Recent advances in the management of Thalassaemia: A Review Update

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Abstract
The discussion of disease management focuses on the use of transfusion therapy and the newly developed oral iron chelators, deferiprone and deferasirox, especially combination of the chelator drugs. It has been also discussed on splenectomy and pediatrician management of endocrinopathies and cardiac complications. In addition, the use of hematopoietic stem cell transplantation has produced cure rates as high as 97%, and the use of cord blood transplantation as well. Major advances have been made in the discovery of critical modifier genes, such as Myb and especially BCL11A (B cell lymphoma 11A), a master regulator of HbF (fetal hemoglobin) and hemoglobin switching. Finally, the year 2010 has brought in the first successful experiment of gene therapy in a β-thalassemia patient, opening up the perspective of a generalized cure for all β-Thalassaemia patients. (J Shaheed Suhrawardy Med Coll, 2014;6(1):31-37)

Key words: Thalassaemia, recent advances, management

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1.0 Introduction
Thalassemia is a group of autosomal recessive disorders resulting from reduced or absent production of β-globin chains from the β-globin locus and these are very heterogeneous at the molecular level¹-². The large majority of mutations are simple nucleotide substitutions or deletions or insertions of oligonucleotides leading to frame shift. Rarely the β-thalassemias are the result of gross gene deletions. Homozygosity for β-thalassemia usually leads to the severe transfusion dependent phenotype of thalassemia major.

Alfa-Homotetramers in β-thalassemia are more unstable than β-homotetramers in alfa- thalassemia and therefore precipitate earlier in the RBC life span, causing marked RBC damage and severe hemolysis associated with ineffective erythropoiesis (IE) and extra-medullary hemolysis³. Ineffective erythropoiesis results in expanded marrow cavities that impinge on normal bone and cause distortion of the cranium, and of facial and long bones. In addition, erythroid activity proliferates in extramedullary hematopoietic sites, causing extensive lymphadenopathy, hepatosplenomegaly and extra-medullary tumors⁴. Over the last 3 decades, profound improvements in the management have been observed. The development of regular transfusion therapy and iron chelation has dramatically improved the quality of life. It has transformed thalassemia from a rapidly fatal disease to a chronic disease compatible with prolonged survival. Today, the life expectancy of patients with thalassemia major has increased from 25 years to over 55 years, mainly due to aggressive transfusion support and chelation coupled with patients' compliance with medical treatment⁵.

In developing world, especially Bangladesh, poor availability of proper medical care, safe and adequate red blood cell transfusions together with high cost and poor compliance with chelation therapy remain major obstacles. Since 1982, hematopoietic stem cell transplantation (HSCT) has become an alternative modality of treatment⁶. It is the only available procedure that may lead to cure. Recently, it has also been demonstrated that cord blood is as effective as, and possibly safer than, bone marrow for transplantation for paediatric patients. On the other hand

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there were hopes for the corrective gene therapy. None of the procedures are conducted in Bangladesh. Technological development and research regarding the recent developments of Thalassemia management are not promising in Bangladesh.

**Treatment of β-thalassemia intermedia**
In β-thalassemia intermedia patients whose ferritin levels are well above 500 g/dL, monitoring of iron excess using only serum ferritin is insufficient and we recommend annual assessments of liver iron concentration (LIC) by liver biopsy or by the more recently applied noninvasive T2* magnetic resonance imaging (MRI). Iron chelation therapy is warranted when LIC exceeds 5-7 mg/g dry weight and to prevent serious endocrine and cardiac complications similar to those seen in β-thalassemia Major(TM) patients. Monitoring for splenomegaly and hypersplenism is mandatory as a possible indication of the need for splenectomy.

**Management of β-thalassemia major (TM)**

**Transfusion therapy**
The decision to initiate a regular transfusion program in a child newly diagnosed with thalassemia must take into account both laboratory and clinical findings. If the child is growing poorly and has developed facial or other bone abnormalities, and/or when Hb levels are <7 g/dL, regular transfusions will be beneficial. Confounding factors that might aggravate the degree of anemia, including folic acid deficiency and acute febrile illness, blood loss, or coinheritance of glucose-6-phosphate dehydrogenase deficiency, need to be addressed simultaneously with transfusion therapy. Before the first transfusion, patients' RBCs are typed for Rh and ABO antigens. At the same time, cytomegalovirus status should be obtained when there is a possibility of curative stem cell transplantation (SCT). Parents and first-degree relatives should not be blood donors for these candidates. Hepatitis B vaccination is given before transfusion therapy, as is hepatitis A vaccine when age appropriate. Transfusions of washed, leukocyte-depleted RBCs are recommended for all the patients to reduce the incidence of febrile and urticarial reactions as well as infectious cytomegalovirus contamination. If they are not available, frozen thawed RBCs should be administered. Once a pre-transfusion Hb level >9-10 g/dL is achieved, transfusions are administered monthly in infancy and subsequently at 2- to 4-week intervals. In clinically stable patients, 8-15 mL RBCs per kilogram of body weight can be infused over a span of 1-2 hours at each transfusion event. If Hb levels are _ 5 g/dL and/or in the presence of heart failure, smaller aliquots of RBCs (5 mL/kg) should be administered to prevent volume overload until the Hb level is gradually increased to 9 g/dL. A clinical record of all transfusion events should be monitored annually to identify hypersplenism. A record of weight, the amount of blood transfused at each visit, and the pretransfusion Hb level is needed to calculate the annual transfusion requirement.

**Cardiac complications**
Cardiac failure and serious arrhythmias are the major causes of life-threatening morbidity and mortality in iron-overload patients. Before the availability of chelation therapy, cardiac disease was inevitable during the second decade and still occurs in older patients or those who are poorly compliant with chelation therapy. Therefore, cardiac function is monitored annually beginning at 7 or 8 years of age by electrocardiogram, echocardiogram, 24-hour Holter monitor, and recently by cardiac T2* MRI, which can detect preclinical cardiac iron accumulation.

**Pericarditis**
Thalassemia patients are susceptible to benign pericarditis, possibly caused by viral and mycoplasmal organisms, bacterial or fungal infections, or associated with the engraftment syndrome in post-transplantation thalassemia patients. "Iron-induced" pericardial siderosis has also been postulated as a causative factor. Diagnosis is made by history and physical signs and is confirmed with serial electrocardiograms and chest x-ray and requires hospitalization if they are symptomatic. Pericarditis is best managed with bed rest and aspirin. Steroids may be helpful with engraftment syndrome and iron chelation with hemosiderosis. When a significantly large pericardial effusion is present, the patient should be hospitalized and observed. Pericardiocentesis and diuretics are recommended to prevent cardiac tamponade. Surgical intervention may be necessary if significant pericardial effusions recur.

**Splenectomy**
After the initiation of a regular transfusion program from an early age, splenomegaly may be averted, but hypersplenism may nonetheless develop, usually in children between 5 and 10 years of age. The therapeutic rationale for splenectomy, particularly in patients with growth retardation and poor health, is to protect against the development of extramedullary hematopoiesis by improving the Hb level, decreasing the transfusion requirement, and consequently reducing iron overload. Therefore, we recommend splenectomy when the calculated annual transfusion requirement is _ 200 to 220 mL RBCs/kg per year with a hematocrit of 70% (equal to 250-275 mL/kg per year of packed RBCs with a hematocrit of 60%)10. The susceptibility to overwhelming infections after splenectomy can be reduced by immunization with pneumococcal and meningococcal vaccines before splenectomy and antimicrobial prophylaxis with penicillin after splenectomy. Fever over 38°C (101°F) developing in splenectomized patients with no focus of infection requires immediate intravenous broad-spectrum antibiotics. However, before recommending splenectomy, one should bear in mind that, in a recent evaluation of 584
patients with TI, significantly higher rates of complications were documented in splenectomized patients.\textsuperscript{13}

**Newer complications**

Newer and previously less often described complications have now been well-recognized. These include hyper-coagulable state, osteoporosis, hepatocellular carcinoma, psychosocial problems.

**Hyper-coagulable state**

Because improvements in the medical management of patients with TM and TI have resulted in significant prolongation of life, previously undescribed complications are now being seen. These include the existence of a hypercoagulable state, particularly in splenectomized patients with TI who do not receive regular transfusions.\textsuperscript{23,24} Prothrombotic hemostatic anomalies, including low levels of coagulation inhibitors, such as protein C and protein S as well as thrombocytosis and platelet activation, have also been observed in these patients.\textsuperscript{23,24} However, until now, there are no recommendations based on clinical trials regarding if, when, or for whom prophylactic antithrombotic treatment is indicated.

**Hepatocellular carcinoma**

Hepatocellular carcinoma (HCC) can complicate liver cirrhosis secondary both to iron overload and viral infections. Italians have published 22 cases of HCC in thalassemia major, 15 of them were males and the mean age of diagnosis was 45±11 years.\textsuperscript{13} Eighty-six percent were infected by hepatitis-C virus and majority were diagnosed after 1993, suggesting that the problem is becoming more frequent with the aging population of thalassemia patients.\textsuperscript{27}

**Osteoporosis**

Although RBC transfusions suppress IE, making skeletal abnormalities less common today than in the past, bone health in thalassemia patients must be monitored to identify age-related low bone mass. Nearly 90.0% of TM patients, including 30.0% of those younger than 12 years, have low bone mass Z-score (< 2.0).\textsuperscript{28} For this reason, beginning in childhood, yearly studies that include bone mineral density as well as studies of calcium, vitamin D3 metabolism, and thyroid and parathyroid function should be performed. Administration of pamidronate has shown a significant increase in BMD of the lumbar spine and it is now recommended that pamidronate at a monthly dose of 30 mg is an effective treatment for thalassaemic osteoporosis.\textsuperscript{29} Alternative treatment includes zolendronic acid in the dose of 1 mg as short i.v. infusion once every 3 months.

**Psychosocial problems**

With most of thalassemia major patients achieving adolescence, psycho-social support has become an extremely important part of patient and family management. There is a great need of meeting a genetic counselor at regular intervals. Unfortunately, there is no formal programme on this front in Bangladesh.

**Oral iron chelators**

In cases of ongoing transfusion therapy, with each RBC unit containing \(\beta\) 200 mg of iron, cumulative iron burden is an inevitable consequence. In TI and TM patients, the rate of transfusional and GI tract iron accumulation is generally 0.3-0.6 mg/kg per day.\textsuperscript{30} Increased GI tract iron absorption can result from severe anemia and IE, which down-regulate the synthesis of hepcidin, a protein that controls iron absorption from the GI tract and increases release of recycled iron from macrophages.\textsuperscript{31-32} To date, there are 3 major classes of iron chelators: hexadentate (deferoxamine [DFO], Desferal), dentate (deferiprone, L1 [DFP]), and tridentate (deferasirox [DFX], Exjade) (Table-1).

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<thead>
<tr>
<th>Compound</th>
<th>DFO</th>
<th>DFP</th>
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<tr>
<td>Molecular weight</td>
<td>Da 657</td>
<td>Da139</td>
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<tr>
<td>Chelating properties</td>
<td>Hexadentate</td>
<td>Bidentate</td>
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<tr>
<td>Recommended dose</td>
<td>30-60 mg/kg/day</td>
<td>75-100 mg/kg/day</td>
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<tr>
<td>Delivery</td>
<td>SC or IV 8-12 h, 5-7 d/wk</td>
<td>Oral 3 times daily</td>
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<tr>
<td>Half-life</td>
<td>8-10 min</td>
<td>1.5-4 h</td>
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<tr>
<td>Excretion</td>
<td>40%-60% fecal</td>
<td>90% urinary</td>
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<tr>
<td>Adverse effects</td>
<td>Ocular, auditory toxicity, growth retardation, local reactions, allergy</td>
<td>Gastrointestinal upset, arthralgia, neutropenia, agranulocytosis</td>
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**Table 1: Comparison of the 3 leading iron-chelating drugs in the management of thalassemia**

Source: Eliezer A. Rachmilewitz and Patricia J. Giardina. BLOOD, 29 SEPTEMBER 2011 VOLUME 118, NUMBER 13; * Subcutaneous=SC; intravenous=IV

**Efficacy of Iron Chelators**

The purpose of an effective iron chelation therapy is to prevent or reduce body iron accumulation. In regularly transfused patients the rate of iron accumulation, originated from destroyed senescent red cells, is 0.3-0.5 mg/kg/day. Therefore, a chelator should be able to remove at least this amount of iron to minimize the risk of iron-induced toxicity. Several studies have shown that DFP, at comparable doses has an efficacy similar to that of DFO, and that iron excretion increases with the dose and with the degree of iron overload.\textsuperscript{33,34} DFP at appropriate doses is able to decrease or stabilize body iron as assessed by sequential serum ferritin or liver iron concentration, despite repeated RBC transfusions.\textsuperscript{35-37} Several independent studies have shown that DFP is more effective than DFO in removing cardiac iron, improving cardiac function and reducing mortality for cardiac disease.\textsuperscript{38-40} The greater efficacy of DFP in removing excess cardiac iron may be due to some pharmacological characteristics of DFP.
such as low molecular weight, neutral charge and lipophility, which facilitate myocyte membrane crossing and chelation of intracellular iron41.

**Deferiprone and Deferoxamine association**

DFP and DFO can be given to the same patient with different regimes: in combination on the same day, either simultaneously (i.e. DFP given before breakfast, lunch and dinner and DFO infused during the day) or sequentially (i.e. DFP as above and DFO infused overnight), or as alternate treatment (i.e. one or the other chelator is given on different days). Combination therapy is considered an intensive chelation regimen and usually DFP is administered every day, while subcutaneous DFO is given 2 to 7 days/week, according to the severity of iron overload. In patients with heart failure, to reinforce chelation, DFO can be given intravenously 24 h/day. The potential advantages of the combined chelation are reported in Table 2. Several single case reports and prospective studies have shown that combined intensive chelation is effective in reducing cardiac siderosis and in improving cardiac function in patients with severe heart iron overload and heart failure42-45. A randomized, placebo-controlled, double blind study suggested that in comparison to the standard chelation with DFO, combination therapy with DFO and DFP, was able reduce myocardial iron (ratio of change in cardiac T2* geometric means 1.5 in combined therapy vs 1.24 in DFO monotherapy, P=0.02) and improve LVEF (2.6% vs 0.6% P=0.05). In a study from Greece, reversal of endocrine complications, with very intensive combined chelation (DFP 75-100 mg/kg/day and DFO 20-60 mg/kg/day), has been reported46. More recently, in a large long-term (5 years of follow-up), multicenter study, 213 patients were randomized to receive DFO for 4 days/week and DFP for the remaining 3 days, or DFP monotherapy for 7 days/week47. In the alternating treatment group serum ferritin showed a significant reduction (P= 0.005) as compared to DFP group. DFO monotherapy versus DFP monotherapy, alternate or combined DFO-DFP regimes, have been evaluated in another long-term multicenter, randomized trial including 265 patients48. None of the patients on DFP alone or in combined treatment died, one death occurred with alternate treatment and 10 deaths with DFO treatment.

**Table 2: Potential advantages of combination therapy**

<table>
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<th>Advantage</th>
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<tr>
<td>Access to different iron pools</td>
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<tr>
<td>Greater efficacy</td>
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<tr>
<td>Dose decrease® toxicity decrease</td>
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<tr>
<td>Chelation of toxic free iron</td>
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<td>Better tolerability and compliance</td>
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**Deferasirox and Deferoxamine Association**

Preliminary promising results have been obtained with iron balance studies, which evaluated the total (i.e. fecal and urinary) iron excretion, in three patients treated with DFX and DFO in combination49. Total iron excretion was synergistic (i.e. higher than the sum of iron excretion obtained with each single drug) in two patients, but less than additive in the third patient, who responded the best to both drugs individually. Alternating treatment with DFO and DFX resulted to be safe and effective in two retrospective studies including a limited number of patients50, 51.

**Cure of Thalassemia: hematopoietic Stem Cell Transplantation (SCT)**

The first curative allogeneic SCT to a thalassemia patient from an human leukocyte antigen (HLA) identical sibling donor was reported in 1982. Since then, >3000 successful transplantations have been reported52. The probability of overall event-free survival has been recently reported as high as 89%-97% for patients with no advanced disease and of 80%-87% for patients with advanced disease53. There are several risk factors, including hepatomegaly > 2 cm, portal fibrosis, and inadequate iron chelation therapy, that can influence the outcome of SCT. Approximately 10% of SCT patients are transfusion-free for years, although they experience persistent mixed hematopoietic chimerness54. This suggests that only a few engrafted donor cells are sufficient for correction of donor phenotype. Approximately 30% subsequently reject their grafts55. Those who deteriorate and require further transfusion support may benefit from a second transplantation with nonmyeloblastic conditioning to restore normal Hb levels54. Another option is to use matched unrelated donor if a matched sibling is not available or when patients are not compliant with conventional therapy. However, 40% developed GVHD and a third had chronic GVHD56. A few patients who failed the first transplantation underwent a second transplantation.

**Cord blood transplantation**

The potential benefits of umbilical cord blood (UCB) treatment are the low risk of viral contamination from a graft, the decreased incidence of acute and chronic GVHD, and easier accessibility. The small size or small number of stem cells in the UBC collection relative to the number required for engraftment are probably the main causes of failure of UCB transplantation; therefore, this procedure is being used mainly in pediatric patients57. The use of UCB from unrelated donors has resulted in only 77% survival and 65% event-free survival, respectively, in 36 thalassemia patients58. The experience with UCB transplantation is encouraging, but additional data are required for definitive conclusions. On the basis of all the available data to date, we think that every patient with a severe form of thalassemia should be offered the option for SCT. Although SCT is the only curative available, its use is still limited in other developing country because of the relatively high cost and the difficulty in identifying suitable donors. In Bangladesh it is not using at all due to available Bone Marrow Transplant center.

**Future therapies for Thalassemia Patients**
Fetal Hb inducers
For many years, a major therapeutic goal has been to decrease the severity of anemia in Beta-thalassemia patients by the pharmacologic enhancement of the fetal globin gene expression to increase gama-globin chain production that would improve the excess Alfa-chain imbalance. Several drugs, including erythropoietin, demethylating agents, such as 5-azacytidine, and short chain fatty acids, such as butyrate, have been studied individually and in various combinations. The short-chain fatty acid butyrate was reported to decrease transfusion requirements in transfusion-dependent -thalassemia patients for 7 years. Erythropoietin administration is capable of increasing thalassemic erythropoiesis, mainly in patients with TI but also in those with E-ß--thalassemia, without increasing HbF. Patients with low endogenous erythropoietin levels have been reported to respond to the combination of erythropoietin and butyrate. Hydroxyurea (HU), which is very effective in increasing HbF levels, has been used extensively for many years in patients with sickle cell anemia (SCA). However, the experience in thalassemia is limited. A substantial decrease in transfusion requirements and/or an increase in Hb levels, which may have been correlated with haplotypes, has been reported during a 6-year follow-up of 149 of 163 patients with ß-thalassemia in Iran subsequent to their receiving a dose of 8-12 mg/kg per day. One of the major concerns is possible effects of HU on fertility, pregnancy or the risk of malignancy. However, the long-term experience with HU in SCA has ruled out these options. Most recently, decitabine and HQK-1001, new fetal globin inducers that stimulate fetal globin induction through the proximal promoter and also exhibit erythropoietic-stimulatory effects, are being studied. Another potential strategy is to develop techniques to silence HbF suppression. Recently, the molecular basis of the HbF to HbA switch identified a variation in chromosome 11-encoding locus BCL11A (B cell lymphoma-leukemia 11A) which was found to be associated with the level of HbF in patients with thalassemia and to be a regulator of gama-globin expression. Knockdown of BCL11A expression resulted in reactivation of HbF expression, which inversely correlated with the level of HbF.

Gene Therapy
Continuous improvements in the traditional care of ß-thalassemia has ameliorated the quality of life and greatly improved the life expectancy. Despite these results, until recently a definitive cure could only be achieved with bone marrow transplantation (BMT) from related or unrelated donors. However, BMT is only available for a minority of patients and bears a significant risk of mortality and morbidity, especially when the donor is unrelated. In the search for a more general and definitive cure, hematologists have pursued alternative strategies aimed at correcting the defective ß-globin gene by either gene transfer of a normal ß-globin gene or substitution of the defective gene by homologous recombination. Although gene therapy would have been theoretically possible soon after the discovery and cloning of the human globin genes, for many years two main obstacles have hampered the progress in this field. The first obstacle has been the extremely complex regulation of the globin genes that has taken decades to at least partially unravel. The second and equally important obstacle has been the lack of an optimal vector for gene transfer into quiescent hematopoietic stem cells (HSC). Murine ß-thalassemia models have been successfully cured with the use of a retroviral vector (TN39) transferring the human ß-globin gene sequence and its promoter region into murine stem cells of TI and TM mice. ß-Globin gene transfer into progenitor hematopoietic cells of humans is also being studied. However, concerns regarding gene transfer include the need for improved efficiency of gene delivery and mastery of vector stability, viral titers, nononcogenic insertion, the variable expression of globin genes, and the variable contributions of the ß-thalassemia phenotype and other modifiers to the effectiveness of gene transfer. Gene therapy is a promising approach to curing thalassemia but is still in the early investigational phase trials.

Prevention
Although, it is not a part of this write up, it is important to stress that the most important advance in the field of thalassemia is its prevention. This has been very successfully achieved in Mediterranean countries i.e., Italy, Greece, Cyprus and Sardinia. There are four important aspects of prevention like Awareness, detection of carrier, effective counseling and prenatal diagnosis. Over last two decades, the programme has been successfully implicated in major cities of India. However, the desired goal of zero birth rate of thalassaemia has remained a distant goal. Bangladesh needs such programme to prevent thalassaemia and to reach the desired goal. However, in the absence of a national thalassemia prevention programme, this still remains a difficult but extremely desirable goal.

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