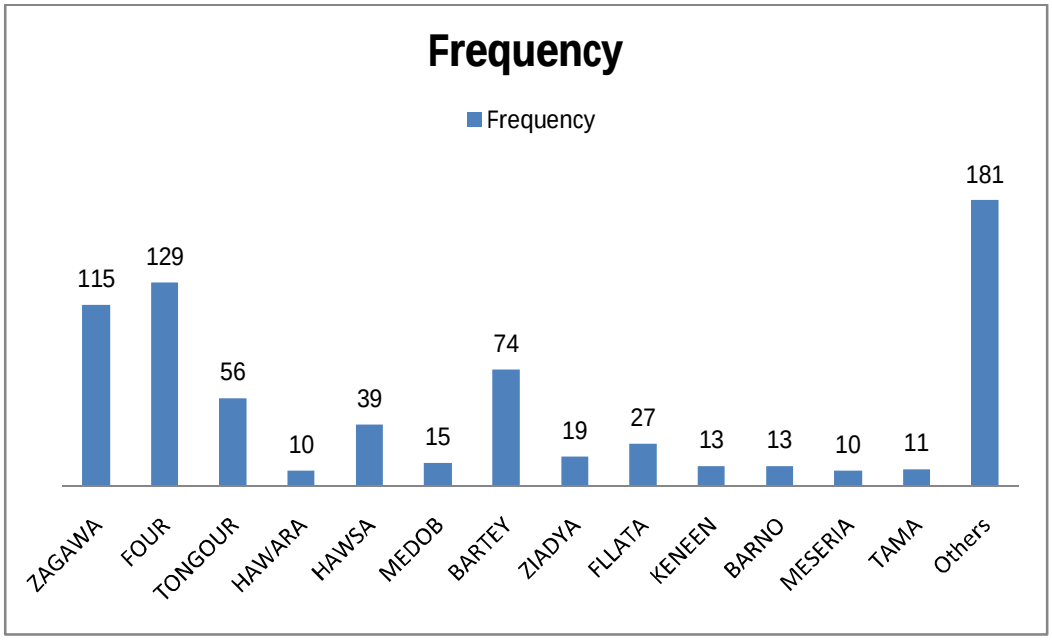

Figure 3.1: Frequency of study group for tribes

Table 3.4: Frequency of localities

Locality	Frequency	%
ALFASHER	195	29.28%
SARF-OMR	15	2.25%
KALMENDO	15	2.25%
ALMALHA	17	2.55%
ALKOMA	19	2.85%
OMBARO	26	3.90%
ALTEWSHA	12	1.80%
ALWAHA	33	4.95%
UM-KADAD	14	2.10%
ALLAYEED	33	4.95%
ALSERAF	21	3.15%
DAR-ALSLam	41	6.16%
TAWELA	56	8.41%
KUTOM	40	6.01%
MALET	17	2.55%
KORMA	52	7.81%
KABKABYA	10	1.50%
ALTENA	33	4.95%
KARNOY	17	2.55%
Total	666	100%

Table 3.5: Distribution of hemoglobin variant among the study tribes

Tribe	Hb_Electro				
	N	S	SS	D	Total
	Frequency	Frequency	Frequency	Frequency	
ZAGAWA	101	11	2	1	115
TAAYSHA	5	0	0	0	5
FUR	108	17	4	0	129
TONGOUR	53	3	0	0	56
HAWARA	8	2	0	0	10
HAWSA	30	5	4	0	39
DAJO	2	0	0	0	2
MOSABAAT	4	1	0	0	5
MEDOB	15	0	0	0	15
KENANA	2	0	0	0	2

MASALEET	9	0	0	0	9
AWLAD MANA	7	0	0	0	7
BARTEY	64	7	2	1	74
NUBA	1	0	0	0	1
ZIADYA	16	2	0	1	19
FLLATA	25	2	0	0	27
MEMA	5	3	0	0	8
HABANEYA	5	0	0	0	5
SHOYHAT	1	1	0	0	2
KAWAHLA	1	0	0	0	1
KENEEN	9	4	0	0	13
ETAFAAT	0	1	0	0	1
BARNO	10	3	0	0	13
TAMA	10	1	0	0	11
BRAGO	8	0	0	0	8
BNI HESAIN	1	1	0	0	2
MARARET	3	1	0	0	4
GEMER	2	0	0	1	3
REZEGAT	6	1	0	0	7
BNI HLBA	0	1	0	0	1
SLEHAB	1	0	0	0	1
KROBAT	4	0	0	0	4
MESERIA	10	0	0	0	10
AWLD RASHED	1	1	0	0	2
ARAB BASHER	2	0	0	0	3
HEMEDIA	0	1	0	0	1
BNI OMRAN	2	0	0	0	2
GALEIA	1	0	0	0	1
DADENGA	1	0	0	0	1
GAWAMA	7	0	0	0	7
SLAMAAT	1	0	0	0	1
SHYGIA	1	0	0	0	1
DANGLA	3	0	0	0	3
MAALYA	1	0	0	0	1
SHRAFA	6	0	0	0	6
HAMR	1	0	0	0	1
TKAREER	1	0	0	0	1
ASERAA	1	0	0	0	1
AWLAD ALREEF	2	0	0	0	2
KHOZAM	1	1	0	0	2

QURAN	4	0	0	0	4
BNI MANSOOR	4	0	0	0	4
BAZAA	5	0	0	0	5
BAGRMA	1	0	0	0	1
DAR HAMID	1	0	0	0	1
WAHEIA	1	0	0	0	1
MANASEER	1	0	0	0	1
KALEMBO	1	0	0	0	1
KTENGA	3	0	0	0	3
Total	579	70	13	4	666

Table 3.6: Distribution of hemoglobin variant according to localities

Locality	Hb_Electro				
	N	S	SS	D	
	Frequency	Frequency	Frequency	Frequency	TOTAL
ALFASHER	160	26	6	3	195
SARF-OMR	13	2	0	0	15
KALMENDO	13	2	0	0	15
ALMALHA	17	0	0	0	17
ALKOMA	14	3	1	1	19
OMBARO	25	1	0	0	26
ALTEWSHA	8	3	1	0	12
ALWAHA	30	3	0	0	33
UM-KADAD	14	0	0	0	14
ALLAYEED	33	0	0	0	33
ALSERAF	20	1	0	0	21
DAR-ALSL	27	11	3	0	41
TAWELA	49	7	0	0	56
KUTOM	38	2	0	0	40
MALET	17	0	0	0	17
KORMA	47	5	0	0	52
KABKABYA	9	1	0	0	10
ALTENA	30	3	0	0	33
KARNOY	15	0	2	0	17
Total	579	70	13	4	666

Table 3.7: Frequency of homozygous form of HbSS

Tribes	Frequency of HbSS	%
Housa	4	10.26 %
Fur	4	3.1 %
Bartey	2	2.7 %
Zagawa	2	1.74%

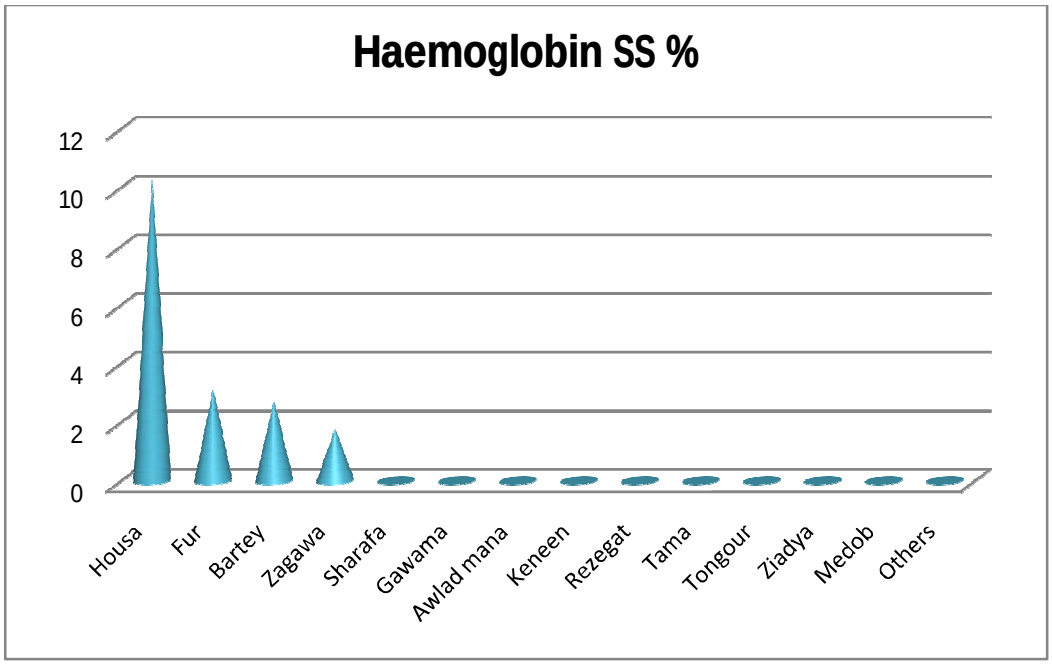

Fig 3.2: Frequency of homozygous form of HbSS

Table 3.8: Frequency of heterozygous form of HbAS

Tribes	Frequency of Hb AS	%
Keneen	4	30.7 %
Barno	3	23.1 %
Rezegat	1	14.3 %
Fur	17	13.2 %
Housa	5	12.8 %
Ziadya	2	10.5 %
Zagawa	11	9.6 %
Bartey	7	9.5 %
Tama	1	9.1 %
Fallata	2	7.4 %
Tongour	3	5.4 %
Others	12	36.1

Table 3.9: Frequency of Hb AD

Tribes	Hb AD Frequency	%
Gemer	1	33.1
Ziadya	1	4.2 %
Bartey	1	1.4 %
zagawa	1	0.8 %

Table 3.10: Mean hematological parameters

Parameter	Hb g/dl	PCV%	RBCs $\times 10^{12}$/L	MCV fl	MCH/pg	MCHC/g	TWBCs $\times 10^{9}$/L	Plts $\times 10^{9}$/L
Mean	12.6±2.19	38.5	4.7±0.8	84.9	27.9	32.8	6.9±2.2	265±8×

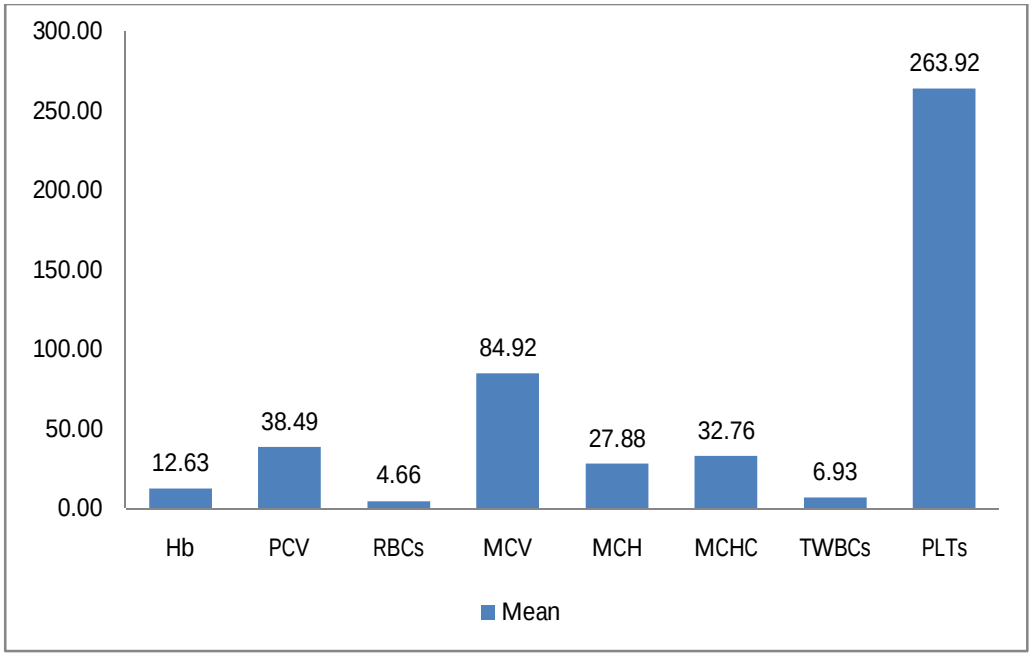

Figure 3.3: Mean hematological parameters

Table 3.11: Distribution of Hb among the group study

HB_gldl	Frequency	%
Below 10	94	14.11%
Above 10	572	85.89%
Total	666	100%

Table 3.12: Distribution of MCV among the group study

MCV_fl	Frequency	%
Less than 80	147	22.07%
80 - 100	508	76.28%
above 100	11	1.65%
Total	666	100%

Table 3.13: Distribution of TWBCs among the group study

TWBCs_× 10^9/L	Frequency	%
abnormal(<4)	140	21.02%
Normal	468	70.27%
Abnormal(>11)	58	8.71%
Total	666	100%

Table 3.14: Distribution of Plts among the group study

PLTs_× 109/L	Frequency	%
Less than 150	87	13.06%
150 - 450	530	79.58%
above 450	48	7.21%
Total	666	100%

Table 3.15: Mean Hb Concentration in Hb variant

AS	SS	AD
Mean Hb	Mean Hb	Mean Hb
11.76 g/dl	6.95 g/dl	12.35 g/dl

Table 3.16: hematological parameter in HbSS

Variable	Total	
	Average	SD
Hb g/dL	6.95	1.04
TWBCs × 10^9/L	18.92	14.94
MCV/ fl	92.19	7.44

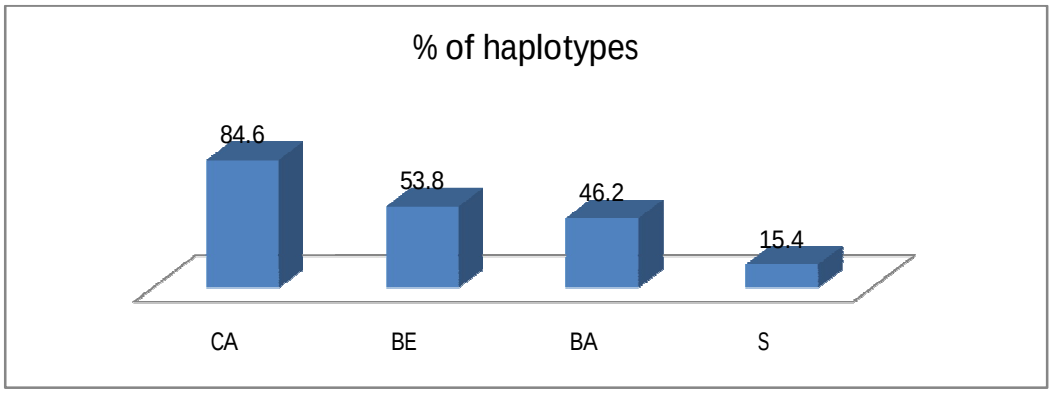

Figure 3.4: Frequency of the haplotypes among the study group

CHAPTER 4

DISCUSSION, CONCLUTION AND RECOMENDATIONS

4.1 Discussion

Prevalence of haemoglobinopathies among the tribes comprising the Northern Darfur population is the primary issue being addressed; this work may act as a pilot study for further research. It was first carried out in Northern Darfur state to assess the prevalence of abnormal Hb amongst the tribes of Northern Darfur state. The overall age of individual with HbSS was found not exceed fifteen years, which may indicate that the children are more affected by the disease than other age groups. Out of hundred and sixty six individuals was enrolled in the study; the male to female ratio was 1:1.2. Most Northern Darfur state tribes were represented within this study, from all localities of the state, that was meant to see if different frequencies were present in different areas. Due to the number of participants per tribe was small , this aim has been reached only partially.

The present study demonstrates the ability of CZE to detect Hb and globin chain variants and indicate its potential value as an aid in the clinical diagnosis of congenital hemoglobinopathies. Eighty three (12.5%) of participants were found with HbSS and HbAS, from these cases, seventy individual (10.51%) had the AS [SCT] gene and the remaining thirteen had the SS gene (1.9 5%). This distribution is lower than it was reported in eastern Sudan, Beja tribes (Gibreel *et al*, 2014*)*, but it is higher than the

distribution were reported in Khartoum area (Elderdery *et al*, 2008, Elderdery *et al*, 2011) and Blue Nile *(* Ahmed *et al*, 1986).

In contrast, Hb AS findings in Arabic countries were as follows: Saudia Arabia 2-27%, Bahrain 11.2 %, Kuwait 6 %, Jordan 0.44%, UAE 1.9% and Yemen 0.95% (El-Hazmi *et al*, 1994, Bashir *et al*, 1991). Thus, the carrier state (SCT) was higher in Saudia Arabia and Bahrain than the prevalence in the current study. Approximately 8% of the Afro-American population are heterozygous for HbAS (Boulware 1998), which was slightly lower than reported in this study.

The prevalence of HbSS in Arabic counties has been detected in varying degrees for instance in the Eastern province of Saudia Arabia 2.6% (Jastaniah 2011), Bahrain 2.1% (Mohammed *et al*, 1992) and Libya 0.37% (Jain 1985), therefore the frequency of HbSS in current study was lower than findings in Saudi Arabia and Bahrain *(* Mohammed *et al*, 1992, Jastaniah 2011) and higher than that reported in Libya (Jain 1985).

Globally, the highest frequency of HbSS was reported in Africa 10.6% (Modell and Darlison 2008); the disorder affecting 11.3% in the Sokoto region of Nigeria and 5% of the total population of The Democratic Republic of the Congo(R. A U *et al*, 2007, Tshilolo *et al*, 2009). These findings are both higher than was found in this study.

The prevalence of HbS among the patients studied was particularly higher when compared with reports of Hb S in Europe, where prevalence varies between 0.00 and 1.19% (Modell *et al*, 2007). And again high when compared to Afro-Americans 1 in 500 are homozygous Hb S (Boulware 2011).

Hb D disease is an autosomal recessive disorder that causes mild haemolytic anaemia. It is most common as heterozygous (HbAD) rather than Homozygous form (HbDD), which is rare (Itano 1951). The current study revealed that HbAD was found in four tribes of Northern Darfur state, this was the first time to be recorded in Northern Darfur state.

This abnormality was reported recently in Eastern Sudan, Beja tribes 3.4% (Gibreel *et al*, 2014) which was higher than found in this study. In contrast, HbAD was found among individuals who lived in India, Pakistan, Ireland, England, Holland, Australia, Iran, Turkey, and China (Agrawal *et al*, 2007, Balgir 2005). The prevalence of Hb-D trait in Uttar Pradesh was 3.1% (Agarwal *et al*, 1989), which is also higher than it was reported here.

Sickle gene was found with high prevalence in certain ethnic groups of Northern Darfur tribes with varying degrees for instance, higher frequency was found in Keneen tribe, and the lowest frequency in Tongour tribe, This elevation among certain tribes may be due to consanguineous marriage, as the high incidence of abnormal Hb among people who prefer to marry consanguineously is already very well known in many countries, including Sudan (Elderdery *et al*, 2011). Obtaining family history is therefore an important factor in gaining a fuller picture of the case. Medical history within the family is particularly important in communities where consanguineous marriage is preferred. Such awareness within communities will help to prevent carrier parents having children with the disease (Elderdery *et al*, 2011, Teeuw et al, 2014).

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 complication. SCT does not cause anaemia and if anaemia is present, it would be attributed to causes (such as iorn deficiency) other than sickling state. Sickle cell anaemia occurs in the homozygous state (HbSS) in which the Hb is nearly all HbS and remainder is HbF. There is no HbA, while in heterozygous sickle cell trait half or less of circulating Hb is HbS, the rest being normal HbA. Sickle cell disease (SCD) is an important cause of morbidity and mortality. The assessment of incidence and diagnosis of HbS married couples or in prenatal state is an important step towards reducing this gene distribution in future generation (Sears, 1978, Pedersen, 2002, Khoury and Massad, 1992). This gene was not found in some Northern Darfur tribes, may be due to smaller sample size of this tribes.

HbSS had consistently low blood Hb concentration, and high mean TWBCs, with low level of Hb F. Usually the anaemia is normocytic normochromic anemia with leucocytosis due to infections and haemolysis.

In Sudan, sickle cell anaemia was first reported in 1926 by Archibald (Archibald, 1926). Three foci of the disease have been described: Western Sudan, with a prevalence rate of up to 30% among the Baggara tribe (Vella, 1964), Southern, where prevalence rate up to 18% was found among the Southern Nilotes and in the Blue Nile province, central Sudan, a prevalence ranging from 0-5% among the indigenous population and up to 16% was found among the immigrant tribes in the area (Foy and Kondi *et al*, 1954,Ahmed et al, 1986).

In generally the clinical picture of patients with Hb S shows great heterogeneity even in the homozygous SS. Generally, severe

illness is seen in Africa while a mild form compatible with normal life is seen among Arabs of eastern Saudia Arabia. High Hb F level in patients with sickle cell anaemia have been found to be associated with mild clinical course of the disease, level of Hb F > 20% needed to protection from severe complication of the disease. The symptoms usually start after the age of six months but have been reported to present as early as three months after birth. Patients with sickle cell disease have an increased susceptibility to bacterial infections. Azactidine and hydroxoyurea have been used to increase Hb F concentration. Blood transfusion, analgesics and other supportive measures are all needed to alleviate the suffering of these patients. However penicillin has been found to be life saving in the patients under five years of age (Biedrzy, 2010). Patients with sickle cell anaemia were known not to survive beyond their 20^{th} birthday. However, improved understanding of disease and more effective use of penicillin and anti malarial prophylaxis at an early age have made remarkable improvement in the survival of patients with sickle cell disease. Allogenic bone marrow graft is the only cure presently available for sickle cell anaemia it is both full of risk and not available for most of the patients(Biedrzy,2010).

The values of hematological parameters are affected by a number of factors even in apparently healthy populations. These factors include age, sex, and ethnic background, body build, social and nutritional.

The hemoglobin concentration was similar to study done in Egypt 15.35 ±1.24 g/dl, but low than reference value that found in British population 150 ± 20 g/l(Bain *et al*,2011) .The mean RBC count in this study was agree with study done in Khartoum 4.6-5.6

$\times 10^6$/ µL (Cong *et al*,2003), and nearly to that found in British population 5.o × 10^6/µL (Lewis.S.M, *et al*, 2001), and Chinese population 5.13× 10^6/µL (Cong *et al*, 2003). But slightly low than reported in American population 6.0 × 10^6/µL (National Centre), and Emirate population 6.7 × 10^6/µL. The mean TWCs in the study population was similar to black population of the South Africa 5.6 ± 1.51×0³/µL (Badenhorst *et al*, 1995).But lowe than that found in British population 7 ± 3 ×10³/µL (Lewis *et al*, 2001). Leucocytosis was detected in some of the study population, most of them were HbSS patients, and it may be due to infections and haemolsis. The mean Platelet count in the study was near to that of black population of the South Africa which were 280×0³/µL (Badenhorst *et al*, 1995). And low than in Khartoum population 277.8±72.4 ×10³/µL(Cong *et al*,2003) and Turkish population 238.50±46.95 ×10³/µL (Tekkeşin *et al*, 2014).

Complete blood components investigation revealed that SCT in the study area, like other SCT individuals, were normal and not suffering or compiling from blood disorder, and if anaemia is present, it would be attributed to causes (such as iron deficiency) other than sickling state .

All the mean of haematological parameters were found within the normal range. The study shows a different pattern in the profile of hemoglobin values, this is because the study population was included AS trait, SS, AD and AA. The difference in mean hemoglobin values between AA and AS, as well as AD was statically not significant. (SS) patients they have extremely low Hb concentration.

Previously published studies indicate that Hb SS is common among Sudanese tribes, particular in western regions (Kordofan

and Darfur). Furthermore, haemoglobinopathesis are also known to be prevalent in the Blue Nile and Northern tribes. Currently, little is known about the frequency of the haplotypes background of beta mutation and its effect on blood profiles among the Sudanese. In this study the Cameroon haplotype was found to be the most frequent one followed by Benin then Bantu and the least one was Senegal. This result is in contrast to other studies in African population in which the Cameroon haplotypes has been reported at lower frequency in sickled patients. The present finding was in agreement with the frequency of those haplotypes found among Sudanese patients with Hb S gene. The Benin haplotypes was lower than that found in North Africa, some Arab countries. The Bantu haplotypes was found at the lowest frequency in current study, these was in marked contrast to studies reported from the Central African population, where over 93% of the patients were found to be homozygous for the Bantu type haplotypes(Elderdery *et al*, 2012). The haplotypes which found in Northern Darfur was similar or near to that of West Africa .The Arab-Indian haplotype was not detected in all study groups.

4.2 Conclusion:

1- The mean age of study group was (21.7 ± 18.3) years.

2- There were 369 (55%) females and 297 (45%) males enrolled in the study.

3- The frequency of Hb disorders was 13.06%.

4- A new Hb variant is apparent in Northern Darfur (Hb AD).

5- Four African haplotypes were identified.

6- The hematological parameters were found within normal range among the study population.

4.3 Recommendations:

Allocate budget for screening this health problem, by encouraging international funds to help those affected population as ways of avoiding the risk of SCA in the future.

Screening should be carried out among infants, pregnant women, adolescents, and young adults. This would promote the control and development of a rational strategy to manage haemoglobinopathies.

To minimize the inheritance of this disease it is better to perform premarital genetic counseling.

To achieve optimal outcome a center for sickle should be establish in Darfur area.

Also we recommended performance of similar study in other Darfur States.

The method used was CZE which is still working but we need more advanced studies with PCR for conformation.

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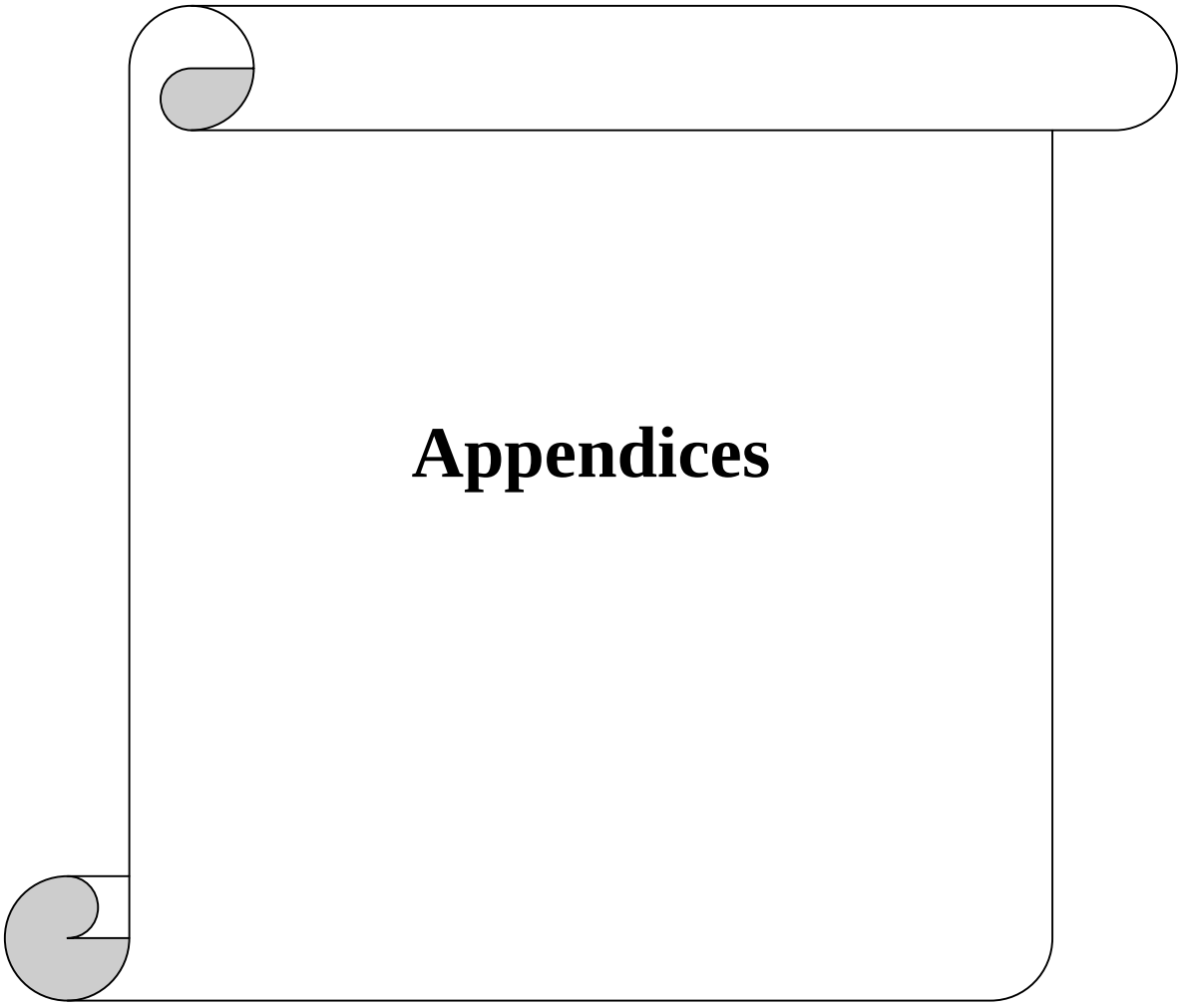


Appendices

Appendix (I) Questionnaire pro-forma for participants

Interviews and questionnaires were designed to collect demographic: age, sex, ethnicity (tribe), family history and clinical data. Results of FBC

Date of collection: ---------- Research ID number: ----------

Name of patient: ---------- Age (in year) ----------

Male ☐ hale ☐

- Tribe: ---------- Residence ----------
- Pregnant: Yes ☐ No ☐

CLINICAL INFORMATION

- Had you any of the following system

Jaundice Fatigue abili☐ Fever ☐ ☐

- Duration of illness: ----------
- Did you see a doctor? Yes ☐ No ☐
- Initial clinical diagnosis: ----------
- How many members of your family have anaemia? ----------
- Women ---------/--------Non pregnant women--------/------Pregnant------/-----
- Men---------/----------children----------/----------
- If any …… Diagnosis….....
- Lab Finding:
- Hb……gm/dl……. %
- PCV……l/l. WBC……cmm PLt……cmm
- MCV……fl MCH……pg MCHC……g/dl, RDW……fl
- Peripheral blood picture:

……
……
…….....……

Electrophoresis comment:

……

Appendix (II) Principle of Sysmex KX-21N analyzer:

Automated blood cell counter use a modifications of the traditional cyanmethaemoglobin method to measure Hb concentration. Automated machines have at least two channels for the cell counting .In one red cells and platelets may be counted and in the other red cells are lysed leaving white cells for analysis. Extra channels are often used for differential white cell counting and reticulocytes. There are two basic methods for cell counting and sizing: electrical impedance and light scattering.

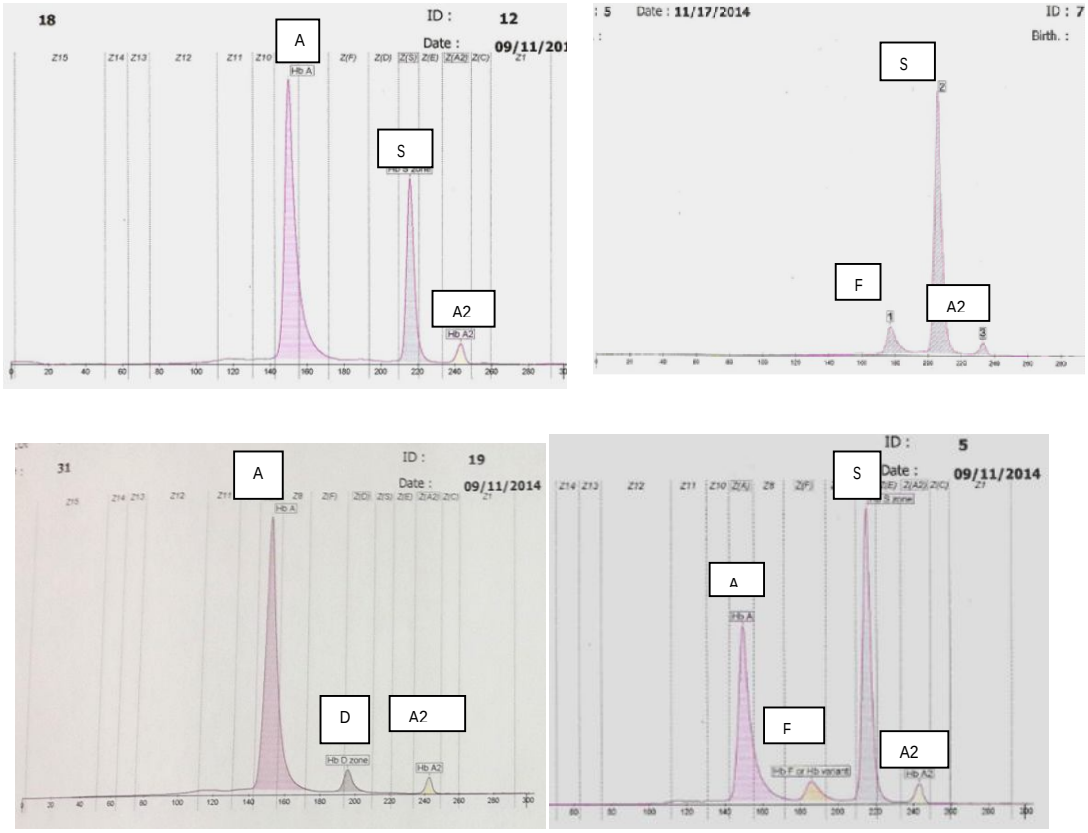
The electrical impedance method relies on blood cells being very poor conductors of electricity .Thus when the cells are passed in stream throw a narrow aperture across which an electrical current is maintained the individual cell create an increase in electrical impedance of a size proportional to the cell volume .In the light scattering method the cells deflect a beam of' light (often a laser beam) and a detector converts the scatter into pulses proportional to cell size. For sophisticated measurements such as the differential white cell count the two methods can be used together with addition of other modalities reliant on biochemical reactions and light absorbance.

The blood is diluted with isotonic diluents. These diluents have two functions, it keeps cells intact and it provides an electrolytic solution capable of 'conducting current. Blood cell analysis comprises diluting whole blood sample with a fluid which functions as diluents. The diluents, (reagent I) provides the ability to analyze portions of dilutes blood sample for different blood cell types, such as red blood cells, platelets, and when used with a lytic reagent (reagent 2), enumerate and differentiation the

leukocyte (WBC). The diluents (reagent 1) include chemical composition of organic buffers anesthetics, and germicides in a somatically balanced neutral solution, consisting of the following: 1- sodium chloride, which allows the diluents to become an electrolyte capable of conducting electrical current in an electronic analyzer and along with sodium sulfate and procaine hydrochloric acid, Provides buffer for pH balance and cell component stabilization (i.e. becoming an isotonic solution. stabilizing blood cell volume and reducing turbidity in the measurement of hemoglobin), 2-Dimethyllolurea a bacteriostatic antiseptic for product preservation against microbial growth.

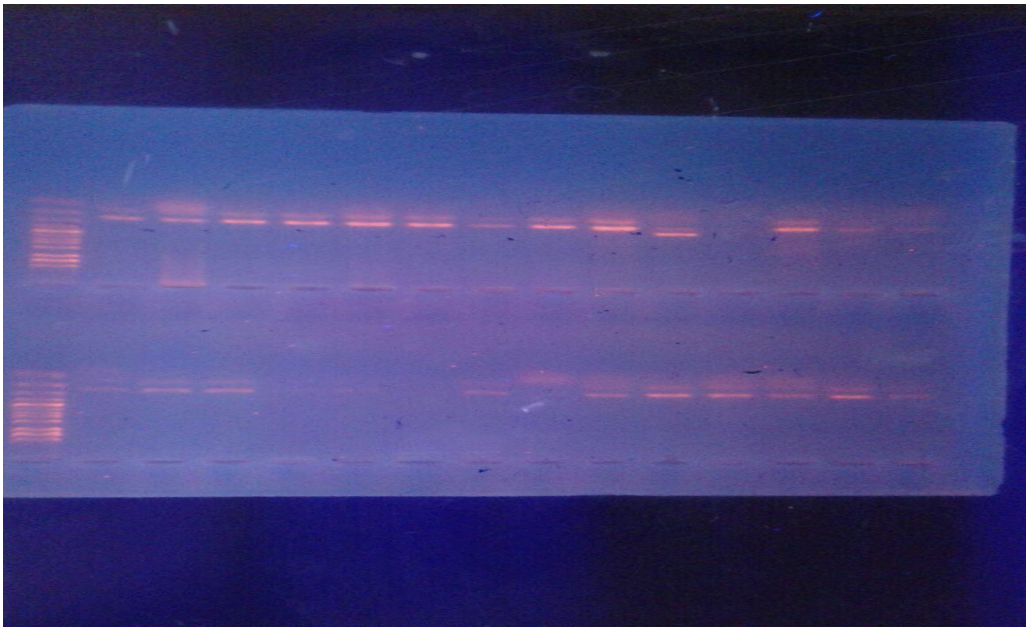
Appendix (III) Principle of the Capillary zone electrophoresis Minicap Flex Piercing (CZE):

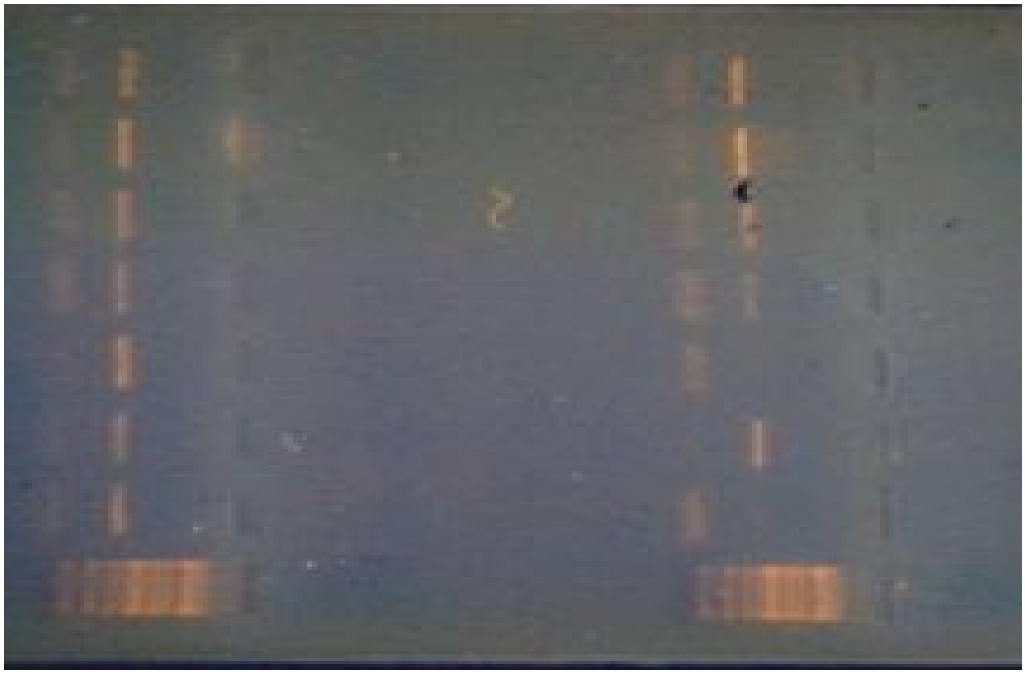
The Minicap system uses the principle of capillary electrophoresis in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH separation also occurs according to the electrolyte pH and electro osmotic flow. The Minicap system has capillaries functioning in parallel allowing tow simultaneous analyses for hemoglobin quantification. The required volume of blood were aspirated automatically, sample diluted with hemolysine solution and aspiration at the anodic end of the capillary. A high voltage protein separation is then performed and direct detection of the hemoglobin is made at 415 nm at the cathodic end of the capillary. By using alkaline pH buffer, normal and abnormal (or variant) hemoglobin are detected in the following order, from cathode to anode: A2, C, A2/O –Arab, E, S, D-punjab, G Philadelphia, F, A, Hope, Bart, J, N-Baltimore and H.



Hb variants by CZE. They are selected for having (a) HbAS + normal level of A_2 (A = 67%, S = 30% and A_2 3%); (b) HbSS + F and A_2 zones (S = 84.5%, F = 12.2% and A_2 3%); (c) HbAD (A = 91%, D = 6% and A_2 3%) and (d)) HbAS (A = 43.1%, S = 48.8% , A_2 = 2.9% and F = 5%).

Appendix (IV) An agrose gel electrophoresis pattern of some RFLP products of β gene cluster





Appendix (V) Identification of haplotypes

N0	Hinc II	XMn	GammG Hind111	GammA Hind111	¥B	Ava11		Hinc II	XMn	GammG Hind111	GammA Hind111	¥B	Ava11	**Haplotypes**	**Haplotypes**
1	-	-	+	+	+	+		-	-	-	-	-	+	Cameron	Bantu A1
2	-	-	-	-	+	+		-	-	-	-	+	+	Benin	Benin
3	-	-	+	-	+	+		-	-	-	-	+	+	Senegal	Benin
4	-	-	+	+	-	+		-	-	-	-	-	+	Bantu A6	Bantu A1
5	-	-	+	+	+	+		-	-	-	-	+	+	Cameron	Benin
6	-	-	+	+	+	+		-	-	-	-	-	+	Cameron	Bantu A1
7	-	-	+	+-	+	+		-	-	-	-	+	+	Cameron	Benin

N0	Hinc II	XMn	GammG Hind111	GammA Hind111	¥B	Ava11		Hinc II	XMn	GammG Hind111	GammA Hind111	¥B	Ava11
8	-	-	+	+	+	+		-	-	+	+	+	+
9	-	+	+	-	+	+		-	-	-	-	-	+
10	-	-	+	+	+	+		-	-	-	-	-	+
11	-	-	+	+	+	+		-	-	+	+	+	+
12	-	-	+	+	+	+		-	-	-	-	+	+
13	-	-	+	+	+	+		-	-	-	-	+	+

Frequencies of haplotypes among tribes with their age and hematological p

Sex	Age	Tribe	Residence	Hb	PCV	RBCs	MCV	MCH	MCHC	TWBCs	PLTs	Hb Elect
F	12 Y	Housa	ALFASHER	7.4	23.4	2.25	104.0	32.9	31.6	6.3	478	SS
F	5 Y	Housa	ALFASHER	7.7	27.5	2.72	101.1	28.3	28.0	11.5	531	SS
F	9 M	Housa	ELFASHER	7.3	23.5	1.31	92.9	28.9	31.1	13.1	443	SS
F	5 Y	Housa	ELFASHER	7.3	21.3	2.37	89.9	30.8	34.3	13.9	423	SS
M	1 Y	Bartey	ALTEWSHA	8.2	22.9	2.70	84.8	30.4	35.8	8.1	174	SS
F	3 Y	Bartey	ALKOMA	5.8	19.2	2.29	83.8	25.3	30.2	35.8	248	SS
F	15 Y	Zagawa	KARNOY	8.0	25.8	2.87	89.9	27.9	31.0	7.5	88	SS
F	5 Y	Zagawa	KARNOY	7.4	22.2	2.21	84.7	28.2	33.3	22.1	402	SS
M	2 Y	Fur	TAWALA	6.6	20.1	1.68	97.6	32.0	32.8	16.8	664	SS
F	10 Y	Fur	TAWALA	8.2	23.6	1.43	90.4	31.4	34.7	14.3	596	SS
M	5 Y	Fur	ELFASHER	5.8	18.4	1.73	106.4	33.5	31.5	61.7	469	SS
M	11 M	Fur	ELFASHER	6.0	23.1	2.75	84.0	21.8	26.0	15.8	448	SS
F	3 Y	Fur	DAR-ALSLAM	4.7	16.0	1.78	89.9	26.4	29.4	14.0	137	SS


Sysmex
KX-21N
When the message "PU Sleeping" is displayed, press the [SELECT] key to return to the analysis result screen, and then the [START] switch to return to the ready.
Ready
SAMPLE No.
ENTER
SELECT
MODE
HELP
SHUTDOWN


minicap
flex piercing


Thermocycler
Biometra
Dyad


WARNING DO NOT FILL ABOVE THE LINE
DESTROY BY INCINERATION
PARAFILM
PARAFILM
CONSORT