REVIEW OF LITERATURE
Historical Review of Sickle Cell Disease:

Prior to first formal report in 1910, SCD must have been misdiagnosed because of its cardinal manifestations were common to other tropical disorders. The first accepted report of the disease in North America appeared in the November 1910 edition of the Archives of Internal Medicine where Dr James Herrick of Chicago described a young Negro student from Grenada in the West Indies in a paper entitled "Peculiar elongated and sickle shape red blood corpuscles in a case of severe Anaemia" (Serjeant & Serjeant 2001).

Mason (1922) was first to use the term ' Sickled cell Anemia '. Herrick in 1923 noted latent sicklers among relatives of patient with this disease. The transformation of the biconcave red cell to the sickle form in vitro was first established by Emmel in 1917. The comprehensive studies of Hahn and Gillespie in 1927 delineated the conditions affecting sickle cell formation in vitro, including pH, temperature, fixatives, tonicity, etc. Hahn applied the term sickle cell trait to the asymptomatic condition associated with in vitro sickling. Diggs et al, (1934) first clearly differentiated symptomatic SCD from silent asymptomatic cases. Diggs and Bibbs (1939) first demonstrated irreversible erythrocyte in peripheral blood smear of sickle cell patient.

Physical chemist Linus Pauling in 1949 deduced that the phenomenon was the manifestation of an abnormal hemoglobin molecule and demonstrated electrophoretically abnormal Hb in sickle cell anemia thus introducing the concept of molecular disease. In the same year Neel (1949) established that sickle cell trait was the heterozygous and sickle cell anemia the homozygous state for the same gene. Ingram et al, (1957) by chemical analysis confirmed that peptides contain less glutamic acid and more valine suggesting that valine has replaced glutamic acid. He demonstrated the difference in the amino acid sequence and the science of molecular biology of the disease began. The 3D structure of Hb was established by Perutz in 1950 at Cambridge.

Recognition of Clinical Feature in Sickle Cell Disease:

In four cases reports subsequent to James Herrick (1910), haemolytic aspect of the disease was recognised. Syndenstricker noted elevated reticulocyte count. Early autopsies
confirmed marked erythroid expansion of the bone marrow (Syndenstricker et al, 1924). At this
he also introduced the concept of haemolytic crisis.

Gall stones associated with increased bilirubin excretion were described in the second
reported case (Washburn, 1911). Increased requirement of folic acid secondary to hemolysis
were noted by Zuelzer and Rytzky 1953.

The contribution of infarction to pathology of SCD was perceived more slowly; when
pulmonary consolidation was described by Graham (1924). Syndenstricker (1930) noted
pulmonary infraction. Multiple Kidney and Lung infarction by Yater and Mollare (1931) and
repeated splenic infarction and fibrosis was demonstrated by Diggs (1935).

Bone infarction causing dactilitis and femoral head necrosis was described by Bauer
(1943). Priapism was recognised as a complication in 1930 (Digg and Ching 1934) and
lesion in central nervous system by Syndenstricker et al in 1924. Serious retinal complication
was recognised by Welch and Goldberg (1966).

Other complications like leg ulceration were reported by King (1936). Yater and
Mollari (1931) observed that pregnancy adversely affects SCD. Retardation of physical and
sexual development was noted by Winsor and Burch (1944).

Origin of the Sickle Cell Disease:

The sickle mutation appears to have arisen spontaneously at least five times in the history
of mankind. Such independent mutations can be recognized by their association with different
beta globin haplotype demonstrated by the analysis of RFLP. Haplotype, defined as pattern of
various DNA polymorphisms along a section of a chromosome, was first mentioned by
Antonarakis to describe the molecular defects of thalassemia (Nagel 1990). DNA haplotype can
be used in a variety of ways such as: a) for the determination of the unicentric origin of a
mutational event for a particular abnormal human globin gene, b) for discriminating between
diverse epistatic events linked to the β gene that may modulate the phenotypic expression c) for
the tracking of gene flow of a particular abnormal β gene.
The first such polymorphism reported was a variation in the recognition site of the restriction endonuclease Hpa I to the 3 side of the β-globin gene (Kan and Dozy, 1978). This work involving the single restriction endonuclease Hpa I and a single restriction site has now been superseded by the use of a series of different restriction enzymes identifying multiple recognition sites of the β-globin gene cluster. This pattern of polymorphic sites or β haplotype provides a much more sensitive and specific genetic marker than the use of Hpa I alone.

The structure of the DNA surrounding the β-globin locus has been found to differ between populations having HbS (Antonarakis et al., 1984), suggesting specific ancestral DNA structures upon which the HbS mutation has arisen later. These differences in DNA structure or polymorphisms are identified by restriction enzymes which have specific recognition sites. The most common enzyme combinations used are HincII, HindIII, XmnI, AvaI, Hpal, and BamHI. These β-globin haplotype, believed to represent independent occurrences of the HbS mutation, have occurred in at least three and possibly four occasions in Africa and are named after the areas where they were first described. They are Benin, Senegal, CAR or Bantu and Cameroon haplotype (Lapoumeroulie et al., 1992). The fifth mutation associated with further foci is associated with the Eastern Province of Saudi Arabia and India termed as the Asian haplotype (Padmos et al., 1991).

**Geographical Distribution of Sickle Cell Disease:**

A) βS Gene in World: It is true that high frequency of the βS gene occurs in equatorial Africa and from there the gene was carried to North and South America, the Caribbean and later to Europe. The highest prevalence of βS is in tropical Africa and among blacks in countries that participated in the slave trade. It occurs with lower frequency in the Mediterranean basin, Saudi Arabia and parts of India.

In the United Kingdom the gene initially came from the Caribbean, but later by direct immigration from Nigeria, Ghana and more recently from other areas of central Africa. In France, the sickle cell population derived from North Africa and former French, Belgian, and Portuguese possessions in West and Central Africa. In Germany, the gene is more frequent among Turkish immigrants. In North America, the sickle cell trait occurs in 8% of the Black population and in the Caribbean, it occurs approximately in 10% of people of African origin. In
both areas the gene is usually of the Benin haplotype migrated during the slave trade that took place between about 1650 and 1830 from Africa. The gene does not occur naturally in Australia but has been imported by the sizeable Greek population in Sydney and some other Eastern cities.

B) $\beta^s$ Gene in India: The $\beta^s$ gene was first recognized in India nearly 50 years ago. Credit for the first authentic documentation of sickle cell gene in India goes to Lehmann and Cutbush (1952), who demonstrated presence of sickle cell trait among tribes of Nilgiri hills of South-India. Subsequent surveys coordinated by the Anthropological Survey of India have indicated high frequencies in huge populations throughout central India, especially in the States of Orissa, Maharashtra and Madhya Pradesh (Roy and Chaudhuri 1967; Negi et al, 1972). It has been observed in families of Dhanudh caste in Manipur. Very high incidence of AS has also been found in Paragh Kondas community of South India (Foy et al, 1956). Existence of SCD in Western India was reported by Sukumaran et al, 1975. In a study in central India, it was found that HbS is present in 22.2% in Moharanas and 11.3% in Telies communities (Shukla and Solanki 1958). In Andhra Pradesh the $\beta^s$ was found to be 3-20%.

The $\beta^s$ gene does not occur in South-East Asia except in immigrants of other areas. The gene has not spread east of Calcutta, although it occurs in Malaysia in Indian people originating from Orissa State.

C) $\beta^s$ Gene in Orissa: Sickle cell anemia was reported among tea garden labourers of upper Assam who originated from tribal population of Orissa and Bihar (Dunlop and Mozumdar 1952). There is high prevalence of $\beta^s$ among Agharia caste of the state (Nanda et al, 1967). Surveys within Orissa have reported 3-41% AS in tribal population of Koraput district (Roy and Roy Chaudhuri 1967). It has been observed that 11.1% in hospital patients of Orissa were sickle cell positive and the gene was found to be wide spread in the State (Kar et al, 1987).

It was also reported that DNA haplotype associated with the $\beta^s$ gene in tribal populations from the east coast of central India are the same $\beta^s$ linked haplotype prevalent among the populations found in eastern Saudi Arabia (Miller et al, 1987). However it is different from the three different haplotype that exist in three separate geographical locations in Africa (Pagnier et al, 1984). Thus, it was concluded that the mutated gene had a unicentric origin of the tribal
people of central and southern India, because of the present disconnection and dispersion of the population bearing this gene.

**Nomenclature and Classification of Sickle Cell Disease:**

The five principal genotype of the sickle cell Disease are:

- **Homozygous sickle cell disease** – Homozygous SCD (SS disease)
- **Sickle cell-Hemoglobin C disease** - SC disease
- **Sickle cell-β⁰ thalassemia** - Sβ⁰ thal (No HbA)
- **Sickle cell-β⁺ thalassemia type-I** - Sβ⁺thal type-I (HbA 3-5%)
- **Sickle cell β⁺ thalassemia type-II** - Sβ⁺thal type-II (HbA 5-15%)
- **Sickle cell β⁺ thalassemia type-III** - Sβ⁺thal type-III (HbA 18-25%)

The sub-types of Sickle Cell β⁺ thalassemia represent phenotypes with different expressions according to the amount of HbA produced. The type-I is a severe defect associated with 3-5% HbA most commonly seen in Indian patients, type-II has higher levels of HbA but remains relatively severe and occurs around the Mediterranean, and type-III has 18-25% HbA, runs a mild course and is the type most frequently seen in patients of African origin.

In addition to these major genotypes, three other less common conditions manifesting the features of SS disease include:

- Sickle cell hemoglobin D Punjab
- Sickle cell hemoglobin O Arab
- Sickle cell hemoglobin Lepore Boston

(Serjeant & Serjeant 2001)

Since all the above conditions result from abnormalities affecting β-chains, further heterogeneity may be introduced into these genotypes by variations in the alpha and gamma chains. In general these conditions do not differ from homozygous SCD. Clinical as well as biochemical heterogeneity in SCD may also result from the interaction with heterozygous and homozygous α⁺ thalassemia.
Variation in the γ-chain synthesis may also contribute to the variability of homozygous SCD with the inheritance of genes determining increased F-cell production in the syndromes of heterocellular persistence of fetal hemoglobin.

All these hemoglobinopathies are indistinguishable from homozygous SCD by the preliminary sickling test and alkaline Hb electrophoresis. The use of advanced laboratory procedures and family studies is often necessary for an accurate diagnosis.

**Genetics and Pattern of Inheritance:**

The abnormal Hb diseases are inherited in a fashion sometimes referred to as autosomal co-dominant rather than as an autosomal recessive manner. Human Hb is a conjugated protein with a molecular weight of 64,500 Da, roughly spherical with a maximum molecular diameter of approximately 6.4 nm. The globin protein of adult Hb is made up of two α and two β polypeptide chains. The gene for α chain is located in a globin gene cluster present in chromosome 16. Similarly, the β globin gene cluster is present in chromosome 11.

A person, who inherits an abnormal HbS gene from one parent and a normal β globin gene from the other, becomes the harmless carrier state known as AS (Sickle Cell trait). Inheritance of abnormal HbS genes from both of the parents results in the homozygous state SS, which causes serious health problem leading to early death.

A person carrying two different abnormal hemoglobin genes is referred to as doubly heterozygous. The inheritance pattern in such situations may take one of two forms, depending on whether the two genes are alleles or non-alleles (i.e. whether they affect the same or different polypeptide chains). For example, children of a man doubly heterozygous for HbS and C/D/E , both of which are β-chain defects and therefore allelic, can inherit only one of these genes. All children, therefore, will be heterozygous for either HbS or Hb variant, but none will be normal, and none will be doubly heterozygous.

If the properties of the Hb are such that symptoms are produced in the heterozygous state, then the pattern of inheritance of the disease is that of an autosomal-dominant trait. If, on the other hand, only the homozygous condition is symptomatic as in SS, a recessive inheritance pattern is observed; and the heterozygous state is called the trait. However, if presence of both
abnormal Hb is expressed significantly together, then it is termed as co-dominance disobeying Mendelian inheritance.

**Structure of Globin**

The exact primary structure of all normal globin chains has been determined based on the DNA sequence of the individual globin genes. The polypeptide chains in hemoglobin differ from one another in amino acid sequences. The α-chain contains 141 amino acids and the non-α-chains, 146. In the β globin gene cluster the δ-chain differs from the β-chain in only 10 of its 146 amino acid residues; whereas γ-and β-chains differ by 39 amino acids. Despite the differences in the primary structure of non-α globin chains, their secondary structures are remarkably similar. Each has eight helical segments designated by the letters A through H. The helixes make up approximately 75% of the molecule-interspersed between them are seven non-helical segments: NA, AB, CD, EF, FG, GH, and HC. This arrangement is important structurally, because the helixes are relatively rigid and linear, whereas the non-helical segments allow bending. The heme pocket is the site of many dynamic interactions involving oxygen binding to Hb. Heme is suspended in a non-polar crevice between the E and F helixes and helixes B, G and H constitute the floor of the pocket. Heme iron forms a covalent bond with the imidazole nitrogen of the proximal histidine at F8.

**Alpha Globin Gene Structure, Expression and Regulation:** The α-globin genes are duplicated and located in the cluster containing an embryonic α-like gene (ζ2) and three pseudogenes (Ψζ1, Ψα1, and Ψα2). A gene (θ) with unknown function, but whose mRNA can be found through all stages of development is part of the α cluster. Several regions of the cluster contain tandem arrays of short GC-rich sequences (minisatellites), identified as hypervariable regions, and many Alu-family repeats. The α complex is arranged in the order in which it is expressed during development: 5'ζ2................. α2- α1. There is a very high homology between α2 and α1 genes; they only differ in the IVS-2 (two base substitutions and a 7-bp insertion/deletion) and intron 3' noncoding region [18 base substitutions and a single-base deletion in the 3' untranslated regions (UTE)]. The embryonic ζ gene shows only 58% homology with the α genes in the coding region.
Fig: 1(a)- Diagrammatic representation of the α and β globin gene clusters

<table>
<thead>
<tr>
<th>Gene</th>
<th>CACCC homology box</th>
<th>CCAAT homology box</th>
<th>TATA homology box</th>
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<tr>
<td>ζ</td>
<td></td>
<td>CCAAT</td>
<td>TATAAAAC</td>
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<tr>
<td>α1 and α2</td>
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<td>CCAAT</td>
<td>CATAAAC</td>
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<td>ε</td>
<td></td>
<td>CCAAT</td>
<td>AATAAAAG</td>
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<td>γ and δ</td>
<td>CACCC</td>
<td>CCAAT/CCAAT</td>
<td>AATAAAA</td>
</tr>
<tr>
<td>β</td>
<td>CACCC</td>
<td>CCAAT</td>
<td>CATAAAA</td>
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<tr>
<td>δ</td>
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<td>CCAAC</td>
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Fig: 1(b)- Sequences showing CACCC, CCAAT and TATA homology in the promoters of globin gene.
The level of transcription of the two α genes differs. The α2 gene encodes two to three times more α-globin than α1. This implies that the globin structural variants of the α2 gene should present approximately 35% of total Hb, whereas the α1 globin gene mutants approximately 15%.

The expression of the α-globin genes is regulated by the sequences in and around the structural genes and by a region located 40 kb upstream from the α cluster. This region HS-40, contains an erythroid-specific DNAase I HS and a 350 bp core element with multiple binding sites for transacting factors (nuclear factor-erythroid 2, GATA-1), several CACCC motifs, and a YY1 transcription factor binding site. The importance of HS-40 as a regulatory element is suggested by the presence of rare deletions of this region that produce α-thalassemia, although both α genes on the chromosome are intact.

Normal individuals have usually four α-globin genes, but as a result of unequal genetic exchange, some may have five or six α genes, while still being phenotypically normal. Multiple arrangements with three to six ζ-like embryonic genes have also been reported.

**Beta Globin Gene-Structure, Function and Expression:** The β globin cluster contains five functional genes, 5' ε- γ -Aγ -Ψβ -δ - β 3' which are arranged in the order of their developmental expression. Upstream of the entire β globin complex is in the LCR, which consists of five DNase 1 hypersensitive sites (designates HS 1-5) distributed between 6 and 20 kb 5' of the ε gene. The LCR plays a critical role in β globin gene expression by maintaining an open chromatin state and acting as a powerful enhancer of globin gene transcription; in its absence, the level of β globin gene expression is low. Four of the sites (HS 1-4) are erythroid specific, encompassing binding sequences for erythroid-restricted transcription factors (GATA-1 and NF-E2), while HS5 is ubiquitous. There is one other hypersensitive site approximately 20 kb 3' to the β gene. The two extreme HS sites flanking the β complex have been suggested to mark the boundaries of the β globin gene domain. The genomic sequence spans 1600 bps; the transcribed region contain three exons separated by two introns or intervening sequences (IVSs). Exon 2 encodes the residues involved in haem binding and αβ dimer formation, while exons 1 and 3 encode for the non-haem-binding regions of the β globin chain. Many of the amino acids involved in globin subunit interactions required for the Bohr effect and 2,3-diphosphoglycerate
binding, are found in exon 3. Conserved sequences important for \( \beta \) globin gene expression are found in the 5' promoter region at the exon-intron junctions and in the 3'-UTR at the end of the mRNA sequences. The \( \beta \) globin gene promoter includes three positive cis-acting elements, a TATA box (positions-28 to 31), a CCAAT box (positions -2 to -76) and duplicated CACCC motifs (proximal at positions -86 to -90, and distal at position -101 to -105). While the CCAAT and TATA elements are found in many eukaryotic promoters the CACCC sequence is found predominantly in erythroid cell-specific promoters. Binding of the EKLF to the CACCC motif appears to be crucial for normal adult \( \beta \) globin expression. In addition to these motifs, the region upstream of the \( \beta \) globin promoter contains two binding motifs for the erythroid transcription factor GATA-1. The importance of these 5'-flanking sequences for normal gene expression is underscored by \( \beta \) thalassemia arising from point mutations in these sequences specifically in and around the TATA box and the CACCC motifs in the -80 to -100 region. An enhancer is also found in intron 2 (Antoniou et al, 1988) and 3 of the globin gene, 600-900 bps downstream of the poly-A site (Trudel & Costantini, 1987).

The 5' UTR occupies a region of 50 nucleotides between the CAP site and the initiation (ATG) codon. There are two prominently conserved sequences in the 5' UTR of the various globin genes (both \( \alpha \) and \( \beta \)) (Collins & Weissman, 1984). One is the CTTCTG hexanucleotide found 8-13 nucleotides downstream from the CAP site, i.e. at positions +8 to +13. The second conserved sequence is CACCATG, in which the last three nucleotides form the initiation codon (ATG). Again, the importance of these sequences in the regulation of the \( \beta \) gene expression is exemplified by several mutations in the 5' UTR causing \( \beta \) thalassemia. The 3' UTR constitutes the region of 132 nucleotides between the termination codon (TAA) and the poly-A tail with one conserved sequence, AATAAA located 20 nucleotides upstream of the poly-A tail.

The developmental regulation and expression of the individual globin genes rely on two mechanisms, gene silencing and gene competition governed by direct physical interactions between the globin promoters and the \( \beta \)-LCR (Carter et al, 2002; Tolhuis et al, 2002) which are dependent on the transcription environment in embryonic, fetal and adult cells. While the \( \epsilon \) and \( \gamma \) globin genes are autonomously silenced at the appropriate developmental stage, expression of the adult globin gene depends on the lack of competition from the \( \gamma \) gene for the LCR sequences. This is supported by the concomitant down-regulation of the cis gene when the \( \gamma \) gene is up-
Fig 2: β-globin chain and Hemoglobin Synthesis from the β-globin gene
regulated by promoter mutations (Wood 2001). In addition, mutations that affect the β promoter, which remove competition for the β-LCR, tend to be associated with variable increases in the γ and δ gene expression (Huisman, 1997).

Pathophysiology of Sickle Cell Disease:

After deoxygenation of HbS molecule, long helical polymers are formed through hydrophobic interaction between the β-6 valine of one tetramer and the β-85 phenylalanine and β-88 leucin of an adjacent tetramer (Schechter, 1982). Initially red cell cytosol converts from a freely flowing liquid to viscous gel as HbS aggregate. With continued deoxygenation state aggregated HbS molecules assemble in long needle like fibre within red cells producing a distorted sickle or holly leaf shape. They adhere to the post venular capillary endothelium leading to significant obstruction and ischemia to the concerned organ. The sickling of cell is reversible phenomenon earlier on oxygenation. However red cell membrane is secondarily affected by repeated and prolonged sickling which ultimately leads to formation of irreversible sickle cell due to irreparable damage to membrane structure.

Physiologic Determinants of Polymerization:

The concept of sickling and sickled cell was central to much of the early thinking on the pathophysiology of SCD. Here are some factors responsible for in-vitro sickling.

Oxygen: Falling in oxygen tension was associated with a rise in sickled cells in SCD. The importance of oxygen tension was first discovered by Hahn and Gillespie (1927) and was further investigated by Lange et al, (1951) and Allison (1956).

Hydrogen Concentration: Recognition of the contribution of pH to sickling may also be attributed to Hahn and Gillespie (1927). Lange et al, (1951) noted the lower oxygen tensions were required for sickling when the pH was high. An acid pH has also been to promote cellular water loss through activation of the KCl cotransport system (Brugnara et al, 1989).

Temperature: The speed of sickling was noted to be temperature related, acceleration by heat being reported by Sydenstricker et al, (1924). Complete inhibition of sickling occurred at temperature close to freezing (1-4 °C) (Allison et al, 1956) but sickling progressed normally when the cells are warmed.
Intracellular HbS Concentration: The importance of intracellular concentration of HbS to the degree of sickling was suggested by observations that red cells from patients with SCD sickled more readily than those from AS. The type of non HbS hemoglobin markedly influenced the degree of sickling (Alison 1957). HbD Punjab was interacting strongly with HbS (Charache et al, 1964), whereas HbF inhibited sickling to a greater extent.

Other Hemoglobins: The influence of other hemoglobins on HbS polymerization is astounding regardless of the oxygen concentration. Both HbA and HbF have an inhibitory effect on gelation. When deoxygenated, these hemoglobins enter the sickle polymer less readily than deoxy HbS, thereby retarding gelation by a dilutional effect because there are 20 surface amino acid differences between βS- and γ-chains and only a single residue difference between βS- and βA-chains. Deoxy HbS molecules copolymerize most effectively with other HbS molecules and in decreasing order with Hb C,D, O Arab, A, J, and F. In vitro observations predict the clinical severity of disorders involving these variants. In contrast, the doubly heterozygous state for Hb S and hereditary persistence of fetal Hb (HPFH), in which red cells contain approximately 70% Hb S and 30% Hb F, is not associated with clinical disease (William & Wilkin 2009).

Clinical Features of Sickle Cell Disease:

The clinical features of SCD are due to change of shape of normal RBC to sickle shape. The patient is asymptomatic from birth to early infancy and symptoms start 4 months onward as HbF level falls and HbS concentration rises. The disease may present as mild painful vaso-occlusive crisis in the form of limb pain or backache to a life threatening condition like acute sequestration crisis or multi organ dysfunctions which may be fatal.

A. Clinical Features in Steady state

B. Crises

A. Clinical Features in Steady State: This is defined as “crisis free period extending from atleast 3 weeks since last clinical event and 3 months or more since last blood transfusion, before the start of new clinical event”. Recent observation has revealed that the word steady state in sickle cell disease is missnomer. It includes
a. Chronic haemolytic anemia

b. Organ dysfunction

a) Chronic Hemolytic Anemia: The sickle cell disease patient suffers from ill health due to shortened red cell survivability. There may be jaundice, cardiomegaly, increased fatigability, reduced exercise tolerance and increased susceptibility to infection. There may be delayed growth, retarded puberty and life expectancy is also reduced.

b) Organ Dysfunction: The syndrome of organ system failure is a common problem associated with critical illness. This syndrome is defined by the simultaneous presence of physiological dysfunction and/or failure of one or more organs. Typically this occurs in the setting of severe sepsis, shock of any kind, as well as severe inflammatory condition such as pancreatitis and trauma. However this condition is not uncommon in SCD where the basis of the organ failure or ischemic organ dysfunction is venous capillary occlusion leading to a state of Multi-Organ Dysfunctions (MODs). There could be single organ dysfunction or concurrent involvement of multiple organs.

B. Crises: Patients of SCD suffer from acute life threatening conditions called crisis. The different forms of crises are

1. Painful (Vaso occlusive) Crises.


3. Aplastic Crises.

4. Hemolytic Crises

1. Painful (Vaso occlusive) Crises: The most characteristic feature of SCD is Sickle cell crisis. The repeated attacks of pain are involving musculoskeletal system, chest and abdomen. The clinical manifestations are sudden in onset and directly attributed to obstruction of microcirculation by intravascular sickling. Vaso-occlusive crisis (VOC) is precipitated by hypoxia, acidosis, dehydration and infection. Various types of VOC’s are Hand-foot syndrome, Painful crises, Avascular Necrosis, Abdominal crises, Hepatic crises, Priapism, Acute chest syndrome and Neurological complication.
2. Acute Splenic Sequestration Crisis: Acute splenic sequestration is the major cause of mortality among children first recognised by Tomlinson (1945). There is sudden enlargement of spleen due to trapping of large proportion of red cell mass that precipitate fall in haemoglobin level with compensatory reticulocytosis and hypercellular marrow. Massive splenic enlargement may result in severe anaemia and circulatory collapse and death. Treatment is urgent blood transfusion. Splenectomy may be done after two attacks of sequestration.

3. Aplastic Crisis: It was first described by Singer et al, (1950) in a 9 year old boy of SCD. Analysis of stored sera from Jamaican cohort study (1971-81) revealed evidence of Human Parvo virus B19 infection among the causes of aplastic crises (Serjeant & Serjeant 2001). In early phase of aplastic crises the peripheral blood reticulocyte and bone marrow normoblast disappear or greatly reduced in number. The process is self limiting within 5-10 days red cell concentration resume to normal.

4. Hemolytic Crisis: It is mostly seen in G6PD (Glucose-6-phosphate dehydrogenase) deficient patients. This crisis is precipitated by oxidants, drugs, mycoplasma infection. It is also called hyper haemolytic crises and hence controversial because at times it is difficult to prove the increase rate of hemolysis (Diggs, 1965). The features of sudden hemolysis are seen in this crises (Konotey et al, 1974). It may lead to megaloblastic changes, if dietary availability of folic acid is deficient (Serjeant, 1994). Folate supplement should be logically given at the time of rapid growth in infancy, adolescence and pregnancy. This crisis is characterised by progressive fall in haemoglobin level that reaches 2 to 3 gm/dl. Reticulocyte count falls below steady state level and MCV get increased. This is treated by oral folate. Response to treatment is known by rapid increase in reticulocyte count and gradual increase in haemoglobin level.

Clinical Heterogeneity of Sickle Cell Disease:

Though sickle cell disease is caused by an identical single base change in their DNA, the severity in the clinical manifestations especially the morbidity pattern varies between and within different population groups and in between individuals. The diversity of the clinical course is attributed to some environmental as well as the interaction of various genetic factors (linked or unlinked). The genetic factors are genotype of the SCD, linked beta globin gene cluster haplotype, XmnI polymorphism and fetal hemoglobin determinants like hereditary persistence of
fetal hemoglobin (HPFH) / δβ-Thalassemia, X-linked FCP locus, co-inheritance of alpha thalassemia and association of other Hb variants and thalassemia. Environmental factors which modulate the phenotype are malaria, tuberculosis, infections, climatic conditions and medical care facilities.

A) Genetic Factors Affecting Clinical Variability of Sickle Cell Disease:

a) Genotype of the SCD: The phenotypic expression of SCD depends upon the genotype of Sickle Cell Syndrome. The term SCD includes a variety of pathological conditions resulting from the inheritance of the HbS gene either homozygously (βSβS) or as compound heterozygous (βSββ) with other interacting abnormal Hb genes. HbS with other allelic hemoglobin disorders (like β-thalassemia, Hb variants HbS, HbC, HbD, HbE etc. and hereditary persistence of HbF) manifest with variable phenotypic expressions. The clinical courses may range from one extreme in which the condition is indistinguishable from severe homozygous SS to a disorder which is completely symptomless and which may be found incidentally during pregnancy, refractory anemia and population screening.

b) Beta globin cluster haplotype: The genetic sequence around the sickle cell mutation constitute the beta globin gene cluster haplotype. Five different haplotypes named Benin, Central African Republic (CAR) or Bantu, Cameroon, Senegal, and Asian-Indian have been identified and are referred to as 19, 20, 17, 3, and 31, respectively (Oner et al., 1992). The most prevalent haplotype in India is the Asian haplotype. However, some rare haplotypes like Bantu and Cameroon; and other rare atypical haplotypes have been reported (Niranjan et al., 1999; Mukherjee et al., 2004) from other states. The degree of anemia, the Hb F concentration, and the preservation of G0 Hb F have been proposed to be haplotype dependent and influence the overall clinical course of the patient.

However, the effects of the haplotypes on the clinical manifestations of the disease are still conflicting. Reider et al demonstrated no effect of haplotype on HbF levels, hemoglobin concentration, packed cell volume or mean cell volume (Reider et al, 1991). Powars et al, reported that the Senegal haplotype is associated with less painful crisis, bone infarction and organ damage, the Bantu haplotype has worse clinical course; severity is intermediate in the Benin (Powars et al, 1991). Studies carried out in Central India (Orissa) and Eastern Province of
Saudi Arabia in sickle cell disease patients linked to Asian haplotype have shown a mild form of disease and elevated HbF levels (Kar et al., 1986; Padmos et al., 1991).

c) XmnI polymorphism: The C→T change at position –158 from the G\textsuperscript{Y} gene, which creates a cleavage site for the enzyme XmnI is called XmnI polymorphism. It has been suggested to be one of the non deletional determinants for high HbF level. Polymorphism at this site in the Senegal and Arab-India haplotypes is strongly associated with high expression of the G\textsuperscript{Y} gene, while absence of this polymorphism has been correlated to the low G\textsuperscript{Y} expression in the two Benin and Bantu haplotypes (Labie et al., 1985). Same author further reported that high G\textsuperscript{Y} expression in β-thalassemia associated with haplotype IX and III is indeed linked to an XmnI-positive site.

d) Fetal hemoglobin level: Fetal hemoglobin (HbF) (α2γ2) is the most thoroughly studied genetic modulator of SCD. Most individuals with SCD are healthy at birth because of high HbF level and become symptomatic later in infancy or childhood after Hb F levels falls and Hb S level raises. Several studies have been undertaken to correlate the effect of HbF on morbidity and mortality of SCD. Observations in different populations have supported the concept that higher HbF level is protective against various complications of sickle cell disease; few studies have demonstrated an adverse effect while some studies suggest no effect (Steinberg et al., 2005).

It was suggested that the threshold level of HbF needed to prevent acute clinical events was about 20% while the threshold to prevent organ damage was 10% (Powars et al., 1984). Studies done in communities with high HbF level in Central and southern India and in the Eastern Province of Saudi Arabia showed that people of these communities tend to have less anemia and mild clinical manifestations (Pembrey et al., 1978; Kar et al., 1986). The fetal hemoglobin level in Indian SCD patients has been found to be high in various studies.

HPFH and δβ-thalassemias are two genetic conditions characterized by persistence of fetal hemoglobin in the adult life in absence of major hematological abnormalities and have been hypothesized to modulate the clinical course of the disease. Coinheritance of these conditions with the sickle cell gene is misdiagnosed as homozygous sickle cell disease unless specific molecular diagnostic test for presence of these conditions/detailed family evaluation is taken up.
It has been hypothesized that association of genetic factors like HPFH and δβ-thalassemia might be responsible for the raised HbF levels (Kulozik et al, 1987).

e) X-linked locus FCP: The F cell production locus, a quantitative trait locus (QTL) at Xp22 is associated with F cell number (Chang et al, 1995, Garner et al, 2002). More than 180 SNPs in 38 candidate genes that might modulate HbF levels were studied in 280 SCD patients. The strongest association with HbF was found with SNPs near the 6q223-23.2 QTL. The phenotype of this locus and the Gβ globin gene -158 C-T polymorphism were estimated to account for about half of the HbF variation in SCD, with the F cell production locus accounting for 35-41% of this variation (Steinberg 2005). Further study showed that it contained five protein-coding genes, some of which displayed a high degree of alternative splicing and AHI1 that spanned about 215kb and genes that did not appear to be protein coding (Close et al 2004).

f) Coinheritance of α- thalassemia: Studies of the interaction of α⁺ thalassemia with SS disease were initially limited by imprecise globin-gene synthesis methods but the findings were essentially confirmed by more accurate technology. This interaction is not consistent and influences a number of hematological indices; the reduced MCHC is likely to reduce intravascular hemolysis, reducing hemolytic rate and increasing the deformity of red cells (Serjeant & Serjeant 2001).

Alpha Thalassemia:

Alpha thalassemia is a common hereditary condition caused by partial or total deficiency α-globin chain results in various forms of α-thalassemia. There are two α-globin genes per haploid genome, four in all. For this reason, α thalassemia is classified according to the total output of each of the α-chain genes that constitute the haploid pairs. A normal α-globin genotype can be represented as α α/ α α. The terms α⁰ and α⁺ thalassemia describe an α-globin haplotype; that is, the state of the two linked α-globin genes on a particular chromosome. In α⁰ thalassemia there is no output of α globin and in α⁺ thalassemia there is some output but usually only the product of a single α-globin locus.
### Classification:

Common Forms of Deletional $\alpha$ thalassemia:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Common name</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha \alpha/\alpha \alpha$</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>$-\alpha/\alpha \alpha$</td>
<td>Silent carrier</td>
<td>Within normal</td>
</tr>
<tr>
<td>$-\alpha/-\alpha$</td>
<td>$\alpha$-thal trait $\alpha$-thal-2 (trans)</td>
<td>Mild microcytic hypochromic anemia; common in African Americans</td>
</tr>
<tr>
<td>$-/-\alpha\alpha$</td>
<td>$\alpha$-thal trait $\alpha$-thal-2(cis)</td>
<td>Same as $-\alpha/-\alpha$ common in Asians</td>
</tr>
<tr>
<td>$--/\alpha$</td>
<td>HbH disease</td>
<td>Microcytic hypochromic anemia of variable severity</td>
</tr>
<tr>
<td>$--/--$</td>
<td>Hydrops fetalis</td>
<td>Lethal</td>
</tr>
</tbody>
</table>

Fig: 3- Deletional $\alpha$-thalassemia (Mechanism)
Classification:

a) \( \alpha^0 \) thalassemias: The \( \alpha \)-thalassemia can also be described at the molecular level. The \( \alpha^0 \) thalassemias are often designated by the particular length of the deletion that removes the both \( \alpha \) globin genes. i.e. the deletion that is common in Mediterranen populations (MED) is different from that which is common throughout South-east Asia(-SEA). These different \( \alpha^0 \) thalassemias can therefore be described as \( \alpha^0_{-MED} \) and \( \alpha^0_{-SEA} \) (Weatherall et al, 2001).

b) \( \alpha^+ \) thalassemias: There are two common forms of \( \alpha^+ \) thalassemia. The 3.7 kb deletion is the result of homologous recombination between misaligned homologous regions of allelic genes (Higgs et al, 1984; Dode et al, 1993; Bayasal et al, 1994). The \( - \alpha^3.7 \) is most prevalent in African American and Mediterraneanean and is also referred to as rightward \( \alpha \)-thal-2. Moreover, the \( - \alpha^3.7 \) allele is heterogeneous and has three different subtypes (I, II, or III) depending on the exact location of the breakpoint of the misaligned allelic genes. The type-I is prevalent in Africans and African Americans where as type-III is prevalent in Asians. The \( - \alpha^4.2 \) kb deletion is the result of misalignment and homologous recombination between the 4.2 kb distant homologous regions of allelic \( \alpha \) genes. It is most common in Southeast Asian (Dode et al, 1993; Winichagaon et al, 1984) and is often referred to as leftward deletion or leftward \( \alpha \)-thal-2.

c) Nondeletional \( \alpha \)-thalassemias: Nondeletional forms of \( \alpha \)-thalassemia are uncommon and have rarely been reported in African Americans. Point mutations in the \( \alpha \)-globin gene can cause \( \alpha \) thalassemia by different mechanisms. Nonsense mutations (such as appoint mutation at \( \alpha \) 116: GAG\( \rightarrow \)TAG; Glu\( \rightarrow \)Term) cause premature termination of translation resulting in the formation of a shortened and an unstable \( \alpha \) globin. Most nondeletional defects causing \( \alpha \)-thalassemia affect the expression of the \( \alpha^2 \) gene. Because the output of \( \alpha^2 \) gene is 2-3 times of the \( \alpha^1 \) gene and hence encodes more mRNA than the \( \alpha^1 \) gene (Liebhaber et al, 1987). Mutations that lead to \( \alpha^2 \) thalassemia usually produce \( \alpha \)-globin chain variants such as \( \alpha^{Seal\,Rock} \) (\( \alpha^2 \): Codon 142: TAA\( \rightarrow \)GAA; Ter\( \rightarrow \)Glu) which is an example of a termination codon mutation (Arcasoy et al, 1999; Olivieri et al, 1987, Liebhaber et al, 1987; Safaya et al, 1988). Hb Constant spring (CS) is a well known example of \( \alpha \) gene mutation that is always associated with the phenotypic expression of \( \alpha \) gene mutation that is associated with \( \alpha \)-thalassemia especially HbH disease (Safaya et al, 1988; Huisman et al, 1988).
Fig 4: Diagrammatic representation of common deletions that can lead to α-thalassemia trait; the shaded blocks indicate the length of the deletion.
d) **Triplication:** The $\alpha^{3.7}$ and $\alpha^{4.2}$ arise by recombination events consistent with a model of interchromosomal crossing over. The events resulting in the production of triplicated $\alpha$ genes correspond to the 3.7 kb deletion ($aaa^{anti\ 3.7}$) or to the 4.2 kb deletion ($aaa^{anti\ 4.2}$). The $aaa$ haplotypes are not associated with $\alpha$-thalassemia unless the homologous chromosomes have deletion of both $\alpha^2$ and $\alpha^1$ genes i.e. $aaa/\_\_$, which is an unlikely event in Africans Americans (Ballas 2001).

**Geographical Distribution of Alpha Thalassemia:**

a) **World:** Reliable data on population frequencies for the various form of $\alpha$-thalassemia are not available. Because of the difficulties in screening for $\alpha$-thalassemia phenotypically, its possible distribution and frequency can be suggested combining the information from the level of Hb Barts in neonates with the limited amount of data that are available from studies at the DNA level. Prior to the introduction of DNA analysis, population surveys for $\alpha$-thalassemia were based entirely on measurement of Hb Barts levels in cord bloods. This assay has certain draw backs that only became apparent when the molecular basis of alpha thalassemia was established.

Although some of the earliest surveys (Silverstrony & Bianco 1962) suggested that $\alpha$-thalassemia is uncommon in Italy but, later studies confirmed that 2.17% newborns from the Naple areas (Iolascon et al, 1982), 1.63% from Apulia (Izzo et al, 1979), 2.7% in Campania (Pinto et al, 1978) had Barts level ranging from 1-3%. In Africa, $\alpha^{3.7}$ deletion has outstanding prevalence ranging from 8-40% and it covers Bantu speaking Africa, Beninian Gulf, Gambia, Kenya, San, Southern Africa, West Atlantic and Zambia (Ramsay et al. 1987; Dode et al, 1988). In Sicily the gene frequency for $\alpha^+\$ thalassemia is 4.1% ($\alpha^{3.7}\text{del-87\%}$) (Fichera et al, 1997). Other countries like Australia, China, Cyprus, Egypt, Saudi-Arabia, Taiwan, Thailand has comparatively less prevalence of $\alpha$-thalassemia than Africa that is ranging from 3-10% (Modiano et al, 1991; Ko et al, 1993). India and Melanesia had highest cumulative frequencies of 50% including maximum alpha 3.7 & 4.2 deletion respectively (Kulozik et al, 1988; Fodde et al, 1988; O Shaughnessy et al. 1990).

b) **India:** Alpha thalassemia has been documented in different areas of India (Brittenham et al, 1980). Common $\alpha$-thalassemia determinant (-$\alpha$) has been characterized by RFLP, PCR and DNA sequencing analysis from various regions of India (Sen et al, 2005).

The $\alpha$-thalassemia mutations i.e. -3.7 and -4.2 deletions are occurred throughout Eastern India. However, it appears to be lower in Arunachal Pradesh and Assam and higher tribals of Bolpur, West
Bengal. It thus appears to be not related to the genetic drift from the Southeast Asian countries and could be due to de novo mutation.

Presence of deletional α+ thalassemias among the Baiga tribes of Central India have been reported by several workers (Talukder et al, 2007). Alpha thalassemia has been found in Rajasthan, Orissa and the Kachari population in Assam (Hundrieser et al, 1987; Kulozik et al. 1988; Choubisa et al, 2000). A non-tribal population study showed prevalence of 60.9% normal alpha chain, 3.80% -3.7 α homozygous mutation, 13.33% of -3.7 α carrier, 5.71% of -4.2 α carrier and -4.2 α homozygous mutation in 0.95% cases (Talukdar et al, 2007). There were 16.19% cases of HbH disease was reported by Sen et al, (2005).

The prevalence of α-thalassemia was much higher in West Central Gujarat (95%) and Nilgiri hills in South India (85.7%) suggesting that the condition is almost genetically fixed in India (Labie et al, 1989; Shaji et al, 2003).

c) Orissa: The prevalence of α-thalassemia in coastal Orissa was found to be 12.6% by Mishra et al, (1991). In a study of SCD patients Kulozik et al, (1988) reported α-thalassemia gene frequency to be 0.29%. Kar (1991) observed frequency of α-thalassemia gene was 0.32 in SCD against 0.28% in AS and 0.12% in AA.

Interaction between Alpha Thalassemia and HbS:

The complex effects of presence of alpha thalassemia in patients with SCD may be the result of two conflicting factors such as (i) reduced polymerization of HbS to less membrane damage, fewer dehydrated and irreversibly sickled cells and improved red cells survivability and (ii) higher Hb concentration leading to increased blood viscosity.

Pathophysiology: The formation of αβ dimers is a rate limiting step in the assembly of Hb. This process is thought to be facilitated by the electrostatic attraction between positively charged α-globin and negatively charged β-globin subunits. βS acquires a positive charge there by reducing their ability to compete with βA chains for α chains and thus less variant Hb than HbA accumulation. In the presence of α-thalassemia in which limited amount of α chains are synthesized in the red cell, these effects are exaggerated. The accumulated levels of positively charged βS are further decreased in proportion to the deficit in α globin chains. The co-inheritance of α-thalassemia may influence the
level of HbA\textsubscript{2} as δ-globin subunits is considerably more positively charged than β-globin subunit (Weatherall et al. 2001).

**Effect of Alpha Thalassemia on Homozygous Sickle Cell Disease:**

Alpha thalassemia affects red cell morphology, being associated with a decrease in irreversible sickled cells and an increase in target cells. Inheritance of α-thalassemia in SCD ameliorates the hematologic features although observations of clinical benefits are less consistent.

**a) Comparison of Hematological Findings in SCD with and without Alpha Thalassemia:** SCD patients with homozygous α\textsuperscript{+} thalassemia had significant change in some hematological indices in comparison to normal α genotype while coinheritance of heterozygous α\textsuperscript{+} thalassemia showed an intermediate effect in all the studies.

**Total Hb Level:** In SCD with 2α gene deletion the total Hb level was found to be significantly higher in comparison to SCD with normal α genotype. A single α gene deletion did not seem to have significant effect in SCD (Mukherjee et al, 1998, Balias 2001). Whereas Natta (1978) and Sejeant et al, (1983) didn’t find any significant difference in total Hb level in SCD with and without α thalassemia. Milner et al, (1986) observed significantly higher Hb in SCD with 2α gene than SCD with normal α genotype in the lower HbF (<10gm/L) group only.

**HCT:** Significantly higher HCT was found in SCD with α\textsuperscript{+} homozygous thalassemia in comparison to SCD without α thalassemia (Serjeant et al, 1983; Mukherjee et al, 1998). Whereas no such findings were observed by Steinberg et al, (1984), Higgs et al, (1982) and Kulozik et al, (1988). Milner et al, (1986) observed significantly more HCT in the lower HbF (<10gm/L) group of SCD with α\textsuperscript{+} homozygous thalassemia.

**RBC Count:** In SCD with 2α gene deletion the total RBC count was found to be significantly higher in comparison to SCD with normal α genotype. A single α gene deletion did not seem to have significant effect in SCD (Milner et al, 1986; Kulozik et al, 1988; Mukherjee et al, 1998). Balias (2001) observed increased RBC survival in SCD with 2α gene deletion.

**MCV:** SCD with both single α and 2α gene deletion showed significant decrease in MCV in comparison to SCD with normal α genotype (Milner et al, 1986; Mukherjee et al, 1998; Balias 2001).
MCH: MCH is found to be low in SCD with 2α gene deletion (Milner et al, 1986; Kulozik et al, 1988; Mukherjee et al, 1998). However, Serjeant and his coworkers (1983) did not observe such findings in their study.

MCHC: Significantly less MCHC was found in SCD with α-thalassemia than SCD without α-thalassemia (Kulozik et al, 1988; Mukherjee et al, 1998; Ballas 2001). The decreased MCHC inhibits the rate of polymer formation, reducing intravascular sickling and resulting in lower reticulocyte counts, fewer irreversibly sickled cells and fewer dense cells (Embury et al 1984; Baudin et al, 1986). Red cell filterability is improved (Serjeant et al, 1983) and red cell survival increased (De Ceulaer et al, 1983). Milner et al, (1986) observed significantly less MCHC in SCD with α homozogous having low HbF (<10gm/L) in comparison to SCD with normal α genotype.

Reticulocyte Count: SCD with α* homozygous thalassemia showed significantly less reticulocyte count in comparison to SCD with normal α genotype (Milner et al, 1986; Kulozik et al, 1988; Mukherjee et al, 1998). Milner et al, (1986) observed nil significant difference in between SCD with and without α-thalassemia having higher HbF (>10 gm/L) whereas the low HbF (<10gm/L) group showed significant difference.

Hb Constitution: In both SCD with and without α-thalassemia Hb constitute HbS, HbF and HbA2.

HbS: No significant difference in HbS was observed between SCD with and without α-thalassemia (Ballas 2001).

HbF: The HbF level in both the SCD with and without α-thalassemia was observed to be variable (Ballas 2001). SCD with α* homozygous thalassemia had significantly lower HbF in comparison to SCD without α thalassemia (Higgs et al, 1982; Kulozik et al, 1987). Whereas HbF was found to be similar in all the above groups (Steinberg et al, 1984).

HbA2: Significantly higher HbA2 was found in SCD with α* homozygous thalassemia in comparison to SCD with normal α genotype (Higgs et al, 1982; Mukherjee et al, 1998; Ballas 2001). It was observed that SCD with α* thalassemia had significant raised HbA2 than SCD without α- thalassemia (Stevens et al, 1986; Steinberg et al,1984; Kulozik et al, 1988). The HbA2 is increased in SCD shows a greater post translational affinity of limited α chain
for delta chain rather than for the $\beta^S$ chain resulting in preferential production of HbA$_2$ (Ballas 2001).

Power et al, (1980) found no relationship between erythrocyte indices in SCD with and without $\alpha$ thalassemia. In a study by Milner et al, (1986) reported that irrespective of $\alpha$ globin genotype patients with high HbF group had higher mean Hb, HCT, MCV and MCH than those in low HbF group. Average HbF levels were lower in patients with concomitant $\alpha$-thalassemia and a high HbF level seems to be uncommon. SCD with $\alpha$ thalassemia had significantly lower bilirubin level comparatively than without $\alpha$ thalassemia (Higgs et al, 1982).

b) Comparison of Clinical Features in SCD with and without Alpha Thalassemia: Clinically the effect of $\alpha$ thalassemia on SCD is not consistent. Heterogeneity among population of patients with SCD, in the ages of the patients studied and the numbers of patients in each study make firm conclusions difficult.

**Painful Crisis:** Effect of $\alpha$-thalassemia was found to be very little in SCD (Platt et al, 1991). Painful crisis has been reported to be more common among the patients with $\alpha$-thalassemia (Steinberg et al, 1984). Another study by Mukherjee et al, (1998) in Indian subcontinent showed homozygote $\alpha^+$ thalassemia has protective effect on painful crisis.

**Stroke:** Prevalence of stroke has been found to be low in SCD patients with $\alpha$-thalassemia (Miller et al, 1988; Balkaran et al, 1992).

**Osteonecrosis:** Influence of $\alpha$-thalassemia observed to be permissive in SCD patients (Steinberg et al, 1984). Ballas (2001) reported that coexistence of $\alpha$-thalassemia increases the incidence of aseptic necrosis in SCD.

**Acute Chest Syndrome:** The role $\alpha$-thalassemia in SCD was found to be also conflicting. Higgs et al, (1982) and Mukherjee et al, (1998) observed the frequency acute chest syndrome in SCD with $\alpha$-thalassemia is low. Whereas Powar et al, (1980) found no such relationship.

**Leg Ulcer:** Leg ulceration was less frequent in SCD with $\alpha$-thalassemia (Higgs et al, 1982; Steinberg et al, 1984; Ballas, 2001).

**Splenic Function:** The effect of $\alpha$-thalassemia on splenic complications is complex. There was a suggestion that acute splenic sequestration (ASS) may be less common in the SCD with $2\alpha$
gene group (Emond et al, 1985) whereas Ballas (2001) observed more acute splenic sequestration in the said group. But preliminary unpublished observations by Higgs et al, (1982) suggest that such patients may be more prone to hypersplenism consistent with observations in older patients. Comparatively more splenomegaly was found in SCD with α-thalassemia by Higgs et al, (1982), Mukherjee et al, (1998) and Ballas (2001).

Cholelithiasis: Alpha thalassemia had a protective effect on Cholelithiasis in SCD patents (Adekile et al, 1996; Haider et al, 1998)

Priapism: Mild protection of α-thalassemia was found in SS disease (Nolan et al, 2004)

Retinopathy: Hayes (1981) and Ballas (2001) observed increased retinopathy in SCD with α-thalassemia.

Growth and Development: No relation of α-thalassemia was found in SCD regarding growth and development.

The clinical complications differed significantly in the SCD with 2α gene from 4α gene, whereas the 3α gene group was observed to be intermediate in all the studies.

The contribution of α-thalassemia is particularly interesting among Indians because of high prevalence in some population group. (Labie et al, 1989; Gupta et al, 1991; Mukherjee et al, 1997). The prevalence of α-thalassemia in the tribal SCD population was 97% as compared to 24% in the nontribal population and most of the SCD patients from the tribal group had only two α genes. In tribals, the MCV, MCH and HbS level was less compared to non tribals. Significantly lower incidence of painful crises was observed in the tribal sample. There was no difference in the incidence of acute chest syndrome (Mukherjee et al, 1997).

g) Association of other Hb variants and Thalassemias: The Inherited abnormalities of Hb may be hemoglobinopathies or thalassemia which leads to modification of α/β chain ratio and thus modulates the HbS polymerization. Some cases β^Sβ^S, β^Sβ and double heterozygote for the β^S gene (β^Sβ^V i.e. HbSC, HbSD, HbSE, HbSHPFH, HbS Beta thal, HbS Delta Beta Thal, Hb lepore etc) may be present in combination with heterozygous or homozygous α-thalassemia condition (Compound heterozygotes) or with normal α globin gene (double heterozygote). Distribution of compound heterozygote is mostly determined by the prevalence and distribution of gene. The
clinical and hematological expressions of all the compound heterozygotes are variable and
determined by advanced molecular biology techniques and DNA sequencing.

The Thalassemias:

The term thalassemia was first used by Whipple and Bradford in 1932. The word is taken
from the Greek word meaning the sea observing that these patients were from Mediterranean origin.

The thalassemias are classified according to their genetic basis by describing the globin
subunit which is synthesized at a reduced rate. The genetic classification of the thalassemia divides
them broadly into $\alpha$, $\beta$, $\gamma$, $\delta\beta$, $\delta$ and $\epsilon\delta\beta$ types (depending on which globin or globins are under
produced).

In 1961 Ingram suggested that there are two major classes of thalassemia, Alpha and Beta in
the same way as there are two types of structural hemoglobin variants for the Alpha and Beta chain.

Beta thalassemia:

Beta Thalassemia is an autosomal recessive disorder characterized by hypochromic
hemolytic anemia and the patients depend on blood transfusion to sustain life. It is caused by
mutations that reduce or abolish the synthesis of $\beta$-globin chains required for the formation of
adult hemoglobin ($HbA, \alpha_2\beta_2$).

Classification:

a) Clinical Classification:

Based on clinical assessment, the $\beta$-thalassemia can be divided into the major forms of
the illness that are severe transfusion dependent and the symptomless minor forms. These can
only be identified by hematological investigation and confirmed by molecular diagnosis. Beta
thalassemia major usually results either from the homozygous inheritance of a particular
mutation or from the compound heterozygous state for two different mutations. It has become
apparent that there are rare forms of moderately severe $\beta$-thalassemia that result from the action
of a single mutant gene.

Another term 'thalassemia intermedia', though it is old fashioned but still retained and is
extremely useful in clinical practice. It describes conditions which, though not as severe as the
### Beta Thalassemia Mutations:

<table>
<thead>
<tr>
<th>Type of Mutation</th>
<th>Consequence</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletional</td>
<td>Absent transcription, unusually high haemoglobin A₂ in heterozygotes</td>
<td>β⁺ thalassaemia</td>
</tr>
<tr>
<td></td>
<td>Reduced transcription</td>
<td>β⁺⁺ thalassaemia</td>
</tr>
<tr>
<td></td>
<td>Reduced transcription, unusually high haemoglobin A, in heterozygotes</td>
<td>Silent β, mild (β⁺⁺) thalassaemia or β⁺⁺ thalassaemia</td>
</tr>
<tr>
<td></td>
<td>Reduced transcription and translation and instability of mRNA</td>
<td>Silent or mild (β⁺⁺) thalassaemia</td>
</tr>
<tr>
<td></td>
<td>Absent transcription</td>
<td>β⁰ thalassaemia (more severe than most other β⁰ thalassaemias)</td>
</tr>
<tr>
<td></td>
<td>Absence of properly spliced mRNA</td>
<td>β⁰ thalassaemia</td>
</tr>
<tr>
<td></td>
<td>Inefficient splicing of mRNA</td>
<td>Silent, mild (β⁺⁺) thalassaemia or occasionally, β⁺⁺ thalassaemia</td>
</tr>
<tr>
<td></td>
<td>Aberrant mRNA is produced in addition to normal mRNA; sometimes a structurally abnormal β chain is produced, which may be highly unstable</td>
<td>Mild (β⁺⁺) thalassaemia, β⁺⁺ thalassaemia or, occasionally, dominant β thalassaemia; haemoglobin E, haemoglobin Malay or haemoglobin Knossus</td>
</tr>
<tr>
<td></td>
<td>Unstable elongated RNA transcript (plus some normal transcript)</td>
<td>β⁺ or mild (β⁺⁺) thalassaemia</td>
</tr>
<tr>
<td></td>
<td>Abnormal processing of mRNA</td>
<td>Silent, mild (β⁺⁺) or β⁺⁺ thalassaemia</td>
</tr>
<tr>
<td></td>
<td>Absent translation (exon 1 and 2 mutations) or translation of aberrant mRNA (exon 3 mutations) leading to a very unstable truncated β chain</td>
<td>β⁺⁺⁺ thalassaemia (exon 1 and 2 mutations) or dominantly inherited β thalassaemia (exon 3 mutations)</td>
</tr>
</tbody>
</table>
major forms, are associated with a more severe degree of anemia than is found in the trait. In practice, this term encompasses a wide spectrum ranging from disorders which are almost as serious as major forms to asymptomatic conditions which are only slightly more severe than the trait. Some heterozygote for thalassemia mutations are clinically and hematologically normal. They are sometimes designated as silent carriers.

b) Genetic Classification:

There are two main varieties of β-thalassemia such as β° thalassemia, in which no β globin is produced and β+ thalassemia in which some β globin is produced but less than the normal. Less severe forms of β-thalassemia are sometimes designated β++ to indicate that the defect in β chain production is mild.

The diagnostic feature of β-thalassemia is an elevated level of HbA2 in heterozygote which is found in most forms of β° and β+ thalassemia. There are, however, less common forms of β-thalassemia in which the HbA2 level is normal in heterozygote. These so-called normal HbA2 β thalassemias are themselves heterogeneous.

Origin and Spread of Beta Thalassemia:

The first evidence that Cooley's anemia is genetically determined was provided by Caminopetros 1936 in Greece. Subsequently study was made by Dameshek (1940). Genetic study was done by Silvestroni and Bianco (1949), Gatto (1947) and Smith (1948). Rich (1952) suggested that there is defect in HbA synthesis with persistent production of HbF in thalassemia. For the first time Kunkel in 1955 identified HbA2. Kelnet and Welnius (1955) observed that in heterozygous thalassemia HbA2 was raised. Later it was observed that HbA2 was normal in some thalassemia major cases.

Prevalence of Beta thalassemia:

a) World: Beta thalassemia is found in a broad belt extending from Mediterranean basin through Middle East to the Far East. It was originally found in Italians, Greek and other people of Mediterranean origin. In Italy it was observed 20% of β-thalassemia near Ferrara (Lovisetto et al. 1955). In Sicily more than 10% people were carrier. α β-thalassemia has been reported in
Turkey, Iran, Pakistan, Syria, Burma and Thailand (Aksoy 1957; Mc Curdy 1961; Ibrahim, 1970; Minnich et al. 1954).

b) India: Beta thalassemia is the commonest single gene disorder in India. The carrier rate in different regions varies between 1% and 17% with a mean of 3.3%. Over 150 different mutations that cause β-thalassemia have been reported from different parts of the world (Baysal and Carver 1995). In Asian Indians, five common and 12 rare mutations have been reported (Varawalla et al. 1992; Jain et al 1994). The frequency of these mutations in the pooled data was IVS-1-5(G→C) 34.1%, the 619-bp deletion 21.0%, IVS-I-1 (G→T) 15.8%, codons 8/9 (+G) 12.1%, and codons 41/42 (-CTTT) 8.7%.

Rare mutations were found in 5.9% subjects of which codon 16 (-C) was the most common (1.6%) followed by the cap site +1 mutation (1%) and those in -88 (C→T) (0.8%) and codons 47/48 (+ATCT) (0.7%). The predominant mutation in most of the states of India was IVS-I-5 (G→C), varying from 85% in the southern states, 76% in Bengal (Eastern India) to 47.6% in Punjab (Northern India) and 13% in migrants from Pakistan. Among migrants from Pakistan, the 619-bp deletion is the most frequent mutation observed (33.3%). The majority of the migrants or their parents had originated from the Eastern (Punjab) part of Pakistan while only 53 (9.2%) were from Sindh in the Western part of Pakistan. Among the Sindhis, the 619-bp deletion accounted for 47.2% of mutant chromosomes (Verma et al. 1997).

Mutations in codons 8/9 (+G) and codons 41/42 (-CTTT) are distributed in all regions of India with a frequency varying between 3% and 15%. Mutations in codon 16, cap+1, codon 15, codon 30 (G→A), and codon 30 (G→C) are present in subjects from all the states. Punjabi and Maharashtra Indians showed minor incidence of varieties of β-thal mutations codon 15 , cap+1,-88(C-G), frame shift (FS)16, FS5, Codon 30, FS 55(+A), IVS-1 3 end (T-G), FS 47/48(+ATCT), Codon 26, IVS-1nt 1(G-A) ranging from 0.7-6% along with the common Asian mutations (Garewal et al. 1994).

c) Orissa: Study by Mishra et al, (1991) observed 8% β-thal trait in Coastal Orissa. In another study from Orissa the prevalence of β-thalassemia was found to be 22% having IVS1-5 G→C as the commonest mutation. (Chhotary et al, 2004).
Sickle Beta Thalassemia (Sβ-thalassemia):

Inheritance of genes for sickle hemoglobin and β-thalassaemia was first described in Italy as microdrepanocytic disease. This was subsequently studied and recognized in many other countries. The places where both genes for sickle cell and thalassemia exist, sickle thalassemia is more common.

The first presumed case of sickle cell thalassemia was reported in India by Neel 1957. Subsequently Chatterjee et al reported an authentic case of sickle cell thalassemia in the year 1959.

Introduction of Hb electrophoresis distinguished main two of type of Sickle Beta Thalassemia (Sβ-thalassemia) i.e. Sβ° thalassemia (Singer et al. 1957) and Sβ+ thalassemia (Smith and Conley 1954; Singer et al 1957) depending on the presence or absence of HbA. Sickle Cell Disease and Sβ-thalassemia disorder normally can not be differentiated with the help of clinical examinations and blood sickling test and as such grouped under generic term SCD.

Genetic aspect of Sβ-Thalassemia:

The typical pattern of inheritance of this disorder is the finding of sickle cell gene in one parent and beta thalassemia gene in the other. Direct transmission of Sβ-thalassemia was described inconclusively in two occasions It is concluded that β structural loci and β-thalassemia loci are alleles or closely linked (Weatherall et al. 2001).

Beta thalassemia gene does not always suppress β chain synthesis completely. Thus cases of Sβ-Thalassemia have a thalassemic blood picture such as low level of HbS and an elevation of HbA2.

Prevalence of Sβ-Thalassemia:

a) World: The occurrence of Sβ-thalassemia is determined by the analysis of distribution and prevalence of the two abnormal genes. It is widely distributed among individuals of Africa and Mediterranean origin. From variable gene frequencies, the crude incidence of both types of Sβ-thalassemia at birth is calculated as approximately 1 in 800 in Ghana and 1 in 5000 in black population of North America. In Jamaica the frequency of Sβ+ thalassemia is approximately 1 in 3000 and of Sβ° thalassemia 1 in 7000 (Serjeant et al, 1979). The disease is well documented

b) India: In Indians Chatterjee et al, (1959); Devi et al, (1969) have documented cases of sickle thalassemia.

c) Orissa: Kar (1991) had observed 8.1% Sβ-thalassemia in Orissa. A hospital based pediatrics study had shown incidence of Sβ-thalassemia to be 6.7% in Orissa (Kar et al, 1997).

Hematological Findings in Sβ-Thalassemia:

In Sβ-thalassemia patients, hematological changes are variable. In the more severely affected cases, there is anemia, with Hb values of 5-10g/dl and an associated reticulocytosis ranging from 10-20%. The red cells show hypochromia and microcytosis with variation in size and shape and variable numbers of target forms. Sickle cells are sometimes seen on the stained film, which may also show Howell-Jolly and Pappenheimer bodies. There is variable number of irreversibly sickled cells in the peripheral blood. There is a reduction in the MCV and MCH, the later values ranging from 20 to 25 pg depending on the particular type of β-thalassemia gene. There may be a slight elevation of the serum bilirubin level.

It has been reported that in the milder forms, minimal degree of anemia with morphological changes of the red cells, MCV and MCH as the only abnormal findings. In severely affected patients, the hematological picture is similar to SCD, the main difference being the fewer target forms and the better haemoglobinized red cells in the later (Serjeant & Serjeant 1979, 1982). In mild forms, the hematological picture may resemble with that of heterozygous β-thalassemia and red cell fragility is usually reduced (Silvestroni & Bianco 1955). The bone marrow shows erythroid hyperplasia with poor haemoglobinization of the red-cell precursors.

Hemoglobin Constitution:

HbA: In the early analyses of the Hb of patients with Sβ-thalassemia, it was noted that two main Hb patterns such as SAF and SF occurred. It also became clear that there is wide variation in the relative amounts of HbA in Sβ-thalassemia. Weatherall (1964) noted the in Afro-
American patients that the level of HbA was usually in the 20-30% range. However, they reported one patient with approximately 5% HbA. In an extensive study of the disorder in Jamaica, Serjeant et al. (1973) suggested that the Hb pattern can be divided into 3 groups such as absence of HbA, or presence of 5-15% and 20-30% HbA. The mean levels of Hb in the latter groups were 12 and 14 g/dl respectively.

The different levels of HbA found in Sβ-thalassemia are associated with different β-thalassemia mutations (Gonzalez-Redondo et al., 1988; Christakis et al., 1991; Kulozik et al., 1991). Inheritance of IVS2-745 C—G mutation in Greek and Turkish populations and IVS1-5G—C in Indian population are associated with (3-5) %, and the IVS1-110 G—A mutation in the Mediterranean population with levels of (8-14) %. On the other hand, in individuals of African origin with the mutations -88 C—T or -29 A—G levels of 18 to 25% HbA are observed. The highest level that has been reported is approximately 45% in compound heterozygote for the β-92 C—T mutation. This results from an extremely mild reduction in β-globin chain synthesis (Divoky et al., 1993; Rosatelli et al., 1995).

HbF: The level of HbF in Sβ-thalassemia is also very variably as reported by Serjeant et al. (1973). It ranges from 0.5 to 21.2% with a mean of approximately 5% in Sβ-thalassemia and means of 4.3 and 7.3% for males and females, respectively in Sβ°-thalassemia.

Unusually high levels of HbF: Although it is unusual to find HbF levels in excess of 10% in patients with Sβ thalassemia, there are many reports of exception. In series of Jamaican and Greek cases reported by Serjeant et al. (1973) and Choremis and Zannos (1957), there were several patients with levels in the 19-25% range. However, they were found in infants or young children. It has been established that there is a decline in HbF levels in the sickling disorders over the first 15 years of life of the patients (Pembrey et al., 1978; Hayes et al., 1985).

Another group of patients with unusually high HbF level was found in the oasis populations of eastern Saudi Arabia (Pembrey et al., 1980) with mild clinical courses. Their HbF values ranged from 4.8 to 27.2% with a mean of 17.3%. The ability to increase HbF production in the presence of the βs gene has been well documented in patients with SCD in this population. The genetic basis is not yet clear but it seems to be a characteristic of both homozygous SCD and heterozygous Sβ-thalassemia among the population of eastern Saudi Arabia. Similar observation
has been reported in Iran (Haghshenass et al, 1977) and in Indian populations in Orissa (Kulozik et al, 1987), that the possible relationship of the high levels of HbF to the β-globin haplotype in these populations, and the Cγ-158 polymorphism. Low levels of HbF i.e. less than 10% are found in African and Mediterranean patients. In Sickle-cell thalassemias after the first few years of life unusually high levels in these populations are sometimes associated with a heterocellular HPFH gene, and high levels are the rule in eastern Saudi Arab and some Indian populations. However there are many exceptions to these generalities. It is not uncommon to find African or Mediterranean patients with HbF levels in the 15-25% range for which there is no obvious explanation (Serjeant 1975; Shaeffer & Moake 1975; Steinberg & Dreilling 1977). In general such patients run a relatively mild course, like those of eastern Saudi Arabia, but again there are exceptions.

**HbA2:** Increased levels of HbA2 in Sβ thalassemic patients have been well documented. In the 56 cases reported by Serjeant et al, (1973), the HbA2 values ranged from 4.7 to 5.4%. The distribution was very similar to that seen in heterozygous β thalassemia. In Sβ0 thalassemia the HbA2 level is enhanced due to less competition by lower cellular concentration of βs globin. The basis of this affinity may be electrostatic in nature since δ chains are more positively charged than βs chains, whereas α chains have a net negative charge (Lehman 1976). Another possibility is that the increase in HbA2 may be due to a decrease in the association of αA globin with βs globin compared to βs chains (Ballas et al, 1997).

An elevated level of HbA2 is not always found in Sβ-thalassemia. One possible explanation for this is that the cells which contain predominantly HbF have a relatively low amount of HbA2, similar to that in homozygous β-thalassemia. Indeed, the differential centrifugation studies reported by Wood et al, (1977) showed that there is an absolute increase in HbA2 in the younger (HbF-poor) cell population as compared with the older (HbF-rich). In 16 Saudi Arabian patients reported by Pembrey et al, (1980) the HbA2 levels ranged from 1.46 to 5.87% and eight of the subjects had levels in the normal range. There was a significant negative correlation between the levels of HbF and A2 in these patients, regardless of whether the HbA2 was expressed as a percentage of the total Hb or of the HbS alone. This finding provides further evidence that the cells which contain relatively high levels of HbF have lower levels of HbA2.
and that the average level of HbA$_2$ in the peripheral blood cells may vary widely depending on the contribution made by the HbF-poor population.

**Clinical Features of Sβ-Thalassemia:**

In all populations in which Sβ-thalassemia is found, both Sβ$^+$ and Sβ$^0$ thalassemia have been observed. The disease is generally milder in American, Jamaican and African populations, in which the mild β$^+$ thalassemia promoter mutations are common. More severely affected Africans have Sβ-thalassemia or the β$^S$ gene interacting with the more severe forms of β-thalassemia. Sβ$^+$ Thalassemia is clinically less severe than Sβ$^0$ Thalassemia. Joint pain in early childhood is the most common presenting symptom in both varieties. In the Jamaican series, the hand-foot syndrome, osteomyelitis, leg ulcers, splenic infarction, jaundice and pneumonia are the common symptoms in majority of patients in early childhood. The hand-foot syndromes, a painful attack of dactylitis leading to swelling of the hands and feet have also been well documented in Jamaica (Serjeant et al, 1973) and Greece (Karpathios et al, 1977).

A large cohort study in Jamaica (Serjeant 1992) and data from the USA from the Cooperative Study of SCD (Gill et al, 1995) have provided valuable informations about the evolution of the clinical and hematological finding in Sβ-thalassemia. The body habit is variable and in some reports growth retardation has been found (Weatherall et al, 2001). In Jamaica, Serjeant et al, (1972) have found that sickle-cell thalassemics are significantly lighter and shorter than non-thalassemics. Patients were shorter, there was delay in skeletal maturation and the mean age of the menarche was increased. In more severely affected patients, there are instances of some bossing of the skull but the gross skeletal deformities characteristic of homozygous β thalassemia are rarely seen (Weatherall et al, 2001).

**Liver and Spleen:** Enlargement of the spleen and liver is usual. However, tendency of splenic fibrosis and atrophy seem to be less marked (Serjeant et al, 1973). In the Jamaican series, half of the patients had hepatomegaly which does not change much with age and about-half the group had palpable spleens. The degree of splenic enlargement is usually moderate but there are well hypersplenism (Rowley & Jacobs 1972). In more severely affected Italian cases as reported by Silvestroni and Bianco (1955), the spleen becomes smaller with increasing age. Retention of function of spleen in this condition relates only to the mild form of Sβ$^+$ thalassemia as seen in
African patients (Barrios et al. 1991). Gross splenomegaly leading to hypersplenism and regular blood transfusion requirement was documented by Serjeant et al., (1973). These complications seem to occur mainly in childhood. Thrombocytopenia due to hypersplenism has also been reported (Rowley & Jacobs 1972).

**Crisis:** Severe VOC episodes including the brain syndrome with convulsions and focal neurological changes have been reported (Monti et al., 1964; Weatherall et al., 2001). Abdominal pain occasionally associated with malena may follow infarction of areas of bowel (Weatherall et al., 2001). Episodes of pulmonary infarction have also been reported (Steinberg & Dreling 1976). During infection particularly associated with parvovirus, there may be aplastic crises in which erythropoiesis is shut down resulting in profound anemia (Serjeant et al., 1973; Brownell et al., 1986). Sequestration crises in which there is rapid splenic enlargement and anemia associated with entrapment of sickled red cells in the spleen have also been reported. (Pearson 1969, Serjeant et al., 1973 and Solanki et al., 1986).

**Infection:** There are limited data on the frequency of infection in the different forms of Sβ-thalassemia.

**Priapism:** Priapism also occurs in Sβ-thalassemia. The pattern and sequelae seem identical to other sickling disorder.

**Bones and Joints:** The sequelae of vascular necrosis of the femoral or humoral heads observed more commonly in Sβ thalassemia lead to variety of complications similar to those that occurs in SCD (Milner et al., 1993). Bone infarction involving the femoral head in four patients has been reported (Serjeant & Ashcroft 1973). Severe bone changes have been observed in Mediterranean patients with this disorder (Miotti & Caramello 1968). Symptomatic effusions into the large joints have also been reported (Van Slyck 1976).

**Leg Ulcer:** Leg ulcers are common in Sβ-thalassemia patients. In the large Jamaican series, Serjeant et al., (1973) have reported leg ulceration in 27% patients of the ages of 10 to 15 years. Leg ulcers seem to be less common in Italian patients (Silvesteroni & Bianco 1955). It is likely that patients’ lifestyles and exposure to trauma play an important role in this complication.

**Ocular Changes:** Ocular changes in thalasemic patients have been reported by several workers (Serjeant et al., 1972, Friberg et al., 1986). In Jamaican patients 14% had some degree of
retinitis and this led to transient visual loss associated with vitreous hemorrhage in some cases. In one case, there was a visual field defect associated with an unusual form of chorioretinal degeneration which resulted from occlusion of the posterior choroidal vessel (Condon et al., 1973).

Renal Complications: As in the other sickling disorders, there appears increase in hematuria in Sβ-thalassemia (Serjeant et al., 1973; Steinberg & Dreiling 1977). The loss of the ability to concentrate urine, characteristic of SCD is not so marked in Sβ-thalassemia (Pearson 1969). However, more recent work in Greece, the disease tends to be more severe because of the low level of HbA that is produced, suggests that progressive renal damage may be common and follow a similar pattern to that of SCD. The investigator concluded that renal involvement in Sβ-thalassemia, at least in the severe form seen in Greece, is much the same as in SCD.

Neurological Complications: There are some reports of episodes characterized by fits and focal neurological sequelae (Monti et al., 1964). Serjeant et al. (1973) mention two patients with neurological complications, one with a subarachnoid hemorrhage following delivery and another with a left VIth nerves lesion. It is not clear whether these observations were causally related to other sickling disorder. The possibility that cerebrovascular accidents in this disease may be associated with the co-inheritance of factor V Leiden has been excluded at least in African populations (Kahn et al., 1997).

Folic Acid Deficiency: Folic acid deficiency leading to megaloblastic erythropoiesis and worsening of the anemia is well documented (Weatherall 1964; Serjeant et al., 1973). This has usually, although not always, been associated with pregnancy.

Fertility and Pregnancy: There is little information about fertility and pregnancy in the patients with this disorder. Presumably pregnancy was less common in the past in these severely affected patients. The most extensive information on this comes from Jamaica. There was a significantly higher incidence of abortion and stillbirth among those with Sβ0 thalassemia. Although pregnancy was well tolerated, complications occurred in the last two trimesters. These included painful crises before or just after delivery, severe postpartum hemorrhage, severe eclampsia and convulsions secondary to a subarachnoid hemorrhage in the postpartum period. As in SCD, there appears to be a likelihood of crises and worsening of anemia during pregnancy.
There is an increased incidence of fetal morbidity and mortality although precise figures are not available.

**Prognosis:** Unfortunately, there is no adequate information about the prognosis in \( \Sigma \beta \) thalassemia. This seems to vary in different parts of the world and probably depends on a wide range of factors including the social-economic background, climate and the genetic variety of disorder. Early reports indicated that the disease is more severe in the Mediterranean population. Silvestroni and Bianco in 1949 reported a high mortality in childhood in a large series of Italian patients. In addition, they reported that successful pregnancies are probably rare in their population. Similarly, Aksoy and Lehmann (1957) mentioned that their Turkish patients had been severely incapacitated due to anemia. On the other hand, the milder forms of \( \Sigma \beta \) thalassemia which is common in the African populations is associated with an extremely good prognosis.

**Comparison between \( \Sigma \beta \)-Thalassemia and Homozygous SCD:**

a) **Comparison of Hematological Indices for \( \Sigma \beta \)-Thalassemia and Homozygous SCD:** There is difference in hematological changes between \( \Sigma \beta \) thalassemia and homozygous SCD. In the latter case the findings are variable as per type of the disease.

- **Total Hemoglobin Level:** In \( \Sigma \beta^0 \) thalassemia (\( \Sigma \beta^0 \) thal) the total Hb level is significantly raised comparatively than SCD (Serjeant et al, 1979) and \( \Sigma \beta^0 \) thalassemia has significantly lower total Hb in comparison to \( \Sigma \beta^+ \) thalassemia (\( \Sigma \beta^+ \) thal) (Serjeant et al, 1982). Whereas Zago et al, (1980) didn’t find any significant difference in between \( \Sigma \beta^0 \) thalassemis and SCD. Kulozik et al, (1991) observed similar level of Hb in \( \Sigma \beta^+ \)thalassemia and SCD.

- **RBC Count:** In the \( \Sigma \beta^0 \) thalassemia T.R.B.C. was found to be significantly higher than SCD (Serjeant et al, 1979; Zago et al, 1980) and less than \( \Sigma \beta^+ \) thal (Serjeant et al, 1982). However study by Kulozik et al, (1991) observed similar RBC Count in \( \Sigma \beta^+ \)thalassemia and SCD.

- **M.C.V.:** Both \( \Sigma \beta^0 \) thalassemia and \( \Sigma \beta^+ \) thalassemia had significantly less MCV in comparison to SCD (Serjeant et al, 1979; Zago et al, 1980; Kulozik et al, 1991). \( \Sigma \beta^+ \) thalassemia had significantly more MCV in comparison to \( \Sigma \beta^0 \) thalassemia (Serjeant et al, 1982).
M.C.H.: Both $\beta^0$ thalassemia and $\beta^+$ thalassemia had significantly less MCH in comparison to SCD (Serjeant et al, 1979; Zago et al, 1980; Kulozik et al, 1991). Serjeant et al, (1982) didn’t find any significant difference in between $\beta^0$ thalassemia and $\beta^+$ thalassemia.

M.C.H.C.: In $\beta^0$ thalassemia, MCHC is significantly raised comparatively than SCD (Serjeant et al, 1979) and $\beta^0$ thalassemia has significantly lower MCHC in comparison to $\beta^+$ thalassemia (Serjeant et al, 1982). Whereas study by Zago et al, (1980) and Kulozik et al, (1991) didn’t find any significant difference in between $\beta^0$ thalassemia and SCD or $\beta^+$ thalassemia and SCD respectively regarding MCHC.

Reticulocyte Count: - Serjeant et al, (1979) found $\beta^0$ thalassemia had more reticulocyte count than SCD; whereas $\beta^+$ thalassemia had significantly lower reticulocyte count than $\beta^0$ thalassemia (Serjeant et al, 1982). Study by Kulozik et al, (1991) observed similar reticulocyte count in both $\beta^+$ thalassemia and SCD.

Hb Constitution:

HbA: In SCD the HbA% range remains up to 5% whereas in $\beta$-thalassemia there is a wide range of HbA% ranging 0% to 45% depending upon the type of associated $\beta$ thal mutation (Serjeant & Serjeant 2001; Weatherall et al, 2001)

HbF: - The HbF level was found to be similar in $\beta^0$ thalassemia, SCD (Serjeant et al, 1979; Zago et al, 1980) and $\beta^+$ thal, SCD (Kulozik et al, 1991). However, $\beta^+$ thalassemia had significantly less HbF level in comparison to $\beta^0$ thalassemia (Serjeant et al, 1982).

HbA2: Both $\beta^0$ thalassemia and $\beta^+$ thalassemia had significantly higher HbA2 in comparison to SCD (Serjeant et al, 1979; Zago et al, 1980; Kulozik et al, 1991). Whereas $\beta^+$ thalassemia had significantly less HbA2 in comparison to $\beta^0$ thalassemia (Serjeant et al, 1982).

HbS: There was similar level of HbS level in patients with $\beta^0$ thalassemia and SCD (Serjeant & Serjeant 2001).
**b) Comparison of Clinical Features of Sβ-Thalassemia and Homozygous SCD:**

Clinically it would be impossible to distinguish Sβ0 thalassemia from SCD, although there are differing frequencies of some complications. Painful crises may be more frequent and splenomegaly and splenic complications are more common than in SCD.

**Painful Crisis:** Painful crisis, dactylitis tend to be more frequent and more severe in Sβ0 thalassemia (Serjeant *et al.*, 1973) than in SCD (Bailey *et al.*, 1991; Platt *et al.*, 1991) whereas painful crisis was found to be similar in the both the above group by Zago *et al.*, (1980). However Kulozik *et al.*, 1991 reported that Sβ+ thalassemia and SCD had similar attack of painful crisis, although the later group had multiple admissions for pain. Both Sβ0 thalassemia and Sβ+ thalassemia had similar incidence of painful crisis whereas significantly more admissions were in the earlier group (Serjeant *et al.*, 1982).

**Bone and Joints:** In Sβ0 thalassemia the pattern of bone involvement is similar to but generally less severe than in SCD and includes medullary infarction (Serjeant *et al.*, 1973) and involvement of the humeral or femoral head (Henderson *et al.*, 1962 and Koneman *et al.*, 1963).

**Splenic Pathology:** Splenomegaly occurs in the majority of Sβ0 thalassemis cases in comparison to SCD (Silverstroni and Bianco 1955) and tends to decline with age as in SCD. Both acute splenic sequestration (Pearson 1969 and Serjeant *et al.*, 1982) and hypersplenism are common and often require splenectomy in Sβ0 thalassemia and are significantly more than SCD (Serjeant *et al.*, 1979). Splenic infarction may occur spontaneously (Serjeant *et al.*, 1973). Both splenomegaly and splenic pain was found to be more in Sβ+ thalassemia than SCD (Kulozik *et al.*, 1991). Sβ0 thal and Sβ+ thalassemia had similar incidence of splenomegaly and the splenectomy was comparatively more in the earlier group (Serjeant *et al.*, 1982).

**Hepatomegaly:** The incidence of hepatomegaly was similar in Sβ0 thalassemia and SCD (Zago *et al.*, 1980). However Sβ+ thalassemia had significantly less hepatomegaly than Sβ0 thalassemia (Serjeant *et al.*, 1982).

**Leg Ulcer:** The leg ulcer was found to be similar in all the three groups Sβ0 thalassemia, Sβ+ thalassemia and SCD (Serjeant *et al.*, 1979; Zago *et al.*, 1980; Serjeant *et al.*, 1982; Kulozik *et al.*, 1991).
Pneumonia: Pneumonia was found to be similar in Sβ° thalassemia and SCD (Serjeant et al, 1979).

Priapism: Priapism was found to be similar in Sβ° thalassemia and SCD (Serjeant et al, 1979) and Sβ+ thalassemia and SCD (Serjeant et al, 1982).

Aplastic Crisis: Aplastic crisis was found to be similar in Sβ° thalassemia and SCD (Serjeant et al, 1979) and Sβ+ thalassemia and SCD (Serjeant et al, 1982).

Other Clinical Features: Cardiomegaly and signs of a hyperdynamic circulation are common in Sβ° thalassemia and consistent with the clinical picture of SCD (Serjeant & Serjeant, 2001). Height and weight are reduced in older children, similar to SCD (Platt et al, 1984), menarche is delayed (Srjeant et al, 1982; Platt et al, 1984) and skeletal development may be retarded (Serjeant et al, 1973).

Interactions between Sβ- Thalassemia and α-Thalassemia:

Phenotype of Sβ-thalassemia can be modified by the co-inheritance of α-thalassemia (Embury et al, 1982, 1984; Higgs et al, 1982). The effect of the co-inheritance of the homozygous state for α+ thalassemia (-α/-α) is not particularly marked and the frequency of acute chest syndrome and leg ulceration is less and persistent splenomegaly is more common.

There has been less opportunity to study the interaction of Sβ-thalassemia with α-thalassemia because of fewer occurrences everywhere. Persistent splenomegaly and splenic sequestration is more common in subjects with four α-globin genes and the co-inheritance of α-thalassemia results in a slightly increased Hb level and decreased reticulocyte response. Interestingly, the MCH and MCV were also higher as has been found in other interactions with α and β thalassemia. Overall, however, the effect of the co-inheritance of α-thalassemia seems to be minimum (Steinberg et al, 1984; Vyas et al, 1988).

B. Environmental Factors Affecting Severity of Sickle Cell Disease:

a) Infections: Infection is the major cause of death in children with SCD. Overwhelming infections caused by encapsulated bacteria, salmonella spp. and Plasmodium falciparum (in malarious areas) are an important cause of morbidity and death in patients of SCD. The most important contributing factors to this increased susceptibility to encapsulated bacteria are: a state
of functional asplenia, an opsonophagocytic defect due to an abnormality of the alternative complement pathway, and a deficiency of specific circulating antibodies. Devitalisation of gut and bone due to repetitive vaso-occlusive crises, saturation of the macrophage system with red cell breakdown products of chronic haemolysis, and underlying splenic and hepatic dysfunction all predispose to salmonella infections.

1. Malaria: Malarial infection is a major determinant of illness and death in patients of SCD. Most common single gene disorders are supposed to be occurring as a result of selection by malaria. J.B.S. Haldane in 1949 suggested that the high frequencies of hemoglobinopathies might be due to selection by malaria.

Although the malaria hypothesis was originally addressed to β-thalassemia, it soon became apparent that the problems posed applied equally to SCD (Silverstrony et al, 1949). The most convincing early evidence for it came from a series of studies on SCD carried out in Africa mostly during the 1950s and 1960s (Motulsky et al, 1964; Allison 1965; Livingstone 1967). Allison obtained three different kinds of evidence regarding interaction between the βS and malaria. First, he demonstrated that children with the Sickle Cell Trait (AS) have a lower parasitemia; second, he showed that adult AS inoculated with P. falciparum did not show infections as frequently as normal individuals; and third, that the frequency of the βS in East Africa is correlated with endemicity of malaria. Although some of this work proved difficult to repeat in detail, later studies confirmed the protective effect of HbS against P. falciparum. In particular, it was found that deaths from cerebral malaria in early childhood were much less common in AS than in normal children.

Recent work (Pasvol. et al., 1978; Friedman et al, 1979) using in vitro culture systems for the malarial parasite has provided clear evidence that the parasite cannot develop in HbS-containing cells maintained reduced oxygen tension similar to that encountered in venous blood or the deep tissues. Indeed, the growth of the parasite is almost completely prevented under these conditions. Because of the reduced rate of development of the parasite at reduced oxygen tensions, it seems likely that the later part of the parasite life cycle will be inhibited and hence some degree of protection afforded to AS. Whether the defective parasite maturation in red cells containing HbS is due to the direct properties of the Hb molecule altering the environment of the cells as they sickle or whether it results from more subtle metabolic changes which occurs
consequent upon sickling and the presence of the parasite in the cell remains to be properly understood.

2. Salmonella Infection: Patients with Sickle cell hemoglobinopathy are more susceptible to osteomyelitis. The commonest causative organism of acute osteomyelitis in general is the Staphylococcus. But it has been claimed that in Sickle cell hemoglobinopathy the Salmonella species is dominate (Ali et al, 1985). Salmonella infections remain a significant cause of morbidity and mortality in patients with SCD. Salmonella dactylitis is the commonest presentation of osteomyelitis in the young child.

3. Tuberculosis: Although pulmonary infection is a common complication of SCD and SCD is frequent in population where the prevalence of tuberculosis is high, the relation between these two diseases is still not clear. Tuberculosis (TB) remains an enormous global health problem worldwide. One third of the world's population is estimated to be latently infected with Mycobacterium tuberculosis. In sub Saharan Africa the prevalence of TB and SCD are both particularly high (Lionnet et al, 2007)

In west Europe, the greatest parts of patients with SCD come from French West Indies and above all from West Africa, where the prevalence of TB is high. So, there are epidemiological, local (sequels of organ damage by vaso occlusive leading to alteration of local host defence) and general (perturbation in immune system, mal nutrition) condition favor the TB in patients with SCD (Almeida et al, 2005)

4. Pneumococcal Infection: People with SCD are especially prone to respiratory infections. These infections are often caused by Streptococcus pneumonia. Infections occur partly due to the spleen not working correctly, but also because damaged tissue and bone resulting from SCD can harbour bacteria. The risk of infection in older children is lower, and the follow-on trial did not show a significant increase in risk when regular penicillin was halted at five years old (Hirst et al, 2002).

Pneumococcal infections used to be the principal cause of death in children with SCD until physicians began routinely giving penicillin on a preventive basis to those who are diagnosed at birth or in early infancy. Spleen damage as a result from sickled red cells, lead to decrease production of antibodies that fight infection. Thus, the bacteria can grow in the blood
stream and cause septicemia. Infants and young children are susceptible to bacterial infections that can kill them in as little as 9 hours from onset of fever. Seventy percent of septicaemias and meningitis among SCD is caused by *Streptococcus pneumoniae*. Septicaemia frequently presents with sudden fever, few prodromal features, and a deceptive appearance of well-being, followed within hours by rapid relentless progression to shock and death. Adrenal haemorrhage is common, and mortality can be as high as 50 per cent, unless intravenous antibiotic, with or without steroid therapy, is promptly initiated. An acute pulmonary involvement, indistinguishable from bacterial pneumonia (the ‘chest syndrome’) is the commonest single complication of SCD at any age. *Str. pneumoniae* is responsible for about half of the episodes. The protective values of the pneumococcal vaccine and long-term penicillin prophylaxis remain to be established in SCD (Onwubalili. 1983).

b) Social class: No controlled data are available which shows that milder disease occurs in patients with better socioeconomic conditions (Konotey-Ahulu 1974). Such amelioration is likely to be multifactorial and influenced by factors such as better nutrition, warmer clothing, more complete immunization, easier access to medical care and better educated and responsive parents. The frequency of painful crises may be reduced by a better understanding of the precipitating factors and sudden falls in Hb requiring transfusion can be minimized by intelligent management of the patient.

c) Diet: Demands of specific nutrients and vitamins such as folic acid, zinc and possibly other trace elements are increased in SCD whereas deficiencies are less likely in patients on well-balanced diet.