

# Thalassemia (Beta - Thalassemia)

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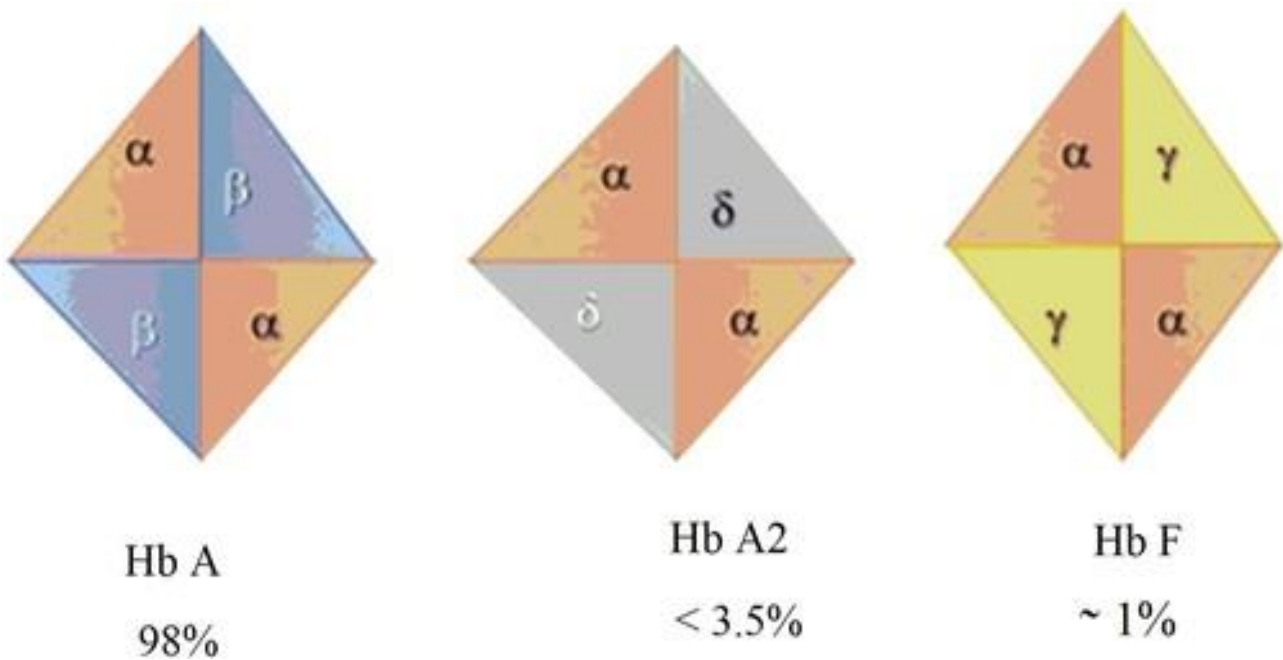
## Abstract:

Thalassemia is an inherited blood disorder characterized by less oxygen carrying protein (Haemoglobin) and fewer red blood cells in the body than normal. There are mainly two types of thalassemia i.e. Alpha and Beta thalassemia about 1-5% of the global population 80-90 million people are the carrier of  $\beta$  thalassemia which is major concern. In this review article fundamental aspects about thalassemia, its history is discussed. This review also focuses upon the types of  $\beta$  thalassemia, pathophysiology, mechanism of transmission, diagnosis of  $\beta$  thalassemia . Finally various treatment strategies and future aspects are addressed.

**Keywords:**  $\beta$  thalassemia, blood disorders.

## 1. Introduction:

Thalassemia is also called as Mediterranean anemia. Thalassemia is an inherited blood disorder that results in faulty synthesis of haemoglobin. Three forms of Hbs are found in normal adults such as HbA, HbA<sub>2</sub>, and HbF that consist of  $\alpha_2$ ;  $\beta_2$ ,  $\alpha_2$ ;  $\delta_2$ , and  $\alpha_2$ ;  $\gamma_2$  subunits, respectively as shown in the Figure 1[1]. Thalassemia can affect on the production of RBC and also affects How long the red blood cells. The name thalassemia is derived from the Greek word “thalassa” meaning “the sea” and “emia” means “anemia”. Because the condition was first described in populations living near the Mediterranean Sea; however, the disease is also wide spreading Africa, the middle East and Asia. Cooley’s anaemia”, as it was first known, was named after Dr. Thomas Benton Cooley, an American pediatrician who was researching childhood anemia when he noticed similar conditions in children of Italian and Greek descent. This form of Thalassemia is now known as beta thalassemia major. In the 1960s, doctors treating thalassemia patients started to transfuse them with fresh red blood cells every month. This alleviated most of the childhood symptoms and led to a major improvement in survival. It is still used as a treatment today. However, since blood contains large amounts of iron, which the body cannot eliminate naturally, most patients died in their teenage years from damage caused by too much iron. Researchers later found that excess iron could be removed from the body by treatment with a drug called desferoxamine. This drug prevented iron-induced heart disease and helped patients live much longer. Recently, two oral drugs have become available to remove iron. They have dramatically improved the quality of life of patients with iron overload from transfusions for thalassemia. Furthermore, specialized imaging tests can now find iron in the heart and allow patients to be treated before they develop iron-related heart failure. Thalassemia have major two types Alpha thalassemia and Beta thalassemia. In this Beta thalassemia is most common than that of the Alpha thalassemia.



**Fig 1: Types of Haemoglobin in normal adult**

Alpha thalassemia is caused due to the deficiency in the synthesis of alpha globulin chain which leads to excessive synthesis of beta globulin chains.  $\beta$ -thalassemia is caused due to the deficiency in the synthesis of beta globulin chain which results by reducing Hb in red blood cells, decreased RBC production and anemia[2]. The genes responsible for making beta globins are located on the chromosome 11 while the alpha globins found on the chromosome 16.[3]

## 2. Types of Beta-thalassemia :

### 2.1 Beta-thalassemia:

#### 2.1.1 Thalassemia major

#### 2.1.2 Thalassemia intermedia

#### 2.1.3 Thalassemia minor (beta-thalassemia carrier)

### 2.2 Beta –Thalassemia with associated Hb abnormalities:

#### 2.2.1 HbC/Beta thalassemia

#### 2.2.2 HbE/Beta thalassemia

#### 2.2.3 HbS/Beta thalassemia

### 2.3. Hereditary persistence of fetal Hb and beta-thalassemia

### 2.4. Autosomal dominant forms

### 2.5. Beta-thalassemia associated with other manifestations:

#### 2.5.1. Beta-thalassemia-tricothiodystrophy

#### 2.5.2. X-linked thrombocytopenia with thalassemia.

### 2.1. Beta thalassemia:

In thalassemia, the unaffected globin genes continue to produce regular globin chains as usual, causing an accumulation of extra mismatched globin in the erythroid precursors. When a person has thalassemia, the unaffected globin genes continue to produce normal globin chains, which causes an overabundance of mismatched globin to build up inside the erythroid precursors. In place of precipitating in the red cell

precursors in the bone marrow and creating inclusion bodies, the free globin chains are unable to form functional tetramers. In the erythroid precursors in the bone marrow as well as in the peripheral red cells after splenectomy, these chain inclusions can be seen using both optical and electron microscopy. The severe intramedullary destruction of the erythroid precursors caused by them is the cause of the inefficient erythropoiesis that underlies all thalassemia[4,5].

**2.1.1. Beta-thalassemia major:**

A hereditary hemolytic anemia called  $\beta$ -thalassemia major necessitates routine red blood cells transfusion, which eventually improve total iron storage [6]. Red blood cells hemolysis from both native and transfused blood serves as primary mechanism for iron build up. The reticuloendothelial(RE) cells of the bone marrow, liver and spleen following their saturation in the myocardial, pancreatic and liver parenchymal cells resulting in significant clinical effects [7,8]. When the people are homozygous (B+/B0, B0/B0) or compound heterozygous (B+/B+) for more severe mutations in the chain, the most severe form of thalassemia, known as Cooley's anemia, develops [9,10]. Usually, it takes 6 months to 2 years to induce. Patients who have significant thalassemia experience severe anemia (heart failure, exhaustion, and cachexia). Hb may be less than 7 g/dl and Hb F may be 90%. When Hb levels dropped, the bone marrow grew to make up for the lost RBCs, but this stunted growth, caused abnormalities in the bones, and enlarged the spleen. Extreme hemolysis causes lithiasis, pulmonary hypertension, and the development of a leg ulcer. Furthermore, this disease is hampered by hypercoagulability[. Regular management with blood or blood product transfusions may lead to iron overload in various organs, which can cause diabetes, hypopituitarism complications in the liver and endocrine glands, such as hypothyroidism, hypopituitarism, hypoparathyroidism, dark metallic skin pigmentation, cirrhosis, cardiac arrhythmia, and myopathy, which can cause 71% of patients with thalassemia major to die [11]. Other side effects of HIV infection include osteoporosis, chronic hepatitis B and C, and blood clots. Additionally, the patients with liver infections are at a greater risk of developing liver cancer[12].

**2.1.2. Beta-thalassemia intermedia:**

A heterogeneous genetic mutation known as intermediate thalassemia causes individuals to have a variable capacity for producing Hb chains (B+/B+, B+/B0). The mutations and are occasionally both present at the same time [9]. Between the ages of 2 and 6 years , it occurs [13]. Anemia in intermediate thalassemia is less severe. Hb levels in this situation range between 7 and 9–10 g/dl, necessitating no blood transfusion. As seen in Table 1, the patient can survive without a blood transfusion or only sometimes needs one [14]. Growth retardation, abnormalities of the bones, and infertility are just a few of the issues that may arise in patients whose bone marrow increases with age. Contrarily, hemolysis increases the amount of iron in several tissues [15].

TABLE 1. Beta-thalassemia genotypes, clinical features/laboratory features

$\beta$ Thalassemia	Globins Chain	$\beta$ Gene	Clinical features	Laboratory features	References
Thalassemia Major (Usually at 4–6	$\alpha_2 \beta_2$ , $\alpha_2 \delta_2$ , $\alpha_2 \gamma_2$	$\beta+/\beta+$ $\beta_0/\beta_0$	Anemia Hepatosplenomegaly Growth retardation	Hb<7g/dl Hb F <90% HbA2 normal	[11]

<b>β Thalassemia</b>	<b>Globins Chain</b>	<b>β Gene</b>	<b>Clinical features</b>	<b>Laboratory features</b>	<b>References</b>
months or child younger than 2 years)	α <sub>2</sub> δ <sub>2</sub> , α <sub>2</sub> γ <sub>2</sub>		Require chronic Transfusion, iron overload	or high HbA absent	
Thalassemia Intermediate (presentation at later age)	α <sub>2</sub> β <sub>2</sub> , α <sub>2</sub> δ <sub>2</sub> , α <sub>2</sub> γ <sub>2</sub>	β+/β0 β+/β+	Milder anemia hepatosplenomegaly	Hb7-10g/dl Hb F >10% HbA <sub>2</sub> (4%–9%) HbA (5%–90%)	[14]
Thalassemia minor (Trait)	α <sub>2</sub> β <sub>2</sub> , α <sub>2</sub> δ <sub>2</sub> , α <sub>2</sub> γ <sub>2</sub>	β+/β β0/β	Asymptomatic Normal to mild anemia No splenomegaly	Hb > 10g/dl Hb F (2.5%–5%) HbA <sub>2</sub> (4%–9%) HbA (>90%)	[18]

### 2.1.3. Beta-thalassemia minor:

When one copy of the globin gene is normal and one copy is defective, it is also known as the thalassemia carrier/trait [B0/B, B+/B;9,16] Most commonly, thalassemia minor develops in children and pregnant women under physiological stress. Due to irregularities in the shape of erythrocytes, it occasionally has moderate anemia but otherwise has no symptoms [17]. Hb levels in people with β-thalassemia minor or carriers may exceed 10 g/dl. If both maternity are carriers, there is a 25% chance of homozygous thalassemia at each gestation [18].

### 2.2. Beta-Thalassemia Associated Hb Abnormalities:

Beta-thalassemia linked to additional Hb abnormalities HbE. Beta-thalassemia interact to produce thalassemia phenotypes that range from moderate form of thalassemia intermedia to a disease i.e. in distinguishable from thalassemia major. Depending upon the symptoms three types are identified:

#### 2.2.1. HbC/β-thalassemia:

Patients with HbC/beta-thalassemia may live free of symptoms and diagnosed during routine tests. When present, clinical manifestations are anemia and enlargement of spleen [13].

#### 2.2.2. HbE/Beta-thalassemia:

It is classified on the basis of severity of the disease

- **Mild HbE/β-thalassemia:**

About 15%of instances of mild HbE/β-thalassemia are found in Southeast Asia. This patient group maintains Hb levels between 9 and 12 g/dl and typically doesn't experience any clinically important issues. Treatment is not necessary.

- **Moderately severe HbE/ $\beta$ -thalassemia:**

The majority of HbE/ $\beta$ -thalassemia cases fall into the group of moderately severe instances. The clinical symptoms continue, and the Hb values are at 6-7g/dl symptoms are same with thalassemia intermedia. Transfusions are not necessary until infectious trigger with anemia.

- **Severe HbE/ $\beta$ -thalassemia:**

The Hb level may range from minimally 4-5 g/dl. This group of patients exhibits symptoms are treated as thalassemia major.

### **2.2.3.HbS/beta-thalassemia:**

The principal process in the molecular pathophysiology of sickling (deoxygenated Hb-S) is polymerization. This is dependent upon the intercellular Hb composition; i.e. dependent on the type and concentration of the other forms of Hb as well as the concentration of Hbs. Consequently, the genotype is the main genetic factor that determines how severe SCD is [19,20,21].

### **2.3. Hereditary persistence of fetal Hb and beta-thalassemia:**

#### **2.4. Autosomal dominant forms:**

Unlike the traditional recessive form of thalassemia, which testing the traditional recessive forms of thalassemia, which results in a decreased synthesis of healthy  $\beta$ -globin chains, some uncommon mutations cause the synthesis of very unstable variations of beta-globin that cause erythroid precursors to precipitate, leading to inefficient haemoglobin causes hemopoiesis. These changes are connected to a manifestation of thalassemia that is clinically evident because they are heterozygous and are consequently called dominant beta thalassemia.[22]

#### **2.5. $\beta$ -thalassemia associated with other manifestation:**

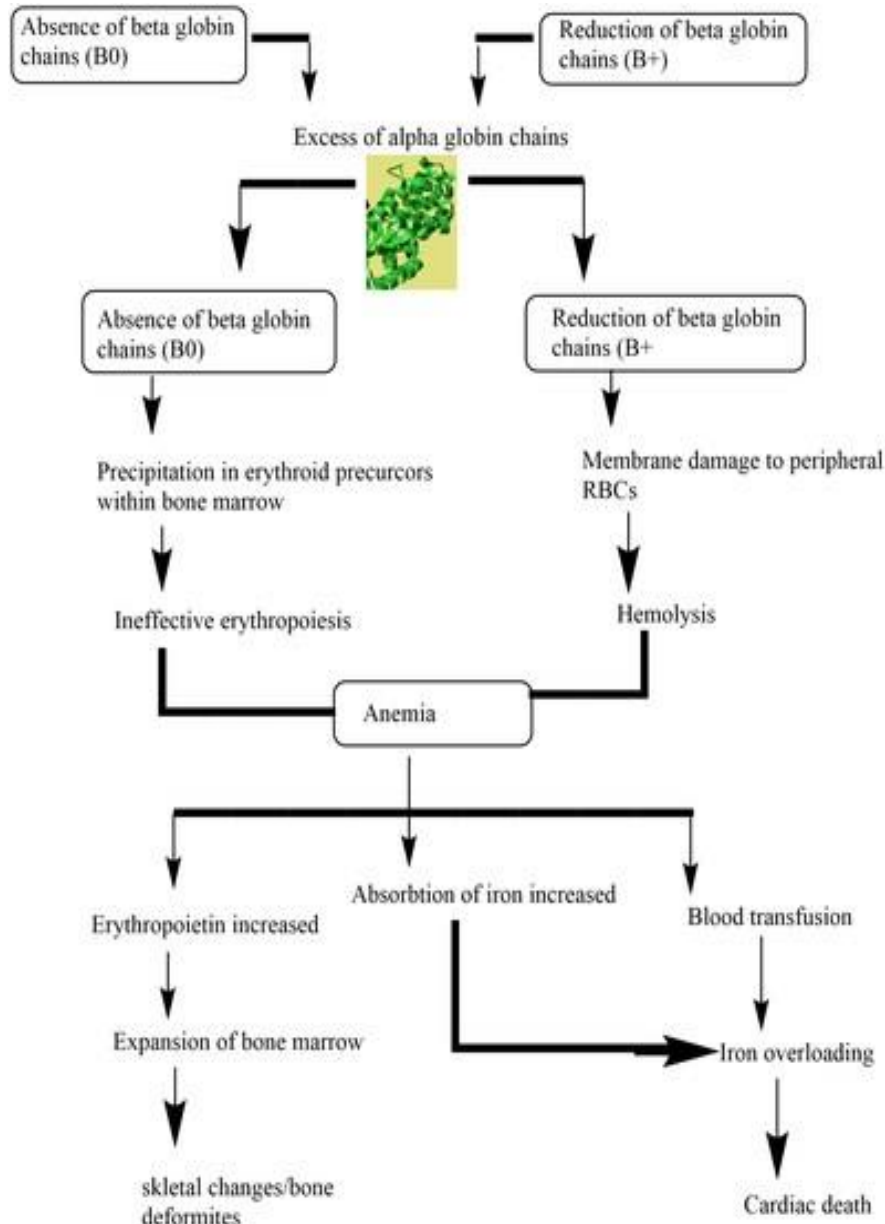
Rarely, the  $\beta$ -globin gene cluster is not where the  $\beta$ -thalassemia deficiency is located. The molecular lessons has been identified either in the gene encoding the transcription factor TFIID ( $\beta$ -thalassemia trait associated with trichothiodystrophy) the X-linked transcription factor GATA-1(X-linked thrombocytopenia with thalassemia) in cases where the  $\beta$ -thalassemia trait is associated.[23,24]

### **3. Pathophysiology of Beta-thalassemia:**

Anemia results from inefficient erythropoiesis, which caused by insufficient ( $\beta_0$ ) or decreased ( $\beta_+$ ) levels of  $\beta$ -globin chain production (fig.2). Excessive unpaired globin chains causes imbalanced and insoluble chemicals to precipitate in erythroid precursors in the bone marrow and injured the plasma membrane (RBCs) also leads to the premature destruction of the erythrocytes. This process is known as erythropoiesis.

Ineffective erythropoiesis or hemolysis which results in anemia by the precursors of immature RBCs and mature RBCs [22].Anemia promotes erythropoietin production, which causes up to 25-30 times the typical bone marrow enlargement and causes abnormalities in the bones. Although the bone marrow speed up RBC production to make up for lost RBCs, this is insufficient to prevent severe anemia. Heme is released when erythrocytes break down which increases the absorption of iron in the digestive system[25].Hepcidin, a protein that regulate the duodenal uptake of iron insufficiently repressed which results in high iron absorption. Iron become overloaded as result of increased erythropoiesis and regular

blood exchange due to the generation of hazardous reactive oxygen species overloaded iron, several organs, especially the heart is affected, including lipid peroxidation of membranes[26].



**Fig.2 : Pathophysiology of beta-thalassemia**

#### 4. HERIDEDITARY TRANSMISSION AND MUTATION OF BETA THALASSEMIA:

Thalassemia is an autosomal recessive congenital disease Children that inherit a mutated single copy of the globin chromosomes from affected parents are required to be heterozygotes. Each offspring of heterozygous parents has a 25% chance of being unaffected and not a carrier, a 25% chance of being affected and a 50% chance of being an asymptomatic carrier at birth [9,27]

The molecular makeup of the thalassemia is variable and functionally crucial areas of the globin gene on chromosome 11 have been found to include more than 200 mutation. It is major point for mutations [28]. Deletions are uncommon in  $\beta$ -thalassemia, which is brought on by absence of or decreased the production rate of globin chain. A list of mutations classified by severity and ethnic distribution, list of mutations shown in table 2 [29,30].

**TABLE2. List of mutations in beta-globin gene**  
**Mutation in β-gene Severity Population**

-101 C→T	β <sup>++</sup>	Mediterranean
-31 A→G	β <sup>++</sup>	Japanese
-619 del	β <sup>0</sup>	Indian
IVS2-nt654 C→T	β <sup>+</sup>	Chinese
-29 A→G	β <sup>++</sup>	African
-28 A→C	β <sup>++</sup>	Southeast Asian
AATAAA AACAAA	to β <sup>++</sup>	African- American

### 5. Diagnosis of beta thalassemia:

Phenotypically, carriers of β thalassemia display a mild form of the disease, which is diagnosed on the basis of a characteristically elevated HbA2 level, reduced mean corpuscular volume [MCV], reduced mean corpuscular hemoglobin [MCH], and slightly increased erythrocytes count [31,32,33]. Carriers of thalassemia may have higher level of HbA2 as a result of transcriptional and occasionally post translational effect. Alteration in gene expression may be included.

#### 5.1. Hematologic diagnosis:

Microcytic anemia is indicated by RBC indices.  $MCV > 50 < 70$  fl and lowered the Hb level (7g/dl) are characteristic of thalassemia major. The mean corpuscular Hb (MCH)  $> 12-20$  pg. Intermittent thalassemia Hb levels in the media are typically between 7-10g/dl [9,34]. RBC morphological alteration in affected individuals included microcytosis, hypochromia, anisocytosis and poikilocytosis (spiculated teardrop and elongated RBCs) and erythroblasts, which are nucleated RBCs. The quantity of erythroblasts influence the level of anemia and is noticeably worsened after less severe RBC morphological alteration are present in carriers than those who are impacted. Normally, erythroblasts are never seen.

#### 5.2. Molecular diagnosis:

Several polymerase chain reaction (PCR) based on techniques are used to detect β-thalassemia mutations [35]. Reverse dot-blot analysis and primer specific amplification, both of which use a set of probes or primer of complementary to the most frequent mutations found in the population from which the patient or carrier originated, two techniques that are frequently used [36]. In the event that the mutation is the dot-spotted, direct sequencing is carried out after denaturing gradient gel electrophoresis [37,38]. Followed by direct sequencing is performed [39].

#### 5.3. Genetic modifiers:

Contrary to expectations, the availability of increasingly precise information on thalassemia mutations at the DNA level resulted in a therapeutic conundrum individuals with the same mutations sometimes

even siblings differ significantly in their levels of clinical severity within the same family. The idea of genetic moderators of thalassemia resulted from this. At first, the basis for the hunt for these modifications was linkage analysis of extensive familial pedigrees [40] or many gene analyses in individuals with distinct manifestation of thalassemia [41]. This topic has been expertly examined [42].

## 6. Treatment of $\beta$ -thalassemia:

### 6.1. Iron chelation therapy:

Each RBC contains 200mg of iron in the case of routine blood transfusions, accumulating iron levels to 0.3-0.6 mg/kg every day [43]. Chelators for iron consist of three groups: deferasirox (DFX), deferoxamine (DFO) and deferiprone (DFP). Taking out one of the most crucial controls of iron is for those people who have blood transfusion [44]. In 2005 approval of Deferasirox (DFX) is highly bioaccumulative chelators that is ingested, for use in transfusional overloaded persons [45]. The half life of DFX is 12-18hrs and recommended dose is 20-30mg/kg orally once a day. The deferoxamine DFO is derivative of streptomyces pilosus with a molecular weight of 657 and 8-10 minutes half life. It enters the parenchymal liver cells. Where it uses the iron chelator deferoxamine to chelate the iron in the bile and plasma. Dose duration varies depending on the patient, depending upon how much iron is overloaded after transfusion. The recommended dose of DFO is 30-40mg/kg daily for each week and 40-50mg/kg in teenagers. Deferiprone infiltrates cell membranes more swiftly than deferoxamine. These compounds are rapidly diffuses across the cell membrane and chelation of intracellular Fe pools are likely responsible for their high activity [46,47]. Deferiprone appears to be able to efficiently remove Fe from the high-affinity Fe-binding sites in serum [48,49]. It is effective at preventing cardiac illnesses induced by iron overload and enhancing cardiac function by removing iron from the heart [50]. The most dangerous adverse effect is agranulocytosis, which has a documented incidence of 0.6 per 100 patient years. [51].

### 6.2. Bone marrow transplant:

for the thalassemia major the only curative treatment for bone marrow transplantation in childhood. Allogeneic bone marrow transplantation is a rational treatment option for  $\beta$ -thalassemia, a genetic condition where the hematopoietic system is the site of the recognized expression of the genetic abnormality. In an untransfused 14-years old with  $\beta$  thalassemia, the first successful transplant was performed. Youngster, who was one month old, and was documented by Thomas in 1982 [52]. In general a hematopoietic stem cells transplant produce great results in low risk individuals, which include liver biopsy revealed no portal fibrosis or hepatomegaly, chelation therapy on regular basis or at most two of these abnormalities [53].

### 6.3. Gene therapy:

The use of a retroviral vector (TN39) transmitting the human  $\beta$ -globin gene has successfully treated murine thalassemia models. The promoter region of the globin gene and its sequence into murine stem cells from mice TI and TM [54,55]. Transfer of the globin gene to the progenitor human hematopoietic cells are also being researched [56,57]. However, concerns about gene transfer include the requirements for increased effectiveness. Virus titers, vector stability, and the accuracy of gene delivery are all



mastered. Nononcogenic insertion, the fluctuating globin gene expression, and the different contributions from the phenotype of thalassemia and other modifiable factors to gene transfer's effectiveness[58].

#### 6.4. Cord blood transplantation:

The reduced risk of virus contamination from a graft, the lower frequency of acute and chronic GVHD, and the ease of accessibility are the potential advantages of umbilical cord blood (UCB) treatment. The small quantity or size of stem cells in the UCB sample compared to the quantity needed for engraftment are most likely the primary reasons causes USB transplant fail; hence this technique mostly applied to people who are younger. Peripheral progenitor cells or bone marrow have been used in conjunction with USB transplantation for certain individuals [59]. Just 77% of cases of USB from unrelated donors have been successful. Only 65% event free survival and 100% survival in 36 thalassemia individuals [60].

#### 6.5. Pharmaceutical drugs for Hb agumentation :

The  $\gamma$ -globin gene is activated by hydroxyurea, which also increases HbF (fetal hemoglobin). One inexpensive and efficient medication that helps certain thalassemia patients reduce the number of blood transfusions needed is hydroxyurea [61]. HbF replaces the dysfunctional hemoglobin by combining two chains with the globin chains [62]. In addition to raising HbF levels, hydroxyurea also raises the body's overall hemoglobin levels. It's efficiency is reliant on the patients genetic composition and it has demonstrated to be a good course of treatment for multiple serious case of thalassemia individuals [63].When treating urea cycle problems, sodium phenylbutyrate was first created as an oral medication to encourage waste nitrogen excretion.[64]

#### 6.6. Thrombosis:

In a recent study, Eldor and Rachmilewitz provided strong clinical evidence supporting patients elevated risk of thrombosis by condensing data from numerous studies. Combined with  $\beta$  thalassemia intermedia and  $\beta$  thalassemia major,  $\beta$  thalassemia hemoglobinopathies, and hemoglobin E/ $\beta$  thalassemia(E/ $\beta$ -thalassemia) [65]. A prevalence was found in a retrospective analysis by Cappellini et al. of 83 patients with thalassemia intermedia and 65 patients with thalassemia major. A 29% incidence of venous thromboembolic events (VTE) [66]. Abnormalities in plasma coagulation factor, exposure to red cell phosphatidylserine, and splenectomy are among the biological risk factors for thrombosis. [65,67,68].

#### 6.7. Other supplements :

Folic acid supplements are used for the treatment of  $\beta$  thalassemia [69]. The creation of purines, pyrimidines, and nucleoproteins is just one of the numerous significant biochemical reactions for which folic acid is a co-enzyme[70,71].

Zinc has been discovered that individuals with thalassemia intermedia have reduced serum zinc levels but not zinc binding capacity [72]. Vitamin C is used for the treatment of  $\beta$  thalassemia in transfusion dependent patient [73]. Vitamin E is also called tocopherol used for the treatment [74]. Magnesium supplements are also being used for treatment [75]. Vitamin B12, also referred to as cobalamin, is essential for blood formation, peripheral nerve function, and nervous system integrity. It's

shortage increases the probability of developing neuropathy [76]. Vitamin B12 is called also called as water soluble vitamin that is play vital role for DNA synthesis, erythropoiesis, and cellular metabolism. [77]. L-carnitine is used for treatment of  $\beta$  thalassemia. It may protect the erythrocytes membrane against oxidative stress and increase the synthesis of HbF [78]. It has been observed that  $\beta$  – chain hemoglobinopathies can benefit from the use of aminobutyric acid , sodium phenyl butyrate, and isobutyramide to increase HbF [79,80,81,82].

## 7. Conclusion:

Thalassemia is a hereditary blood illness that impacts the synthesis of haemoglobin, potentially leading to various health issues such as anemia and fatigue. Treatment choices are contingent upon the kind and degree of thalassemia, which can range from moderate to severe. As the patients do not take proper treatment as early as possible then it leads to the death. In place of participating in red precursors in the bone marrow and creates inclusion body. Because of this the free globin chains are unable to form functional tetramers. Iron chelating agent i.e. deferoxamine if take rapidly by IV infusion then it cause hypertension, blistering and peeling. Deferasirox is oral chelating agent which reduces serum ferritin levels after long term use. It also results in elevation of enzymes. Agranulocytosis and neutropenia are most serious side effects of deferiprone. These treatments are not sufficient for  $\beta$  thalassemia. We have need to enhance the treatment strategies of  $\beta$  thalassemia.

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