1. INTRODUCTION

1.1 Thalassemia Historical Concepts

The name thalassemia derived from a combination of two Greek words: *thalassa* meaning the sea (Cooley *et al.*, 1925, 1927; Bradford and Dye, 1936) that is the Mediterranean and anemia (“weak blood”). Another term found in literature, although infrequently, is Cooley’s anemia and it was believed to be endemic. Prof. Cooley Thomas, a pediatrician in the USA who first described the clinical characteristics of this disorder in patients of Italian origin 1925. The name thalassemia was coined by the Nobel Prize winning pathologist George Hoyt Whipple (1878-1976). Whipple and Bradford (1936) studied the erythroblastic anemia of Cooley and associated pigment anomalies simulating hemochromatosis.

Thalassemia is the name of a group of genetically inherited blood disorder passed down through families in which the body makes an abnormal form of hemoglobin, the protein in red blood cells that carries oxygen. It results in excessive destruction of red blood cells, which leads to anemia. It is not infectious and cannot be passed from one individual to the other by personal or any other contact, or through blood transfusion, food or air (Wikipedia, 2008). Individuals with thalassemia major have severe anemia and hepatosplenomegaly. Without treatment, affected children have severe failure to thrive and shortened life expectancy. Treatment with a regular transfusion program, chelation therapy, bone marrow transplantation and medication aimed at reducing transfusion iron overload, allows for normal growth and development and extends life expectancy into the third to fifth decade (Gene Reviews, 2009).

1.2 Worldwide Distribution

Thalassemia is a major health problem, placing an immeasurable emotional, psychological and economic burden on millions of people around the World (Panos, 2005; Riewpaiboo *et al*. 2010). Recent data indicate that about 7% of the World’s population is a carrier of a hemoglobin disorder and that 3,00,000-5,00,000 children are born each year with the severe homozygous states of these diseases (WHO-March of Dime, 2006). Global distribution of different types of thalassemia is showed in Figure-2.

**Alpha Thalassemia:** It occurs most commonly in persons from Southeast Asia, the Middle East, China, and in those of African descent Worldwide, there are about 26 million alpha thalassaemia carriers people of Southeast Asian origin, with an incidence of 5-15%. Alpha
thalassaemia also occurs in the Mediterranean area (Cyprus, Greece, Turkey and Southern Italy) and parts of the Middle East, with an incidence of around 1%. It is very rare in north Europeans (Renzo Galanello et al, 2005).

**Beta Thalassemia:** It is a complex disease, rare in the United States but common in Africa, the Mediterranean region, the Middle East, the Indian subcontinent, China and throughout southeast Asia in a line stretching from Southern China down the Malaysian peninsula to the Indonesian Islands (Lookopoulos and Kollia, 2001; Bernini, 2001). Beta thalassemia is also known as Mediterranean anemia. It is estimated that over 300,000 affected children are born each year, most with sickle cell disease, while about 60,000-70,000 are born with, beta thalassemia major (Weatherall and Clegg, 2001). It is caused by mutations that result in the reduced or non-production of beta globin chains. Hence, beta thalassemia it is one of the most significant single gene disorders globally (Renzo Galanello et al., 2005).

**Hemoglobin S (HbS):** It occurs at gene frequencies of up to 20% in parts of Africa (Cameroon, Guinea, Uganda and Kenya, Zaire), Saudi Arabia and parts of India. The sickle cell gene has been reported at lower gene frequencies of up to 5% in Nepal; Mediterranean countries such as Turkey, Syria, Lebanon and Greece; Portugal and the coast of North Africa; Iran and the Middle East (Renzo Galanello et al, 2005).

**Hemoglobin E (HbE):** It is abnormal hemoglobin found in Southeast Asians, especially among the Khmer, Laotians, the Zhuang in Guangxi province of the People's Republic of China, India and Sri Lanka. It is most concentrated on the border between Laos, Cambodia and Thailand, an area known as the HbE triangle. It is estimated that 30 million Southeast Asians are heterozygous for HbE and one million are homozygous (Renzo Galanello et al, 2005).

### 1.3 Thalassemia in India

India is a large Southeast Asian country with a population of over one Billion. An estimated 1-3% of the populations are carriers of beta thalassemia, a figure rising up to 17% in some ethnic groups (Sukumaran and Master, 1973; Modell and Petrou, 1983). About 6,000 children are born with thalassemia major each year, more than 30% of births with a major thalassemia syndrome in South East Asia (Modell and Petrou, 1983). Madan Sharma et al. (1998) observed that 10% of the World incidence of Thalassemia. In India, prevalence of
Thalassemia is very high in Punjabi, Sindhi, Gujarati, Bengali, Parsee, Lohana and certain tribes community, i.e. Northern, Western and Eastern parts, while it is much less in the south of India (Shah, 2004).

1.4 Genetic classification of Thalassemia

It depends upon the basis of the type of the affected globin chain. It can be classified into following types (Table-3).

Table - 3. Different types of thalassemia and related disorders (Weatherall 2001).

<table>
<thead>
<tr>
<th>Types of Thalassemia</th>
<th>Disorders (affected globin chains)</th>
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<tbody>
<tr>
<td>α- thalassemia</td>
<td>α&lt;sup&gt;+&lt;/sup&gt;</td>
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<td></td>
<td>α&lt;sup&gt;-&lt;/sup&gt;</td>
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<td></td>
<td>Deletion (−α)</td>
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<td>Non deletion (−α&lt;sup&gt;T&lt;/sup&gt;)</td>
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<tr>
<td>β- thalassemia</td>
<td>β&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>Normal Hb A2</td>
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<td>Type 1 (Silent)</td>
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<td>Type 2</td>
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<td>δβ thalassemia</td>
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<td></td>
<td>(δβ)&lt;sup&gt;-&lt;/sup&gt;</td>
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<td>(Aγδβ)&lt;sup&gt;-&lt;/sup&gt;</td>
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<tr>
<td>δ thalassemia</td>
<td>δ&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>γ Thalassemia</td>
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<td>εγδβ Thalassemia</td>
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<td>Non deletion</td>
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<td>Linked to β – globin genes</td>
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<td></td>
<td>Gγβ+, A γ β+</td>
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<tr>
<td></td>
<td>Unlinked to β – globin genes</td>
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The Hb is made of two proteins, namely alpha globin and beta globin. Thalassemia occurs when there is a defect in a gene that helps control production of one of these proteins. There are many forms of thalassemia. Each type has many different subtypes. Both alpha and beta thalassemia includes thalassemia major (TM) and thalassemia minor (BTMi).

A) α (Alpha) Thalassemia
In this type, people whose hemoglobin does not produce enough alpha protein. It affects the production of normal hemoglobin - a key constituent of human red blood cells. This type of thalassemia is commonly found in Africa, the Middle East, India, Southeast Asia, southern China, and occasionally the Mediterranean region (Cooley's Anemia Foundation, 2010). There are different types of alpha thalassemia that range from mild to severe:

Silent Carrier State: This condition generally causes no health problems and symptom free thalassemic person, because the lack of alpha protein is so small that the hemoglobin functions normally (The Swedish Information Centre for Rare Diseases, 2005).

Hemoglobin Constant Spring: This is an unusual form of Silent Carrier state that is caused by a mutation of the alpha globins. It is called Constant Spring after the region of Jamaica in which it was discovered. With this condition no health problems (Cooley's Anemia Foundation, 2010).

Alpha Thalassemia Trait or Mild Alpha Thalassemia: In this condition, there is lack of alpha protein and Patients has smaller red blood cells and a mild anemia, the condition is symptoms free (Cooley's Anemia Foundation, 2010).

Hemoglobin H Disease: In this condition, there is lack of alpha protein, which causes severe anemia and serious health problems such as bone deformities, enlarged spleen, and fatigue. Abnormal hemoglobin H destroys red blood cells (Cooley's Anemia Foundation, 2010) and causes anaemia.

Hemoglobin H-Constant Spring: This condition is more severe than hemoglobin H disease. Individuals with this condition tend to have a more severe anemia and suffer more frequently from enlargement of the spleen and viral infections (Cooley's Anemia Foundation, 2010).
**Homozygous Constant Spring:** This condition is a variation of hemoglobin H-Constant Spring that occurs when two Constant Spring carriers pass their genes on to their child. This condition is less severe than hemoglobin H Constant Spring and more similar to hemoglobin H disease (Cooley's Anemia Foundation, 2010).

**Hydrops Fetalis or Alpha Thalassemia Major:** In this condition, there are no alpha genes in the individual's DNA, which causes the gamma globins produced by the fetus to form abnormal hemoglobin called hemoglobin Barts. It is the most severe α-thalassemia, the homozygous state for α⁰-thalassemia. All four α-globin genes are not functioning and no α-chains are produced. It causes severe anemia leading to the death of the fetus (Wintrobe and Lee 1999). Individuals with this condition die before or shortly after birth. In some extremely rare cases where the condition is discovered before birth, in utero blood transfusions have allowed the birth of children with hydrops fetalis who then require lifelong medical care and blood transfusions. (Cooley's Anemia Foundation, 2010).

**B) β (beta) Thalassemia**

It affects the production of normal hemoglobin, and does not produce enough beta protein. It is found in people of Mediterranean descent, such as Greeks and Italians, and is also found in the Arabian Peninsula, Africa, Iran, Southeast Asia and Southern China. There are three types of beta thalassemia that range from mild to severe according to their effect on the body (Cooley's Anemia Foundation, 2010).

**Beta-Thalassemia Minor (BTMi) or Thalassemia Trait:** In this type, the lack of beta protein causes no problems in the normal functioning of the hemoglobin. A person with this condition simply carries the genetic trait for thalassemia with no health problems other than a possible mild anemia (Cooley's Anemia Foundation, 2010).

**Beta-Thalassemia Intermedia (BTI):** It is a condition intermediate between the major and minor forms. In this type, the lack of beta protein in the hemoglobin causes a moderate to severe anemia and significant health problems, including enlargement of the spleen and bone deformities (Cooley's Anemia Foundation, 2010). However, there is a wide range in the clinical severity of this condition, and the borderline between thalassemia intermedia and the most severe form, thalassemia major, can be confusing. Affected individuals can often
manage a normal life but may need occasional transfusions that are at times of illness or pregnancy, depending upon the severity of their anemia (Gene Reviews, 2010).

**Thalassemia Major (TM) or Cooley’s anemia:** TM or β-thalassemia occurs when similar gene defects affect production of the beta globin protein (Linda, 2010). This is the most severe form of beta thalassemia in which there is complete lack of beta protein in the hemoglobin, which causes a life-threatening anemia that requires regular blood transfusions and extensive ongoing medical care. These extensive, lifelong blood transfusions lead to iron-overload which must be treated with chelation therapy to prevent early death from organ failure (Cooley's Anemia Foundation, 2010). The low haemoglobin concentration lowers the oxygen level in the blood stream. This is a serious condition that increases the risk of heart failure and is fatal if not treated. For unknown reasons, iron absorption in the gastro-intestinal tract is often enhanced in individuals with TM. This may lead to iron overload and subsequent organ damage (The Swedish Information Centre for Rare Diseases, 2005).

In TM the body tries to compensate for the impaired maturation process by accelerating the space for red blood cell production in the bone marrow. The liver and the spleen, which do not normally produce red blood cells, are also activated. As a result of this extreme activity, the bone marrow cavities expand and the liver and spleen are enlarged. The blood volume increases and, as a consequence, the heart is under great pressure.

**Other Abnormal Types of hemoglobin**

Other “abnormal” types of adult hemoglobin, also known as structural hemoglobin variants, have been identified and these include: mainly (Cooley's Anemia Foundation, 2010).

a. **Hemoglobin S (HbS)**  
b. **Hemoglobin E (HbE)**  
c. **Hemoglobin C (HbC)**  
d. **Hemoglobin D (HbD)**  
e. **Hemoglobin Lepore**

Structural hemoglobin variants can combine with β-thalassemia to produce other related clinically significant blood disorders including:

a. **Hemoglobin E (HbE) / β-thalassemia**  
b. **Hb Lepore / β-thalassemia**  
c. **HbS / β-thalassemia**
There are many forms of thalassemia. Each type has many different subtypes. Both alpha and beta thalassemia includes thalassemia major (TM) and thalassemia minor (BTMi).

1.5 Pathophysiology

**Alpha-Thalassemia**

**Aliases:** ATR, deletion type; ATR-16 syndrome; Alpha-Thalassemia Mental Retardation syndrome; Deletion Type. (Gene Reviews, 2009).

**Gene(s) involved:** HBA1, the gene encoding alpha-globulins, HBA2, the gene encoding alpha-globulins found in the telomeric region of the short arm of chromosome 16. (Gene Reviews, 2009). There are four α-thalassemia syndromes currently recognized; silent carrier, α-thalassemia minor, HbH disease, and hydrops fetalis with Hb Bart’s (Wintrobe and Lee 1999).

**Beta-Thalassemia (BT)**

It is probably the most common single gene disorder causing a major genetic health problem in the World (Khattak and Saleem, 1992). Beta thalassemias are due to mutations in the HBB gene on chromosome 11, also inherited in an autosomal-recessive fashion. The severity of the disease depends on the nature of the mutation. Mutations are characterized as (β0) if they prevent any formation of β chains; they are characterized as (β+) if they allow some β chain formation to occur. In either case there is a relative excess of α chains, but these do not form tetramers: rather, they bind to the membrane of red blood cells; producing damage of the membrane and at high concentrations they form toxic aggregates (Mendelian Inheritance in Man, 1999). It occurs when similar gene defects affect production of the beta globin protein. It is an autosomal recessive genetic disease, caused mainly by point mutations within and near the β globin gene.

Nearly 200 different mutations have been described in patients with β-thalassemia and related disorders. BT is a hereditary anemia characterized by absent to decreased synthesis of β-globin chains resulting in imbalance between α- and β-chains and consequent ineffective erythropoiesis and hemolysis (Schrier, 1997; Olivieri, 1999). BT is characterized by decreased synthesis of β globin chains during erythroid differentiation, usually due to a mutation in the β globin gene that may impair transcription, mRNA processing, or translation. As a result, balanced hemoglobin synthesis and overall erythropoiesis are hampered, producing microcytic and hypochromic erythrocytes (Urbinati, et al. 2006). Beta thalassemia
patients will exhibit many chronic symptoms throughout their lives, including iron over-load from repeated transfusions (Urbinati, *et al.* 2006), making this disease of significant interest to life science and health science researchers.

**Delta (δ) Thalassemia**

Alpha and beta chains being present in hemoglobin about 3% of adult hemoglobin is made of alpha and delta chains. Just as with BT, mutations, affect the ability of this gene to produce delta chains (Gene Reviews, 2009). A mutation that prevents formation of any delta chains is termed a delta mutation, whereas one that decreases but does not eliminate production of delta chain is termed a delta mutation. When one inherits two delta0 mutations, no hemoglobin A2 (alpha2, delta2) can be formed. In this case the genetic counseling is important because a child who is the product of parents each of whom has BT trait has a one in four chance of having beta thalassemia major (Wikipedia.org, 2008).

**Abnormal hemoglobin**

1. Hemoglobin E (HbE),
2. Hemoglobin S (HbS),
3. Hemoglobin C (HbC),
4. Hemoglobin D (HbD),
5. Hemoglobin Lepore.

**Structural Hemoglobin Variants combine with β-thalassemia**

**Haemoglobin E (HbE)/β-thalassaemia:** It is abnormal hemoglobin, particularly amongst people of Southeast Asian ancestry, such as Thais, Vietnamese and Cambodians. If one parent carries the β-thalassaemia trait and the other parent carries the HbE trait, there is a 25% chance in each pregnancy that the child will be born with HbE/β-thalassaemia. It is a severe anemia and its symptoms are usually similar to β-Thalassemia Intermedia but which may be as severe as those seen in TM. The increasing frequency and extreme clinical heterogeneity of the hemoglobin (Hb) E/β-thalassemia syndrome has led to clinical concerns (Weatherall, 2001).

**HbS/β-thalassemia or Sickle cell disease:** Sickle-cell disease was one of the first human genetic variants associated with a specific molecular defect (Pauling *et al.* 1949). Sickle cell disease can occur when an individual inherits the abnormal hemoglobin HbS from both parents or if one parent carries HbS and the other β-Thalassemia. It affects millions of people.
It is a serious inherited genetic disorder, lifelong disease with abnormal shaped red blood cells. People who have sickle cell anemia are born with it. Normal red blood cells are move easily through blood vessels to carry oxygen to all parts of the body but in sickle cell anemia, the body produces abnormal shaped blood cells like a sickle or crescent they don't move easily through blood vessels and block the flow of blood to the limbs and organs. This causes pain, organ damage and anemia. Effective treatments exist for the symptoms and complications of the disease, but there is no cure, although in selected cases bone marrow transplantation may offer a cure.

**Hemoglobin C (HbC) and Hemoglobin D (HbD):** The DNA analysis, heterozygote state for HbC/beta (0)-thalassemia (Fr 8/9 mutation). The studies on the parents showed that mother was a compound heterozygote for HbD(Punjab) and HbC while father had beta-thalassemia trait. It is the first confirmed report of HbC from India (Kumar *et al.* 2007). HbC/beta-thalassemia exhibits a great range in terms of diversity of phenotypes and spectrum of severity. Patients with HbC/beta-thalassemia may live free of symptoms and be diagnosed during routine tests (Galanello and Origa, 2010).

**Hb Lepore/β-thalassemia:** A combination of Hb Lepore with β-thalassemia results in a severe clinical condition resembling β-thalassemia major and is inherited in the same way as the one described above for HbE/β-thalassemia. When one parent carries the β-thalassemia trait and the other parent the Hb Lepore trait there is a 25% chance in each pregnancy that the child will be born with Hb Lepore/β-thalassemia.

### 1.6 Symptoms

The most severe form of alpha thalassemia major causes stillbirth (death of the unborn baby during birth or the late stages of pregnancy). Children born with TM are normal at birth, but major symptoms in early childhood are anemia and mild jaundice. There is always some degree of hepatosplenomegaly, bone changes are variable and range from none to severe deformity, identical to that seen in β-homozygous thalassemia (Patil, 2006). Modell (1976) and Costin *et al.* (1979) explained that the child who is not transfused fails to thrive and shows growth retardation early in life, in association with severe anemia and hypersplenism, he also observed the poor musculature, reduction of body fat, poor appetite and lethargy in thalassemic children.
TM symptoms include fatigue, weakness of the body and shortness of breath. The affected person will have a pale appearance of his skin and he would seem to be more irritable than normal. The skin may also take a yellow discoloration and the abdomen may seem to be protruding. Other thalassemia symptoms are slow growth, dark colored urine and facial bone deformities, shortness of breath, yellow skin coloring. Such thalassemia symptoms or signs may occur at birth or might take about two years of life, to have their occurrence. There are some who may not even experience any such symptoms, if they have one hemoglobin gene affected (Buzzal.com, 2011). Persons with minor forms of alpha and beta thalassemia have small red blood cells, but may not have any symptoms.

1.7 Medical history

**Pedigree Analysis:** A pedigree is a powerful tool in which a diagram of family relationships (uses symbols to represent people and lines) to represent genetic relationships. These diagrams make it easier to visualize relationships within families and help us determine the mode of inheritance (dominant/recessive, autosomal/sex-linked) of genetic diseases using either genotype (alleles present in the gene) or phenotype (Lange et al, 1976; Bennett et al., 1995). It is a Table, chart, diagram, or list of an animal's ancestors, used in genetics in the analysis of Mendelian inheritance, and in the prediction of productivity and breed quality in the offspring (Schuette and Uhlmann 1998; Pratt et al. 2000; Svishcheva, 2007). Physicians can use several approaches to collect family information and construct a pedigree. The most traditional approach is direct questioning of the patient or family informant.

**Trait Analysis:** The following pedigrees will be used to determine whether the trait is autosomal dominant or autosomal recessive. In tracing autosomal alleles, if both parents have the disorder and the offspring do not, the condition is autosomal dominant. If neither parent shows the disorder but some of their children do, the condition is autosomal recessive. A carrier is an individual who appears to be normal, but who is capable of passing on a gene for the disorder. If the characteristic is dominant, there can be no carriers because only a single gene is needed to show the disorder.

**Orofacial complications study:** In thalassemic children, orofacial complications are seen, mainly dental and facial abnormalities include spacing of teeth, open bite, protrusion of maxilla, saddle nose and rodent face maxillary protrusion, anterior teeth spacing, anterior open bite, and deep bite and mucosal discoloration. Bimaxillary protrusion and other
abnormalities are also frequently seen in thalassemia major cases (Salehi et al., 2007). Pratima Raju et al. (2009) are evaluating the oral manifestations as correlates in TM cases in current dental practice in central part of India.

1.8 Testing

Prenatal testing

Prenatal testing is available for many important birth defects. It has been introduced worldwide to prevent severe thalassemia (Kan et al., 1974; Wong et al., 1978; Beris et al., 1995). The aim of prenatal diagnosis is to discover birth defects, syndromes, genetic deficiencies and other disease in the fetus. The testing can be invasive or non-invasive. This enables medical treatment of any birth defects timely or for preparation of treatment of any malady later on (Banerji, 1999). The prenatal diagnosis is performed with several molecular methods by using chorionic villi (CV) sampling, amniotic fluid, and cord blood (Altay and Babak, 1995). Thakur et al. (2000) pointed out the prenatal diagnosis of beta-thalassaemia and other haemoglobinopathies is very essential for thalassemic patients. In India, Colah et al., (2005) studied the prenatal diagnosis of sickle syndromes and Aditi et al., (2004) pointed out the profile of beta-thalassaemia in eastern India and its prenatal diagnosis. There are three types of tests that can determine whether an unborn child has thalassemia.

i) Sampling of amniotic fluid (Amniocentesis)

The foetal cells present in the amniotic fluid are aspirated and then analyzed in the laboratory to determine whether the fetus has thalassemia. This test is used when the pregnancy is quite advanced. It poses no significant risk to the mother but in some. However, in some cases, the test may cause a miscarriage from a few days to a few weeks after the test (Aditi et al., 2004).

ii) Cordocentesis (sampling of fetal blood)

Under ultrasound guidance, a fine needle is inserted through the abdomen into the fetal umbilical cord. About 2–3 ml of blood is aspirated and fetal blood is separated out in the laboratory. In skilled hands 100% pure fetal cells are obtained from the first attempt in the majority of cases. Causes of failure in obtaining pure fetal blood include early gestational age, less than 18 weeks, maternal obesity and posterior placenta. Early gestational age is also the most important cause of occurrence of serious complications in cordocentesis. Globin chain separation with gel electrophoresis is the usual laboratory method of detection (Aditi et al., 2004).
iii) Chorionic villus sampling (CVS)
CVS can be performed somewhat earlier than amniocentesis, at about 10–11 weeks' gestation. Using ultrasound as a guide, the specialist obstetrician removes a small sample of the chorionic villi—cells that contain the same genetic information as the fetus and which will eventually form the placenta. As with amniocentesis, CVS poses no significant risk to the mother. However, there is again a small risk of a miscarriage (Caughey et al., 2006).

Postnatal Diagnosis
If prenatal testing is not conducted in a pregnancy at risk of thalassemia or a sickle cell disorder, testing of the child should be performed to allow early diagnosis and referral to a pediatric hematology centre, if indicated. Although molecular testing can be done at any age, the timing of hematological testing depends on the type of hemoglobin abnormality in question (Sylvie et al., 2008).

Hematological methods
i) Hematological indices.
These hematological parameters are measured by electronic equipment, cell counter used to assess the size, volume of red blood cells and the amount of hemoglobin. Thalassemia is diagnosed when the size and volume of red blood cells and the concentration of hemoglobin inside them are significantly reduced, with hemoglobin levels between 2–6g/dl.

ii) Blood film and RBC morphology.
Microscopic examinations, the red blood cells appear paler (hypochromic) and smaller (microcytic) than normal and majority have abnormal shapes and sizes.

iii) Hemoglobin electrophoresis
This is a process that separates the different proteins that make up a hemoglobin molecule, i.e. HbA, HbA2 and HbF. A diagnosis of thalassemia is indicated where levels of fetal hemoglobin are higher than normal and may vary between 20-90%. HbA2, which usually accounts for up to 3% of normal adult hemoglobin, may be non-existent, reduced, normal or slightly elevated. Hb electrophoresis and HPLC also detect other hemoglobinopathies (S, C, E, OArab, Lepore) that may interact with beta-thalassemia (Galanello and Origa, 2010).
iv) Molecular methods.
Investigation of hematological parameters as well as of genetic mutations to the α, β and γ genes are essential steps, both in confirming a diagnosis of thalassemia and in deciding treatment.

1.9 Common Indian mutations
Nearly 28 mutations in beta globin gene have so far been recorded in India among which eight account for 95% of the cases (IVSI-5 (G→C), IVS1-1 (G→T), CD 8/9 (+G), CD 41/42 (−CTTT), CD 15, HbE, HbS and del 619bp). Complete absence of beta globin on the affected allele (β-gene mutation−619 del, IVS1-nt1 GTA, IVS1-nt5 GTC) found in India (Galanello and Origa, 2010).

1.10 Blood transfusion therapy in Thalassemia patients
i. Confirmed laboratory diagnosis of Thalassemia major
ii. Laboratory criteria: Hb < 7g/dl on 2 occasions, > 2 weeks apart (excluding all other contributory causes such as infections)
iii. Laboratory and clinical criteria, including:
   Hb > 7g/dl with: facial changes, poor growth, fractures, and extramedullary haematopoiesis.
   Patients with beta thalassemia major should receive leucoreduced packed red blood cells with a minimum haemoglobin content of 40g. Reduction to 1 X 106 or less leucocytes per unit is considered the critical threshold for eliminating adverse reactions attributed to contaminated white cells and for preventing platelet alloimmunization. Washed red cells may be beneficial for patients with thalassemia who have repeated severe allergic transfusion reactions. Saline washing of the donor product removes plasma proteins that constitute the target of antibodies in the recipient. In addition, washing of red cell units may remove some erythrocytes from the transfusion product, and it is therefore valuable to monitor post-transfusion haemoglobin levels to ensure attainment of the targeted Hb level Cappellini et al. (2008).

1.11 Adverse reactions to transfusions
Blood transfusion is a tissue transplantation which can cause serious adverse transfusion reactions; it can cause morbidity and death to patients who receive a blood transfusion (TRIP report, 2006). Alloimmunization is a frequent problem that can be prevented by transfusing blood matched for the patient’s extended red blood cell phenotype (not just the ABO and RhD antigens). An alloantibody screen should be performed prior to each transfusion (Elliott
and Laurice, 2008). In 1989 Walker et al., pointed out the alloimmunization by blood transfusion. Sirchia et al., (1995) studied the Red cell alloantibody in thalassemia major patients. Blumberg and Gettings (2003) find out that the WBC reduction of RBC transfusions is associated with decreased incidence of RBC alloimmunization. With the growing knowledge of the immune effects of current blood transfusions and limited data on the immune status of Thalassemia patients, a large study addressing the complex interaction of these factors is needed. Red cell alloimmunization in routinely transfused patients of beta thalassemia major was studied by Gupta et al., (2010).

The management of patients who develop antibodies requires use of blood matched by extended red cell antigen phenotype. The risk of transfusion-transmitted infections, while low, is still a concern for known and emerging pathogens, and annual monitoring for hepatitis B, hepatitis C, and HIV is necessary. People with Thalassemia Major are at major risk of infections (Cimaz et al., 2001; Weiner, 2003) and the risk of bacterial infections increases with the severity of anaemia, splenectomy, acquired viruses such as HIV, syphilis, hepatitis B and C, iron overload and defective adaptive/innate immune response (Piga, and Monasterolo, 2001). Common blood reactions associated with transfusions are Non-hemolytic reactions, Allergic reactions, acute hemolytic reactions, autoimmune hemolytic anemia, Delayed transfusion reactions, Transfusion related acute lung injury (TRALI) and graft vs. host disease (Hira et al., 2011; Elliott and Laurice, 2008). Chronic blood transfusion in thalassemic patients is a double-edged sword. Ultimately thalassemic patients die either due to transfusions or due to lack of it, with the result that they seldom survive beyond the age of 25 years (Neeraj et. al., 2010).

1.12 Transfusion-induced Malarial and other protozoan parasites
Malaria is a serious, sometimes fatal, parasitic disease characterized by high fevers, shaking chills and flu-like illness, and is caused by a parasite that is transmitted from one human to another by the bite of infected Anopheles mosquitoes. The disease now occurs in more than 90 countries Worldwide, and it is estimated that there are over 500 million clinical cases and 2.7 million malaria caused deaths per year, 75% of them are African children. The infection with P. falciparum, if not promptly treated, may be fatal. P. vivax, they are found Worldwide but most commonly in India, Central and South America. The incubation period in the human body is approximately 8-13 days for the symptoms of the disease to become apparent. Infection by this parasite can sometimes lead to life-threatening rupture of spleen. They
remain latent in liver and become active later to infect the person (Emedicinehealth.com, 2006).

1.13 Transmission of infectious agents (Viral and bacterial)
Bacterial contamination of blood can occur during collection, it can grow during storage at room temperature and during refrigeration. Platelet products carry the greatest risk, because they are stored at room temperature. Transfusing contaminated blood, results in septic shock and death. Infections are major complications and cause of death of severe thalassemic patients.

Protozoan infections due to *Babesia* have been implicated in a haemolytic febrile state in splenectomized patients and malaria is repeatedly reported as more severe in asplenic people with an increased risk of death (Boone and Watters, 1995). Many viruses are infected via blood transfusion thalassemic children’s Hossain (2007). Even in countries where residual risk of transmission through blood of clinically significant pathogens like HIV, HBV, HCV and Syphilis has been reduced to minimal levels, problems continue to emerge because: a limited range of known pathogens is targeted in mandatory donor screening (excludes HPV B-19, HCMV, EBV, HAV, *Yersinia enterolitica*, parasites, e.g., malaria). In many regions of the developing World, where thalassemia is most prevalent, continued transmission of hepatitis B, hepatitis C and HIV underscores the importance of promoting the quality of national blood transfusion services, including voluntary blood donations, careful donor selection and screening, and public health services’ provision of necessary immunization. Hepatitis C and HIV are no longer major threats in the North American blood supply (Cappellini *et al.* 2008).

1.14 Transfusion-induced other complications and diseases
**Iron Overload:** The iron in the transfused blood cells builds up in a condition known as "iron overload" and becomes toxic to tissues and organs, particularly the liver (Berry and Marshall, 1967) and heart. Iron overload typically results in the patient's early death from organ failure. It is monitored by estimating serum ferritin levels regularly and if the levels exceed, the patient should be started on iron chelating agents (Shah, 2004). It is the major cause of morbidity for thalassemic patients (Porter, 2001). Even non-transfused patients develop iron overload secondary to increased intestinal absorption of dietary iron, it causes mortality and organ injury. Patients who are not on a transfusion regimen are also prone to
iron overload due to significantly increased intestinal absorption of iron secondary to ineffective erythropoiesis. Under normal circumstances, in humans, iron is transported bound to a carrier protein called transferrin, it transports iron into certain tissues (Cappellini et al., 2000). Because the iron is bound to this protein, other tissues are protected from the toxic effects of free iron. Patients on chronic transfusion rapidly acquire much more iron than which can be bound by transferrin, and free iron levels increase in the blood. There are two goals of iron chelation therapy: the binding of toxic non-transferrin bound iron in the plasma and the removal of iron from the body. In general, significant iron loading of the liver can be detected after about six months of monthly transfusions, while cardiac loading takes about eight to ten years. However, once it starts, iron loading of the heart is very rapid. Evidence of liver damage can occur after about four years of transfusions. Under full chelation with deferoxamine, about 50 percent of liver iron can be removed in four to six months. It takes about 17 months to remove half of the heart iron.

**Compliance with chelation therapy:** It is vital for the thalassemic patient's long term survival. However, many patients find the administration of Desferal so difficult that they do not keep up with it or abandon treatment altogether. If they do not have access to another chelating option, this is extremely dangerous. Lack of compliance with chelation therapy leads to accelerated health problems and early death (Cooley's Anemia Foundation, 2010).

**Splenectomy:** The use of splenectomy in thalassemia has declined in recent years. This is partly due to a decreased prevalence of hypersplenism in adequately transfused patients. There is also an increased appreciation of the adverse effects of splenectomy on blood coagulation. When the spleen continues to enlarge, hypersplenism results and corrective measures such as splenectomy are required. Thromboembolic phenomena, both venous and arterial, are not uncommon in patients with thalassemia, particularly in patients who have undergone splenectomy and who undergo transfusion infrequently. splenomegaly is a common complication caused mainly by massive erythropoieses (Cappellini et al., 2000a). After splenectomy the symptoms of chronic haemolytic jaundice is disappear, but the red blood cells hardly ever regards fragility, thus suggesting that the fragility is an inherent peculiarity.

**Liver Disease:** The principal iron storage pools are located in the liver, spleen, and bone-marrow (Bothwell et al., 1979; Barry. 1973). Thakemgpol and Fucharoen, et al., (1992)
observed the liver tissue injury secondary to iron overload in beta thalassemia/hemoglobin E disease. Liver toxicity can occur as a direct consequence of iron toxicity, from transfusion-acquired hepatitis, and/or from other causes of liver disease such as medications, liver toxins, autoimmune reactions, or metabolic disease (Wilson’s disease, alpha-1 antitrypsin). Liver function and hepatitis serology should be routinely screened in thalassemia patients on chronic transfusion as described below. Due to blood transfusions, many patients with β-thalassemia are infected with either hepatitis C virus (HCV) or hepatitis B virus (HBV), particularly those who were born before the 1990s (Aach et al., 1991). The risk for hepatocellular carcinoma is increased secondary to liver viral infection, iron overload, and longer survival (Borgna-Pignatti et al 2004).

**Endocrine Dysfunction:** Endocrine dysfunction due to iron deposition and toxicity to the endocrine tissue is a common complication of iron overload, causing significant morbidity. Gonadal failure, sterility, and growth failure are common, as well as osteopenia and osteoporosis. Diabetes mellitus may also develop in patients with iron overload. The high rate of endocrine disturbances indicates the importance of regular follow-up of TM patients with regard to endocrine complications of the disease (Yeşim et al. 2002).

**Cardiac Dysfunction:** Cardiac disease is the major cause of death in TM patients with iron overload. The liver and heart have different rates and mechanisms of iron uptake and elimination. Cardiac complications are a main feature of the clinical spectrum in transfusion dependent beta thalassemia (Piomelli et al., 1974). They are the leading cause of death and have been well documented only in patients with TM (Ferrara et al., 2004). The prominent finding in this condition is left ventricle (LV) dysfunction, which is attributed mainly to iron overload, cardiomyopathy and leads gradually to cardiac failure and cardiogenic death (Vogel et al., 2003). Extensive iron deposits are associated with cardiac hypertrophy and dilatation, degeneration of myocardial fibres, and in rare cases fibrosis (Nadeem, et al., 2004). As a result, measurements of ferritin and liver iron do not completely predict cardiac risk; high values are associated with future cardiac iron accumulation, but low values may not necessarily be reassuring.

**Low bone mass (osteoporosis):** Besides bone disfigurement as a result of massive bone marrow expansion, decreased bone density is remarkable, due to osteoporosis and osteomalacia. Bone fracture is common. Bone healing follows blood transfusions; bone
disease is an increasingly recognized cause of serious morbidity in thalassemic patients (Jensen et al., 1998). Often it is detected late and efforts to prevent it and detect it early will reduce morbidity substantially. Patients commonly present with bone deformities, scoliosis, chronic bone pain, osteoporosis, fractures, growth failure, or nerve compression. Failure to achieve a peak bone mass, normally during late adolescence is an important factor contributing to low bone mass (Filosa et al., 1997).

1.15 Dental evaluation
The teeth can be significantly affected in patients with thalassemia, but proper transfusion therapy can prevent many of the changes. However, close dental and orthodontic monitoring is crucial. In addition to regular annual dental care, thalassemia patients should be evaluated by a dentist to determine if bony changes requiring orthodontic treatments have developed. Patients, who are non-transfused, under transfused or who begin transfusion at a later stage in the disease may have some malformations of the facial bones due to marrow expansion. This can affect growth of the teeth and cause malocclusion schedule (Cappellini et al. 2008). Weel et al. (1987) described a case of thalassemia major with gross dental and jaw deformities, which was operated to achieve a satisfactory aesthetic and functional result. Siamopoulou-Mavridou et al. (1992) evaluated flow rates and chemistry of parotid saliva to dental caries and gingivitis in thalassemia major patients.

1.16 Psychosocial Support
Thalassemia imposes a significant intrusion in the lives of patients and their families. The effects are many, sweeping from financial hardships and absence from school and work to significant problems with self-image and self-esteem. All of these issues have a tremendous impact of the effectiveness of therapy and on the quality of life of patients. BT is chronic in nature and requires costly lifelong care and management strategies; they cause significant health care and psychosocial burdens on the patient, the family, the health care system and the community (Weatherall and Clegg, 1981; Modell et al., 2000). Canatan et al., (2003) he observed psychosocial burden of β-thalassemia major in Antalya, South Turkey. Increase risk of psychosocial and behavioral problems in thalassemic patients and their parents indicated the importance of a lifelong psychosocial support for the prevention of mental health issues Masera et al., (1990). The patients and their parents, who were more conscious of the illness, were more worried but more compliant with the therapy and need stronger psychiatric support (Khurana et al., 2006).
1.17 Treatment

In recent years, the treatment of thalassemia in India, both government agencies and non-governmental organizations (NGOs) have initiated programmes to deal with the problem, coordinated with National thalassemia control policy. Treatments for thalassemia depend on the type and severity of the disorder. People who are carriers or who have alpha or beta thalassemia trait have mild or no symptoms. They need little or no treatment.

**Regular Blood Transfusions:** The most common treatment for all major forms of thalassemia is red blood cell transfusions, to maintain Hb above 10gm% is the mainstay of treatment, but this regimen leads to facial and skeletal deformities, poor growth as well as cardiac problems due to chronic anemia and hypoxia. These transfusions are necessary to provide the patient with a temporary supply of healthy red blood cells with normal hemoglobin capable of carrying the oxygen that the patient's body needs. Since the deficiency in thalassemia is that of red cells only packed red cells and not whole blood should be transfused and that too using a leucocyte filter to avoid any allergic reactions or antibody formation which may create problems during future transfusions.

Blood transfusions are usually required every 3-4 weeks, to maintain pre transfusion hemoglobin above 10 gm% and post transfusion hemoglobin at about 12 gm%. Transfusions should be given in an outpatient setting and in a thalassemia care centre which has medical staff trained to care for these patients. This is beneficial to the patients as they meet other patients with similar illness, leading to better psychological acceptance of the disease and its treatment. With repeated transfusions there is always risk of transmitting viral infections like hepatitis B and C and HIV. The advent of HIV has put the importance of safe blood transfusions into sharp focus. It has been recorded that the risk of transmission of HIV from an infected donation transfused to a recipient is well over 90%. According to World Health Organization (WHO) 10% of all HIV infections in developing countries is a result of transfusion with infected blood products (NACA, 2008).

**Iron Chelation Therapy:** The hemoglobin in red blood cells is an iron-rich protein; regular blood transfusions increase iron concentration in the blood. This condition is called iron overload, it damages the liver, heart, and other parts of the body. For preventing this damage, iron chelation therapy is needed to remove excess iron from the body. The heme needs to be degraded to iron, biliverdin IXalpha, and carbon monoxide (Sassa, 2004). To remove excess
iron, patients undergo ‘Iron chelation therapy,’ in which a drug is introduced into the body which binds with excess iron and removes it through the urine or stool. For many years, the only FDA-approved iron chelator was Desferal, which has to be administered through a painful and difficult infusion process. When using Desferal, a needle is attached to a small battery-operated infusion pump and worn under the skin of the stomach or legs five to seven times a week for up to twelve hours (Cooley's Anemia Foundation, 2010). The only treatment options for removing excess iron were chelation (Vichinsky, 2001). While phlebotomy is a very effective way of removing iron, it is not appropriate for patients with thalassemia except after bone marrow transplantation. Thalassemia patients who are not transfusion dependent cannot maintain an adequate hemoglobin level and become symptomatic after phlebotomy. Iron is very toxic to tissue. Detoxification of excess iron is probably the most important function of chelation therapy. Oral chelator’s deferasirox and deferiprone are useful for transfusional iron overload in thalassemia major patients (Neufeld, 2006). It is clear that certain symptoms of iron overload, such as cardiac arrhythmia and heart failure, can be improved well before local tissue levels of iron have decreased by the continual presence of a chelator in the plasma. Galanello et al, (2006) Clinical evaluation of deferasirox, a once-daily oral chelating agent was useful in pediatric patients with beta-thalassemia major.

**Splenectomy:** It is indicated when hypersplenism sets in as indicated by increase in the transfusion requirements. Splenectomy may also be done if massive enlargement of the spleen produces intolerable discomfort. Splenectomy increases the risk of serious infections and hence should be avoided till 6 years of age. The patient should be immunized with pneumococcal, meningococcal and H influenza vaccines at least 2-4 weeks prior to splenectomy. Oral penicillin 250 mg once daily should be given for at least 5 years post splenectomy. Even minor infections should be treated with antibiotics promptly in a splenectomized patient and he should be hospitalized if fever does not subside within 48-72 hours (Shah, 2004).

**Allogenic bone marrow transplantation:** It is currently the only therapy to cure thalassemia in a patient who has an HLA identical sibling donor. It means freedom from transfusions, iron chelation, and all the complications that come with it. Since the procedure requires a sibling who is HLA identical, it can only be applied to a small percentage of patients. It is associated with high morbidity and, in some cases, even mortality. Moreover it is very
expensive-cost of a bone marrow transplant in India could be 800,000-10, 00,000 Rupees and hence many patients cannot afford it (Shah, 2004).

**Gene therapy:** It is being tried by replacing the defective globin gene with a normal functional gene but it is technically difficult and not yet available as a therapeutic option.

**The Compliance Problem:** This can be achieved by increasing awareness about thalassemia, by screening siblings and parents of the patient to identify carriers of the disease, screening the communities in which thalassemia is very common, screening the couple before they plan to have a baby and prenatal diagnosis if the woman is pregnant i.e. testing the fetus for thalassemia major and aborting it if found to have the disease.

**Medication:** Folic acid supplements: Folic acid is a B vitamin that helps build healthy red blood cells. The patient need to take folic acid supplements in addition to blood transfusions and/or iron chelation therapy. Insufficient folic acid can aggravate the anaemia in thalassemia intermedia patients. Folic acid is found naturally in food such as meat and green vegetables. Popular chelators include deferoxamine and deferiprone, of the two, deferoxamine is preferred; it is more effective and is associated with fewer side-effects (Maggio, et al., 2002). Deferasirox (DFX) is a once-daily, orally administered iron chelator that a large program of clinical trials has shown to be effective in adults and children (Cappellini et al., 2006; Galanello and Origa, 2008). The drug treatments are given for thalassemia depends upon patient’s history and complication.

**Diet and vitamin:** Nutritional stunting as the result of reduced nutrient intake is an important cause of growth failure in young children with thalassemia and is responsive to nutritional support in thalassemic children (Fuchsa et al. 1997). The interaction between nutritional status and malaria disease is complex and often controversial in thalassemia patients. Nutritional deficiencies (macro- or micro-nutrient) are thought to lead to malnutrition with subsequent susceptibility to malaria infection (Alice, 2005). Macrocytosis (MCV > 100 fl) is associated with vitamin B12 deficiency and/or foliate deficiency. Anemia secondary to myelodysplasia is usually macrocytic and associated with thrombocytopenia or leucopenia. Rarely, hemolytic anemia is associated with macrocytosis due to the increase in reticulocytes (Irwin, 2010). However, there is no evidence that iron-poor diets are useful in thalassemia major. Only foods very rich in iron (such as liver, many baby foods, breakfast cereals and
multivitamin preparations contain added iron, along with other vitamin supplements) should be avoided. Since many factors in thalassemia promote calcium depletion, a diet containing adequate calcium (e.g. milk, cheese, dairy products and kale) is always recommended (Galanello and Origa, 2010).

The citrus fruits, guava, amla, etc. which are rich in vitamin C should be avoided. Food with high vitamin C content should preferably be taken after cooking to reduce vitamin C content. Folic acid (5 mg per week) should be given to patients receiving no or irregular transfusions, this is because of relative folate deficiency due to increased folate consumption. However, patients receiving regular blood transfusions ordinarily do not require folic acid unless actual deficiency state exists. A normal diet is recommended, with emphasis on the following supplements: folic acid, small doses of ascorbic acid (vitamin C), and alpha-tocopherol (vitamin E). Iron should not be given, and foods rich in iron should be avoided. Drinking coffee or tea has been shown to help decrease absorption of iron in the gut.

**Patients Education:** Patients and their parents and caregivers should be made aware of the nature of their disease, the fact that it is inherited, and the need to comply with the treatments as scheduled to avoid serious complications. Education and employment status of children and adults with thalassemia in North America (Pakbaz *et al.*, 2010).