The beta-thalassemia is an inherited disorder of autosomal recessive gene disorder caused by impaired synthesis of one or more globin chains. The impairment alters production of hemoglobin (Hb). Thalassemia causes varying degrees of anemia, which can range from significant to life threatening. People of Mediterranean, Middle Eastern, African, and Southeast Asian descent are at higher risk of carrying the genes for thalassemia. These hereditary anemia are caused by mutations that decrease hemoglobin synthesis and red cell survival. These hereditary anemia caused by decreased or absent production of one type of globin chain either α or β globin chain. These hematologic disorders range from asymptomatic to severe anemia that can cause significant morbidity and mortality. It was first recognized clinically in 1925 by Dr. Thomas Cooley, who described a syndrome of anemia with microcytic erythrocytes. Then it was called Cooley’s anemia. Later Wipple and Bradford renamed this disease as “Thalassemia”. Because it was found in the region of the Mediterranean Sea (thalasa is an old Greek word for sea). Beta-thalassemia includes three main forms: Thalassemia major variability referred to as “Cooley’s Anemia” and “Mediterranean Anemia”, Thalassemia
intermedia and Thalassemia minor also called "beta-thalassemia carrier", "beta-thalassemia trait" or "heterozygous beta-thalassemia". Apart from the rare dominant forms, subjects with thalassemia major are homozygotes or compound heterozygotes for beta^0 or beta^+ genes, subjects with thalassemia intermedia are mostly homozygotes or compound heterozygotes and subjects with thalassemia minor are mostly heterozygotes. Thalassemias can cause significant problems because these are inherited disorders, newborn screening and prenatal diagnosis are important in management of patients. This topic will review the clinical features of thalassemia while focusing on genetic modifier factors, pathophysiology, iron overload, complication, management, diagnosis and lifestyle in beta-thalassemia.

**EPIDEMIOLOGY**

It is estimated that 1.5% of the world’s population are carriers of β-thalassaemia with an estimated 60,000 new carriers born each year. Southeast Asia accounts for about 50% of the world’s carriers while Europe and the Americas jointly account for 10-13% of the world carriers. Beta thalassaemia is widespread throughout the Mediterranean with uneven distribution in Greece and Italy, but it is less common at the western end of the Mediterranean and appears to be little in France except in those of Italian or Spanish descent. The disorder is however common in the Middle East and west Asia, and it is probably the commonest inherited haemoglobin disorder in India. Beta - thalassaemia is reported to be between 3-7% in most of North Africa.

**CLINICAL FEATURES**

The β-thalassemias include four clinical syndromes of increasing severity: two conditions are generally asymptomatic, the silent carrier state and β-thalassemia trait, and usually result from the inheritance of one mutant β-globin gene, and two require medical management, thalassemia intermedia and thalassemia major. The more severe forms most often result from homozygosity or compound heterozygosity for a mutant β-globin allele and, occasionally, from heterozygosity for dominant mutations. Homozygous or compound heterozygous β-thalassemia usually presents no diagnostic problems. The early onset of anemia, characteristic blood changes, and elevated fetal hemoglobin concentrations are found in no other condition. The diagnosis can be confirmed by the demonstration of the β-thalassemia trait in both parents. This condition is characterized by mild anemia, reduced mean cell volumes and mean cell hemoglobin concentrations and elevated concentrations of the normal minor adult component of hemoglobin, hemoglobin A2. Thalassemia major and thalassemia intermedia have no specific early clinical correlate but encompass a wide spectrum of clinical and laboratory abnormalities. Patients referred to as having thalassemia major are usually those who come to medical attention in the first year of life and subsequently require regular transfusions to survive. Those who present later or who seldom need transfusions are said to have thalassemia intermedia. After thalassemia is diagnosed, patients who appear not to require immediate transfusion may benefit from a period of observation and folate repletion, particularly if the disease is diagnosed after the age of one year. This approach will allow the identification of patients in whom early growth and development are normal and whose well-compensated anemia may be exacerbated only by infection, folate deficiency, or increasing hypersplenism. With advancing age, even patients with mild forms may have serious complications, including osteopenia, iron loading in tissues, and ectopic marrow expansion.

**GENETIC MODIFIER FACTORS**

More than 200 mutations have been so far reported; the large majority is point mutations in functionally important regions of the beta globin gene. Deletions of the beta globin gene are uncommon. The beta globin gene mutations cause a reduced or absent production of beta globin chains. Modifier genes are defined as genetic variants that lead to differences in disease phenotype. In homozygous β-thalassemia, primary genetic modifiers, affecting the clinical severity of the disease, include genetic variants able to reduce the globin chain imbalance, therefore resulting in a milder form of thalassemia. These factors are the presence of silent or mild β-thalassemia alleles associated with a high residual output of beta globin, the coinheritance of alpha thalassemia and/or of genetic determinants able to sustain a continuous production of gamma globin chains (HbF) in adult life. Some beta-thalassemia mutations (i.e. deletion and non-deletion delta β-thalassemia, deletions of the 5' region of the beta globin gene) increase "per se" the gamma globin gene output. Other mutations increasing HbF production are those associated with deletional and non-deletional hereditary persistence of fetal hemoglobin linked to the beta globin gene cluster. Recently, the genome-wide association approach, particularly studying quantitative trait loci (QTL) which cause elevated HbF, have revealed genetic elements (i.e. polymorphism in BCL11A gene and in the HBS1L-CMYB intragenic region) unlinked to beta globin gene cluster, able to modify the severity of the homozygous beta zero thalassemia. The clinical phenotype of homozygous beta-thalassemia may also be modified by the co-inheritance of other genetic variants mapping outside the globin clusters. These secondary genetic modifiers influence mainly the complications of the thalassemia phenotype. Several secondary genetic modifiers have been identified in the recent years. Less consistent data have been reported for genes involved in iron metabolism (i.e. C282Y and H63D HFE gene mutations), probably because their effect on iron overload is hidden as a result of treatment, and for genes.
associated with bone metabolism. Recently, a polymorphism in glutathione-S transferase M1 gene has been associated with an increased risk of heart iron overload in thalassemia major. In some instances, heterozygous beta-thalassemia may lead to the thalassemia intermedia phenotype instead of the asymptomatic carrier state. Most of these patients have excess functional alpha globin genes (alpha gene triplication or quadruplication) which increase the imbalance in the ratio of alpha/non-alpha globin chain synthesis.

**PATHOGENESIS**

The imbalance between the alpha and beta globin chains of haemoglobin results in thalassaemia. The reduced amount or absence of beta globin chains in beta-thalassaemia result in a relative excess of unbound alpha globin chain that precipitate in erythroid precursors in the bone marrow, this interferes with maturation of the red cells and its destruction in the bone marrow (ineffective erythropoiesis), and it also results in marrow expansion. The resultant hypertrophy of erythroid marrow is characterized by deformation of the bone of the face; it could also result in osteoporosis with pathologic fracture of long bones. The red cell membrane is not unaffected since structural abnormalities of the membrane cause premature destruction of the red cells hence a shortened life span. Peripheral haemolysis contributing to anaemia is less prominent in thalassaemia major than in thalassaemia intermedia, and occur when alpha globin chains induce membrane damage to the red cell. The resulting anaemia stimulates the production of erythropoietin with consequent intensive but ineffective expansion of the bone marrow which causes the said bone deformities (frontal bossing, with enlarged maxilla). Prolonged severe anaemia along with increased erythropoietic drive result in extramedullary erythropoiesis and hepatosplenomegaly, it can also result in the formation of erythropoietic masses which may primarily affect not only the spleen and the liver, but also the lymph nodes and spine. Haemolysis sometimes results in gallstones but this also occurs more commonly in thalassaemia intermedia than major. Although individuals with thalassaemia intermedia are at risk of iron overload, secondary to increased intestinal absorption, hypogonadism, hypothyroidism and diabetes are not common in them. Recent research has revealed that beta-Thalassemia intermedia is not a mild disease and is associated with greater morbidity and a wider spectrum of organ dysfunction and complications than previously thought.

**DIAGNOSIS OF β-THALASSEMAIA**

**CLINICAL DIAGNOSIS**

It is usually suspected in an infant younger than two years of age with severe microcytic anemia, mild jaundice and hepatosplenomegaly. Thalassemia intermedia presents at a later age with similar but milder clinical findings. Carriers are usually asymptomatic, but sometimes may have mild anaemia.

1. **Hematologic diagnosis**

Is characterized by reduced Hb level (<7 g/dl), mean corpuscular volume (MCV) > 50 <70 fl (femtoliters) and mean corpuscular Hb (MCH) >12<20 pg. In peripheral blood smear affected individuals show RBC morphologic changes (microcytosis, hypochromia cells) and nucleated RBC (i.e., erythroblasts). The number of erythroblasts is related to the degree of anemia and is markedly increased after splenectomy. Thalassemia intermedia is characterized by Hb level between 7 and 10 g/dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Thalassemia minor is characterized by reduced MCV and MCH, with increased Hb A2 level.

2. **Qualitative and quantitative Hb analysis**

By cellulose acetate electrophoresis and DE-52 microchromatography or High-performance liquid chromatography identifies the amount and type of Hb present. The Hb pattern in β-thalassemia varies according to β-thalassemia type. In beta-thalassemia, homozygotes HbA is absent and HbF constitutes the 92-95% of the total Hb. In beta-thalassemia homozygotes and beta/beta0 genetic compounds HbA levels are between 10 and 30% and HbF between 70-90%. HbA2 is variable in beta thalassemia homozygotes and it is enhanced in beta thalassemia minor.

3. **Molecular genetic analysis**

The prevalence of a limited number of mutations in each population has greatly facilitated molecular genetic testing. Commonly occurring mutations of the beta globin gene are detected by polymerase chain reaction (PCR)-based procedures. The most commonly used methods are reverse dot blot analysis or primer-specific amplification, with a set of probes or primers complementary to the most common mutations in the population from which the affected individual originated.

**IRON OVERLOAD IN β-THALASSEMAIA PATIENTS**

When red blood cells senesce, transfused red cells are phagocytized by reticuloendothelial macrophages where the hemoglobin is digested and the iron is freed from the heme. With a continuous increase in the iron load because of frequent transfusions e.g. thalassemia major and sickle cell. Quantitatively, one unit of packed RBCs that used in the transfusion regimen contains approximately 200 mg of iron. Thus, with regular blood transfusion a 6 years old thalassemic patient (who has receiving 60-75 units of packed RBCs) is expected to accumulate 12-15 grams of excess iron, compared to 3-4 grams found in normal nontransfused adults. The excess iron in the cytosol of the macrophages starts...
spilling out into the plasma where transferrin binds the released iron\(^2\). However, as transferrin is increasingly saturated with iron, iron storage in hepatocytes starts. As the storage capacity of the hepatocytes and the macrophages gets saturated, circulating iron surmounts the binding capacity of transferrin. Therefore, non-transferrin-bound-iron (NTBI) starts circulating in the plasma and is deposited in cardiac myocytes, hepatocytes, pituitary cells, and pancreatic cells. Reactive oxygen species produced by the metabolism of NTBI play a central role in inducing cellular dysfunction, apoptosis, and necrosis\(^3\).

Iron distribution is modulated by the synthesis of hepcidin, a hepatic peptide, whereby increased hepcidin synthesis decreases iron release from enterocytes, hepatocytes, and macrophages through binding to ferroportin, the iron exporter, and causing its internalization\(^2,3,33\). Even though ineffective erythropoiesis is significantly improved by transfusions in transfusion-dependent thalassemia, hepcidin suppression might contribute to iron overload, especially later in the transfusion-to-transfusion intervals. Hepcidin can be used as a molecular predictor for the severity of cardiac iron overload and can be used as a future target for therapy in β-thalassemia major patients. It has been suggested that the production of growth differentiation factor 15 (GDF15) and possibly other proteins, such as twisted-gastrulation 1 (TWSG1), contributes to the inhibition of hepcidin synthesis and thus promotes iron absorption despite systemic iron overload\(^3,33\). Nevertheless, more recent studies argue against the role of GDF15 in hepcidin suppression\(^3,34\). Kautz et al suggested that, upon erythropoietic stimulation, bone marrow and spleen erythroblasts increasingly produce erythroferrone, which, upon secretion into the circulation, directly acts on the liver to inhibit hepcidin production\(^3\).

In β-thalassemia major patients had increased iron deposition in tissues, this was detected at the laboratory level by elevated serum ferritin and increased iron concentration in liver biopsy, in addition to the decreased magnetic relaxation time T2* radiologically. T2* is very sensitive in detecting preclinical myocardial iron overload\(^3\).

**COMPLICATIONS OF β-THALASSEMIA**

Transfused thalassemic patients may develop complications related to iron overload. Complications of iron overload in children include growth retardation and failure or delay of sexual maturation. Later iron overload-related complications include involvement of the heart, liver (fibrosis and cirrhosis), and endocrine glands (diabetes mellitus, hypogonadism and insufficiency of the parathyroid, thyroid, pituitary, and, less commonly, adrenal glands\(^1,34,40\)). Complications are splenomegaly, chronic hepatitis (resulting from infection with viruses that cause hepatitis B and/or C), human immunodeficiency virus (HIV) infection, venous thrombosis, and osteoporosis\(^3\). The risk for hepatocellular carcinoma is increased in patients with liver, viral infection and iron overload. Individuals who have not been regularly transfused usually die before the second-third decade; survival of individuals who have been regularly transfused and treated with appropriate chelation extends beyond age of 40 years. Cardiac disease caused by myocardial siderosis is the most important life-limiting complication of iron overload in β-thalassemia. In fact, cardiac complications are the cause of the deaths in 71% of the patients with β-thalassemia major\(^2\).

**MANAGEMENT OF β-THALASSEMIA**

1. **Prevention strategies**

Prevention of β-thalassemia is based on public awareness of the disease, detection of carriers, genetic counseling, and prenatal testing\(^3\).

2. **Blood transfusion**

Patients with thalassaemia major are transfusion dependent but this is not so in thalassaemia intermedia. The most difficult therapeutic choice that needs to be made when treating a patient with thalassaemia intermedia is whether or not to initiate a chronic transfusion programme\(^2\). RBC transfusions on a regular basis in beta-thalassemics aim at suppressing the erythropoietic response which is induced by tissue hypoxia\(^4\). It should also be noted that transfusion regimen initiated in childhood to favour growth, can be discontinued after puberty. Patients with thalassemia major require periodic and lifelong blood transfusions every 2-3 weeks to maintain a hemoglobin level higher than 9.5 gm/dl and sustain normal growth.

3. **Chelation therapy**

Since the body has no effective means of effectively removing iron, the only way to remove excess iron is to use iron chelators. The major step forward in improving survival and reducing complications was the introduction, of the chelating agent deferoxamine, used as a subcutaneous infusion. Two oral chelators, deferiprone and deferasirox, have recently become available, making therapy easier and more efficacious. Compliance, although improved by the switch to oral therapy, still presents a problem and is the major obstacle to effective prevention of iron overload. The orally active chelators seem to be more effective in gaining access to the chelatable iron pool of cardiomyocytes, binding labile iron, and attenuating reactive oxygen species formation\(^6\). Other study showed that the combination therapy is associated with lower risk of mortality\(^7\).

4. **Stem Cell Transplantation**

Haemopoietic stem cell transplantation is available for patients who have a related or unrelated human leukocyte antigen (HLA)-identical donor. The probability of cure ranges from 90-95% for recipients of grafts from relatives 21 to 80-85% for those receiving...
grants from an unrelated donor, although the probability even in these cases approaches 90% when donor matching is as strict as that between HLA-identical siblings. The transplant related mortality, even in the best conditions, average 5%, a risk that is worth running probably only in severe transfusion dependent disease.

5. Gene Therapy
This offers a potential cure for β-thalassaemia and would represent an ideal alternative to both conventional therapy and bone marrow transplantation. Gene therapy however poses some challenges among which is the instability and poor expression of retroviral vectors carrying the human β-globin cassette. Considerable progress has now been made using lentiviral vectors which stably transmit the β-globin expression cassette. HbF reactivation by 5-azacytidine, Butyrate and Hydroxyurea (Hydroxyurea) has been found to be equally effective in thalassemia patients. Recombinant Human Erythropoetin (rHuEPO) can increase haemoglobin level in some patients with thalassaemia intermedia.

6. Splenectomy
If the annual red cell requirement exceeds 180-200 ml/Kg of RBC (assuming that the Hematocrit of the unit of red cells is about 75%), splenectomy should be considered, provided that other reasons for increased consumption, such as hemolytic reactions, have been excluded. Other indications for splenectomy are symptoms of splenic enlargement, leukopenia and/or thrombocytopenia and increasing iron overload despite good chelation.

LIFESTYLE IN β-THALASSEMIA
If the disease is fully compensated by ideal treatment, an individual with thalassemia major can enjoy a near-normal lifestyle and experience normal physical and emotional development from childhood to adulthood. Patients with thalassemia do not have specific dietary requirements, unless they have special prescriptions. Patients already have a heavy treatment schedule and it is counterproductive to add further restrictions without the likelihood of clear benefit. During growth, a normal energy intake with normal fat and sugar content is recommended. During adolescence and adult life, a diet low in highly refined carbohydrates may be useful in preventing or delaying the onset of impaired glucose tolerance or diabetes. Increased iron absorption from the intestinal tract is characteristic of thalassemia. The amount depends on the degree of erythropoiesis, the Hb level and other potential independent factors. However, there is no evidence that iron-poor diets are useful in thalassemia major; only foods very rich in iron should be avoided. Since many factors in thalassemia promote calcium depletion, a diet containing adequate calcium is always recommended and phosphorus and 25-hydroxy vitamin D and assessment of bone mineral density for the early detection of osteoporosis or osteopenia.

Patients with thalassemia who remain untransfused or are on low transfusion regimens have increased folate consumption and may develop a relative folate deficiency. Iron overload causes vitamin C to be oxidized at an increased rate, leading to vitamin C deficiency in some patients. Fifty mg of vitamin C in children <10 years and 100 mg >10 years at the time of DFO infusion may increase the ‘chelatable iron’ available in the body, thus increasing the efficacy of chelation. Vitamin C may increase iron absorption from the gut, labile iron and hence iron toxicity and may therefore be particularly harmful to patients who are not receiving DFO, as iron mobilized by the vitamin C will remain unbound, causing tissue damage. The effectiveness and safety of vitamin E supplementation in thalassemia major has not been formally assessed and it is not possible to give recommendations about its use at this time. Conditions requiring special attention include splenomegaly, severe heart disease and osteoporosis. There is no reason for patients with thalassemia to skip or delay standard recommended vaccinations. To prevent and minimize the risk of infection, immunization with vaccine is recommended. It is now universally recognized that thalassemia, like other chronic diseases, has important psychological implications. The way in which the family and the patient come to terms with the disease and its treatment will have a critical effect on the patient’s survival and quality of life, and a general acceptance by the patient of his/her own condition constitutes the key to normal development from childhood to adulthood. A key role for treating physicians and other health care professionals is to help patients and families to face up to the difficult demands of treatment, while maintaining a positive role.

REFERENCES


