CURRENT MANAGEMENT OF HOMOZYGOUS BETA THALASSEMIA

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Thalassemia syndromes are a group of inherited disorders in which major advances have been made in the understanding of the disease processes, molecular biology and genetics leading to several breakthroughs such as antenatal diagnosis, bone marrow transplantation and management over the last three decades. Over 180 million people in the World and over 20 million people in India carry the thalassemia gene(1). A beta thalassemia belt extends from mediterranean area and continues through Turkey, Iran, Southeast Asia and Southern China with the highest gene frequency (5-15%) in the Italian and Greek population. Incidence of the thalassemia trait in our country varies between 3-15% in North Indians to 1-2% in the South. Its prevalence is high in certain communities such as Punjabis who migrated from West Pakistan, Sindhis, Lohana, Agri, Gaud Saraswats, Mahars, *etc*. It has been estimated that over 6000-8000 babies are born in India every year with thalassemia major and unfortunately 80-90% of these children die either undiagnosed because of inadequate facilities, poor management and/or financial constraints.

Underlying mechanism in thalassemia is secondary to reduced or absent beta chain production resulting in (*i*) precipitation of alpha chains resulting in reduced red cell survival, (*ii*) increased production of fetal hemoglobin, and (*iii*) ineffective erythropoiesis. Major complications of beta thalassemia result primarily from (*i*) anemia, (*ii*) excessive hematopoiesis, (*iii*) chronic hemolysis, and (*iv*) iron overload due to increased gastrointestinal absorption and transfusions (*Table I*).

Management

Current management aims to prevent the effects of thalassemia, to ensure normal growth and development and to prevent the disease by genetic counselling and antenatal diagnosis. The mainstay of current therapy is blood transfusion, iron chelation, splenectomy and supportive therapy.

Blood Transfusion

In sixties, blood transfusion was administered to prevent serious complications of anemia and the hemoglobin was maintained around 6 g/dl with the fear, that more blood will be required to maintain hemoglobin at higher levels which may result in excessive transfusional iron overload. These children suffered from chronic and recurrent symptoms of anemia, facial

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TABLE I—*Effects of Beta Thalassemia Major*

(*i*)	*Anemia*
	Tachycardia, cardiomegaly, chronic hypoxia, leading to stunted growth, poor psychomotor development, congestive heart failure and death
(*ii*)	*Excessive hematopoiesis*
	Cranio-facial bone changes, spinal cord compression leading to stunting of child, pathological fractures. Lymphadenopathy and hepatosplenomegaly due to extramedullary hematopoiesis, abdominal distension. Increased iron absorption from gut
(*iii*)	*Chronic hemolysis*
	Gallstones, leg ulcers, jaundice
(*iv*)	*Iron overload*
	Liver disease—hepatic fibrosis, cirrhosis Endocrine diseases—Hypogonadism, hypothyroidism, diabetes, hypopituitarism, hypoparathyroidism

deformities, poor growth and various endocrinopathies. Life expectancy varied between 10-15 years. This form of therapy resulted in increased iron absorption from the gut secondary to excessive erythropoiesis.

In late 1960s and early 1970s, children were managed with hypertransfusion to ameliorate the symptoms of chronic anemia. This was aimed to prevent anemia and to maintain circulating hemoglobin (Hb) levels sufficient to suppress endogenous erythropoiesis. Hemoglobin in these regimes was maintained above 10 g/dl(2-5). Main advantages of this regime were: (*i*) improved physical and psychological well being; (*ii*) normal growth and development; (*iii*) decreased incidence of cardiomegaly; (*iv*) decreased hepatosplenomegaly and facial deformities; (*v*) fewer orthodontic and orthopedic problems; (*iv*) decreased iron absorption from gut; (*vii*) fewer infections; and (*viii*) delay or prevention of hypersplenism. There were overwhelming advantages of these regimes and iron toxicity did not occur at an earlier age. Initial blood requirements were more, however, overall blood requirement to maintain hemoglobin at higher levels was nearly identical.

In some centres a more aggressive transfusion programme has been followed to maintain hemoglobin above 12 g/dl (supertransfusion)(6,7). Initial studies have revealed decrease in blood volume and erythropoietic response. Long term studies are essential for complete evaluation.

The authors' policy is to maintain hemoglobin above 10 g/dl. Majority of children require transfusion every 3-4 weeks which is done in a day care centre to minimize the hospital stay. A single transfusion consists of 15-20 ml/kg of washed packed cells along with frusemide, which raises the hemoglobin by 4-5 g/dl. Sensitization against minor blood groups (observed in 5-15% of these cases) can be prevented by complete blood typing including minor blood groups. Sensitization to serum proteins and or leucocyte antigens can be avoided by using washed red cells, which removes 100% of serum proteins and 95% of leucocyte antigens. Leucocyte filters also accomplish this objective but are expensive. Red cells frozen in glycerol are also free of serum proteins and leucocytes and may be administered after thawing and several washings. Febrile reactions can be easily managed by paracetamol or antihistaminics. Some children on regular blood tranfusion therapy develop isoantibodies to minor blood group antigens such as Kell,

Duffy, C, c, E, *etc*. In such situations red cell elute may be tested against a panel of antigens to identify the offending antibody and to select appropriate donor blood(8). Only Coomb's tested and meticulously cross matched blood should be administered to these children. Plasmapheresis may be undertaken to reduce antibodies and compatible blood may be obtained from a regional centre. However, splenectomy under such circumstances may be useful.

The treating pediatrician should maintain a record of: (*i*) pre- and post-transfusion Hb levels, (*ii*) frequency and amount of blood transfused each time, and (*iii*) transfusion reactions and treatment required, if any. Generally these children need 150-200 ml/kg body weight/year. Children need to be investigated for hypersplenism or the development of immune antibodies if the requirements exceed 300 ml/kg/year.

Transmission of Viral Infections

This is a major risk in these children. Hepatitis B vaccine should be administered to all newly diagnosed patients and to older patients who lack demonstrable antibodies to prevent infection(9,10). Non-A, non-B hepatitis (Hepatitis C) is a major transfusion hazard with an incidence of 6 to 8% per transfusion. It may progress in nearly 50% of cases to chronic hepatitis and in 25% to cirrhosis(11). Identification of the Hepatitis C virus and development of serological tests for screening of donors is likely to reduce its incidence(12,13). Risk of developing human immunodeficiency virus (HIV) infection has been reduced to 1/150,000 per unit transfused by appropriate screening and testing of blood products(14). Currently, effective methods are not available to prevent other diseases such as CMV, toxoplasmosis and diseases caused by the Epstein Barr virus.

Neocyte transfusion

An innovative technique has been developed to transfuse neocytes to children through continuous flow cell separator. Average survival of these neocytes is 90 days in the recipient and therefore, it results in reduced blood requirements and iron overload(6). The high expense involved in this treatment modality has prevented its use even in the developed countries.

Splenectomy

Patients with thalassemia intermedia and major invariably develop splenomegaly due to extra medullary hematopoiesis, reticuloendothelial hyperplasia and iron deposition. However, development of hypersplenism and splenomegaly can be prevented or reduced if hypertransfusion therapy is started early in life(3,4). Indications of splenectomy are given in *Table II*. Splenectomy predisposes these patients to greater risk of severe pneumococcal, meningococcal or *Hemophilus influenza* infections(15,16). Infection secondary to other microbes may occur in these

TABLE II—*Indications for Splenectomy*

1.	Persistent increase in blood transfusion requirements by 50% or more over initial needs for over 6 months
2.	If blood requirements exceed 300 ml/kg/yr
3.	Evidence of leucopenia and thrombocytopenia
4.	Reduced red cell survival of 15 days or less by chromium 51 studies
5.	Radionuclear evidence of splenic sequestration

children. Such children should be given pneumococcal, influenza and hemophilus vaccines 2 weeks prior to surgery. Postsplenectomy patients should be under a penicillin prophylaxis programme. It has been observed that compliance decreases with increasing periods. Children not on regular penicillin prophylaxis should be advised to start amoxycillin/erythromycin/co-trimoxazole at the onset of fever/URI/symptoms of any infections. They should be hospitalized for appropriate management if they have high fever, pneumonia or other definite infections. Aspirin has been advised to prevent platelet aggregation and pulmonary micro embolisation. Splenic embolisation and partial splenectomy have been advocated by some authors to preserve splenic functions and to avoid post splenectomy complications(17).

Chelation Therapy

The inevitable consequence of repeated blood transfusion is development of hemochromatosis. Cumulative iron stores are related to the number of transfusions. Each unit of packed red cell contains 200 to 250 mg or iron and by 10 years of age about 25 to 30 g of iron gets deposited in various parts of the body. Repeatedly transfused and inadequately chelated patients develop hepatocellular damage, skin pigmentation and growth retardation even in the first decade. This is followed by pubertal failure during adolescence, insulin dependant diabetes mellitus, hypothyroidism, hypoparathyroidism, compensated adrenal insufficiency, cardiac failure and arrythmias(18).

There is no evidence that iron is toxic when bound to storage or transport proteins. Toxicity of iron begins when iron load exceeds binding and tissue storage capacity and enters the labile or free pool(19). Storage capacity of the tissue or organ is dependant upon its capacity to generate storage proteins. Thus iron is predominantly stored in the liver. Since the heart has minimal capacity to store iron, it is thus more susceptible to toxicity.

Assessment of Iron Stores

Presently, this is determined by serum ferritin levels. Though it is not an ideal method as serum ferritin levels have a linear correlation only when iron stores vary between 1-3 g. Its level rises in presence of hepato-cellular damage either secondary to iron overload or hepatitis(20). Iron stores can accurately be measured by grading stainable iron in liver biopsy specimens, but this is not practicable. Recently, non invasive methods have been developed which include computerized tomography(21) and magnetic resonance imaging(22). However, these correlate poorly with various pools of iron and the clinical picture and offer little guidance for chelation therapy.

Chelation Therapy

The human body has no mechanism to excrete the excess iron. Objectives of chelation therapy are: (*i*) to chelate excess of intracellular iron; (*ii*) to bind free extracellular iron, and (*iii*) to reduce the iron burden to minimal levels. Desferrioxamine (Desferal) is safe and effective, however the drug needs to be administered as a continuous intravenous (IV) or subcutaneous infusion (SC)(23-25). Desferrioxamine has a half life of 5 to 10 minutes after IV injection. It penetrates the cell and combines with the intracellular labile iron pool and the complex is then excreted by the kidney. Optimal chelation programme should result in a negative iron balance and therefore, the drug needs to be admini-

stered daily. Its effectiveness is dependent upon body iron stores and chelatable iron pool(25). It is administered in doses of 25-50 mg/kg/day SC over 10-12 hour infusion. Approximate cost of effective therapy is Rs. 1000/kg body weight/year. The cost for the infusion pump varies between Rs. 10,000 to 15,000. In older thalassemics who have accumulated significant iron stores, aggressive therapy (400-500 mg/kg over 48 hours) is essential prior to each transfusion in addition to daily therapy. It is recommended that chelation therapy should be initiated by 3-4 years age by daily administration of drug with the help of a portable infusion pump at a concentration of 250 mg/ml via a 26 or 27 gauge butterfly fixed SQ into the thigh or abdomen.

Toxicity of Desferal is minimal. Local side effects include pain, irritation, adominal discomfort and urticaria. Audio-visual neurotoxicity has been reported on prolonged use(26). Cardiac toxicity has not been observed if chelation therapy is initiated early. However, progressive cardiac dysfunction secondary to hemosiderosis has been observed when chelation therapy was initiated after 10 years of age.

Compliance of chelation therapy decreases significantly with advancing age primarily because of daily SC infusion of over 8-10 hours. Secondly, most parents cannot afford the cost of the drug which is Rs. 1000/kg/year for their children. Therefore continuous search for orally effective, cheap, specific and safe iron chelator is being undertaken all over the world for the last two decades. Large number of compounds have been tried in experimental models but none have yet proven safe and effective(28) (*Table III*). Efforts for developing an acceptable oral chelator have not been successful because of poor gastrointestinal absorption, poor bioefficiency,

TABLE III—*Oral Iron Chelators*

2-3 Dihydroxy Benzoic acid
Cholyhydroxamic acid
Phenol Derivatives: HBED and Dimethyl HBED
Desferrithiocin
Pyridoxal-isonicotinoyl hydrazone (PIH)
1,2-Dimethyl 1-3-Hydroxy pyridinone (L1)

higher incidence of toxicity either due to the chelator itself or secondary to redistribution of iron. Among the various oral iron chelating compounds 1,2-dimethyl-3-hydroxpyrid-4-one (L1) has been tried on short term and long term basis in various centres in the world(27). It has no evidence of ear, eye, bone marrow toxicity and is specific for iron chelation as urinary excretion of Ca, Cu, Zn, Mn and Mg is not increased even on full doses.

It causes mild nausea and altered taste in mouth. It has been observed that iron excretion increases with increasing doses. Serum ferritin levels fell by nearly sixty per cent over 16 months when used in full doses(28). Recently, it has been reported that about 30% of patients develop joint pains (mainly knee, ankle, elbow) and low backache within 3-6 months of administration of full doses of L1. Pathogenesis of this so called L1 arthropathy syndrome is not well understood. However, it appears to be dose dependent. Long term studies on L1 are essential to determine the pathogenesis of arthropathy, its ideal dose, long term side effects, if any.

HBED [N N' bis (o-hydroxybenzyl) ethelene diamine-N N-diacetic acid] is an effective oral chelator agent with low affinity for bivalent elements (Ca, Cu, Mg, Zn, Fe) and can be administered orally. Experimental trials of HBED/dmHBED

have been successful without any toxicity. Results of human trials are awaited.

Desferrithiocin has been isolated from *Streptomyces antibioticus*. It is more effective than Desferrioxamine and has achieved negative iron balance in animals on oral administration. However, the drug has significant side effects such as anorexia and loss of body weight, renal and neurological dysfunction. However, no long term side effects were seen in monkeys.

Vitamin C

It has been observed that thalassemic children with hemosiderosis have vitamin C deficiency and some of them may even present with scurvy. Thus, vitamin C supplementation is essential. It increases Desferal induced urinary iron excretion either by direct release of iron from stores or by reducing the ferric iron to ferrous intermediates(29). Cardiac toxicity has been observed in patients receiving 500 mg of vitamin C and intramuscular desferal(30,31). Cardiac toxicity is believed to be secondary to free iron radicals, which damage the cell membrane by lipid oxidation. Therefore, it is suggested that not more than 200 mg of vitamin C should be administered to children on chelation therapy.

Vitamin B_{12} and Folic Acid

Megaloblastic anemia is frequently observed and is often secondary to folic acid deficiency. One to 5 mg of folic acid should be given to these children. Our earlier studies have revealed low vitamin B_{12} levels(32). Thus at our Institute we recommend a B complex syrup containing physiological doses of vitamin B_{12} and 5 mg folic acid daily to these children.

Prevention of Thalassemia

Thalassemia may be prevented either by marriage counselling or by preventing the birth of thalassemic children. Large epidemiological studies are essential to determine ethnic groups at high risk so that screening tests are made feasible for detection of the thalassemia trait before marriage in individuals from such ethnic groups. However, in view of poor health education, the arranged marriage system, social customs and habits of our country, marriage counselling is not a practical possibility.

The other method to prevent beta thalassemia is by preventing the birth of thalassemic children which is feasible by antenatal diagnosis.

Antenatal Diagnosis of Beta Thalassemia

Two major strategies are presently being applied: (*i*) Estimation of the relative rates of globin chain synthesis in fetal red cells, and (*ii*) DNA studies.

(*i*) *Estimation of Relative Rates:* For estimation of the relative rates of globin chain synthesis fetal blood is obtained by ultrasound guided cannulisation of the umbilical vein at around 16 to 18 weeks of gestation and the ratio of beta : gamma chain synthesis is determined by radio labelling techniques(33). Normally, beta: gamma biosynthetic ratio is 0.11 ± 0.05 between 16 to 23 gestational weeks while the ratio is less than 0.025 in homozygotes for beta thalassemia(34). Currently, this method is being practiced in the Mediterranean countries such as Greece and Cyprus. The major disadvantages of this technique include a long period of uncertainty for the mother and difficult therapeutic abortion, if essential.

(*ii*) *DNA Studies:* With better understanding of the molecular biology of the thalassemias, a limited number of muta-

tions responsible for the condition, and availability of the polymerase chain reaction (PCR) it is now possible to diagnose homozygous beta thalassemia in the fetus as early as 8 to 10 gestational weeks. Nearly 100 μg of fetal DNA may be obtained by chorionic villus sampling and the beta globin gene in this DNA isolated and amplified to more than 10 fold by the polymerase chain reaction. Subsequent analysis is performed by (*i*) the classical restriction endonuclease enzyme digestion of the fetal DNA followed by Southern blotting and/or (*ii*) Linkage analysis of the restriction fragment length polymorphisms (RFLPs) of the beta globin gene cluster, or (*iii*) the newer technique of reverse dot hybridization where mutation specific oligonucleotide probes are immobilized separately on a nylon membrane. A biotin primed, and PCR amplified product from a genomic DNA is then hybridised to this membrane. Any hybridisation to any specific probe is thus rapidly detected by the presence of biotin in the oligonucleotide dots(35,36).

Using PCR amplification followed by dot hybridization and/or restriction endonuclease analysis, prenatal diagnosis is nearly always available within 3-7 days of sampling. The important role of PCR techniques is in decreasing the time required for diagnosis and increasing the accuracy of detecting thalassemic mutations.

Newer Approaches

Bone Marrow Transplantation

The principle involved in bone marrow transplantation (BMT) is to ablate the patients erythropoiesis and replace by grafting normal erythropoietic elements from syngenic or allogenic marrow. First successful transplant was undertaken by Thomas *et al.* in 1982 from an HLA identical sibling in a 16 month old thalassemic patient(39). Recently, Lucarelli *et al.* from Italy have observed that overall survival and event free survival after one year of BMT was 82 and 75% respectively(32). Among various prognostic factors heptomegaly, portal fibrosis and poor chelation therapy were associated with a poor prognosis. It appears that BMT holds the promise of cure in these children. However, the procedure is expensive and beyond the reach of majority.

Manipulation of Hb F Synthesis

Increased gamma globin synthesis will reduce the imbalance of alpha chains, thereby modifying the clinical phenotype of beta thalassemia is a distinct theoretical possibility. Several drugs including 5-azacytidine, cytosine arabinoside, hydroxyurea, vinblastine and busulfan have been shown to increase Hb F synthesis in experimental animals and humans. Among these 5 azacytidine, hydroxyurea and busulfan have been tried in a few thalassemic patients with limited success(39,40). Possible mechanism by which these drugs increase the Hb F production include: (*i*) hypomethylation of DNA near the gene, thus affecting the gene expression and (*ii*) late stage perturbation of erythroid marrow progenitor cells thereby augmenting the gamma chain synthesis.

Gene Therapy

Transfer of cloned gene into tissue culture cells has been achieved with regulated expression of newly introduced gene in the laboratory. Human beta globin gene has been transferred into mice resulting in increased expression when these cells are allowed to undergo erythroid matura-

tion(41,42). This technology is still at the experimental stage involving recombinant DNA technology. If successful this will revolutionize the management of thalassemia and other genetic disorders.

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