CHAPTER 6

Future Aspects
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Wheat Grass Juice Therapy

The treatment of transfusion dependent β-thalassemia imposes a considerable burden on the family and institutional resources. In economically challenged nations, basic management (red cell transfusions, iron chelation) is a distant dream for the majority, who, consequently, endure a poor quality of life. In chronic illnesses, seeking remedy in alternate systems of medicine is a common practice. After learning of the potential benefits, some patients started consuming wheat grass juice, which has been promoted as a supplementary health food/tonic for many years. At least 3 of these children perceived an increase in the interval between transfusions with the desired level of hemoglobin being maintained for a longer period. The observations were significant and prompted us to scientifically evaluate the effects of wheat grass juice therapy in patients with transfusion dependent β-thalassemia.

Consumption of wheat grass juice was found to have beneficial effect on the transfusion requirements in 50% of patients in this pilot study.

The criteria employed for determining the benefit of wheat grass juice was taken arbitrarily as a decrease in blood transfusion requirement by 25% or more. We felt that this cut-off was appropriate for judging the response. Most patients, who had a response by the designated criterion, also had an increase, albeit small, in the mean pre-transfusion hemoglobin whilst consuming wheat grass juice. This was true in all responders, except in three. In one case, No. 4, pre-transfusion hemoglobin remained the same, whilst a modest decline of 0.2 and 0.3 g% were observed in cases, respectively. The decrease in hemoglobin in these two patients was miniscule, in comparison to the decrease in amount of transfusion as well as the increase in
interval between transfusions. The mean interval between transfusion visits in all responders, the maximum increment being of 122%. This reflects the postponement of the scheduled transfusion visit due to satisfactory hemoglobin value in between the transfusions. In three patients, reduction in the amount of blood transfused, exceeded 40% of the pre-wheat grass juice period. Poor compliance in 14 of 38 patients (37%) could be attributed to lack of sufficient motivation. The process of growing, harvesting and extracting juice was too laborious, especially in summer months for most parents of defaulters. Other factors that influenced their decision to discontinue were high expectations coupled with the lack of discernible benefit in the early months after entry into the study. Strict scientific evaluation will undoubtedly, recognize a bias towards ‘responders’ when a significant proportion of cases were rendered ‘inevaluable’ for failing to adhere to the criteria in the study protocol. It is difficult to refute this viewpoint especially when ‘therapy indiscipline’ was observed in those who perceived no discernible benefit.

I do not wish to speculate on the mechanism of beneficial action of wheat grass juice in transfusion dependent thalassemics. Chlorophyll makes up >70% of the solid content of wheat grass juice. Both chlorophyll and hemoglobin share a similar atom structure. The only difference in the two molecules is that of the metallic atom element. Hemoglobin consists of iron, while in chlorophyll the metallic atom is magnesium. The believers of alternative system of medicine claim that as chlorophyll and hemoglobin are alike in atom structure, intake of wheat grass juice enhances hemoglobin production. This sounds too simplistic and needs to be proven. Wheat grass juice has iron and what consequences will this have on the iron overload needs to be evaluated. To add to the quandary, wheat grass juice has been documented to be effective for an unrelated condition-ulcerative colitis, in a randomized
double-blind placebo-controlled trial from Israel.

We conclude that wheat grass juice has the potential to lower transfusion requirements in thalassemics. The observations of our pilot study may prompt investigators to conduct similar studies worldwide. The ensuing years should throw light on the mechanism of action of wheat grass juice, adverse effects, particularly in relation to iron status and the appropriate doses. Wheat grass juice extract is available commercially, as tablets and capsules, in a few countries. These preparations should eliminate the inherent variations in obtaining wheat grass juice. Efforts are underway to initiate another study to evaluate the efficacy of dried extract of wheat grass. The findings may help in circumventing the cumbersome procedure of extracting fresh wheat grass juice.

### 6.2 Blood Transfusion

Regular transfusions are needed for all thalassemics who cannot maintain Hb above 7 g/dl. The goal of transfusion therapy is to keep the Hemoglobin (Hb) level at a minimal baseline level. Such a regimen (hypertransfusion regimen) is currently universally accepted since maintaining a near normal baseline Hb level suppresses the bone marrow almost completely, thus causing reduction in bone marrow volume. Thus, there is not much additional blood requirement as compared to a patient whose bone marrow is greatly expanded because of chronic hypoxia resulting from infrequent transfusions. Maintenance of normal Hb level prevents chronic hypoxemia, hypervolemia, hypersplenism, and promotes normal physical activity and growth. This also checks excess gastrointestinal iron absorption and iron overload. Transfusion therapy may also be important in some intermedia patients as they suffer from hypoxia, though of a lesser degree.
By second or third decade they may develop enormous cosmetic defects and increased bone fragility and pseudotumors due to extra-medullary hemopoiesis leading to spinal cord compression.

The current recommendation is to maintain a mean Hb level of 12 g/dl. This can be achieved by not letting the Hb fall below 9.5-10 g/dl (4). The average rate of Hb fall is 1 g/dl per week. Thus raising the post-transfusion Hb to 14 g/dl will allow a 4 weekly transfusion regime with a mean Hb level of 12 g/dl (*Table II*). For packed cells to raise the Hb by 1 g/dl the blood volume required is 3 ml/kg. Thus in a monthly regime, 12 ml/kg of packed cell is required which is equivalent to 20 ml/kg of whole blood. In 2 weekly regime smaller amounts will be required for the same result. For convenience, a 3 weekly transfusion regime is mostly practiced with pretransfusion Hb level of 10.5 g/dl and post transfusion Hb level of 13.5 g/dl. If the patient is hypersplenic, more frequent transfusions are required. Similarly caution should be exercised to see that post-transfusion Hb level does not exceed 15.5 g/dl. Higher Hb levels increase blood viscosity, reduce tissue oxygenation and increase the risk of thrombosis. The rate of transfusion should be around 5-7.5 ml/kg/h. However, in patients with cardiac problem or severe anemia, no more than 5 ml/kg should be given at one time and rate of transfusion should not exceed 2 ml/kg/h.
Table 6.1 – Pre-transfusion Hb Required to Maintain a Mean Hb Level of 12.0 g/dl in Relation to Transfusion Interval

<table>
<thead>
<tr>
<th>Weeks between transfusions</th>
<th>Pre-transfusion (g/dl)</th>
<th>Hb Post-transfusion (g/dl)</th>
<th>Hb Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11.00</td>
<td>13.0</td>
<td>12.0</td>
</tr>
<tr>
<td>3</td>
<td>10.5</td>
<td>13.5</td>
<td>12.0</td>
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<tr>
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<td>10.0</td>
<td>14.0</td>
<td>12.0</td>
</tr>
<tr>
<td>5</td>
<td>9.5</td>
<td>14.5</td>
<td>12.0</td>
</tr>
<tr>
<td>6</td>
<td>9.0</td>
<td>15.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

The type of blood to be used for thalassemics is of utmost importance. Blood component therapy has become the order of modern day practice. Thus a thalassemic child should receive group and type specific packed red cells which have been passed through white cell filter. This prevents unnecessary infusion of plasma proteins and white cells and thus prevents nonhemolytic febrile transfusion reactions. Filters are also important as these minimize antigenic exposure, which is important because of future prospects of bone marrow transplantation. Ideally the filters should be used from the outset with each transfusion. However, when resources are limited, use of filters may be limited to only those patients who have recurrent febrile transfusion reactions. Air introduction into the filters can cause major deterioration of function and this is to be avoided. The commonly available filters are Sepacell (Japan), Pall (USA), Leucostop (Italy) and Imugard (Netherlands) and the cost varies from Rs. 600/- to Rs. 1500/- per filter. Care should also be taken to prevent minor red cell incompatibility as well as hemolytic transfusion reactions which may occur due to allo-immunization to minor blood groups. Fortunately majority of thalassemia patients do not develop red cell antibodies and standard cross matching suffices. Prior
to transfusion blood should be screened to prevent infections like hepatitis B, hepatitis C, HIV, malaria, syphilis, cytomegalovirus, etc. In fact hepatitis B vaccination is now a routine and must for all HbsAg negative thalassemics. However hepatitis C screening is still not mandatory in India.

Evaluation of the transfusion treatment should be a priority in order to rationalize the transfusion therapy. At each transfusion, pre and post transfusion Hb should be recorded, the weight and hematocrit of the blood transfused should be monitored. Serum ferritin, liver function tests, growth monitoring, cardiac and endocrine evaluation should be done at regular intervals. A chart as shown in Fig. 1 should be maintained for each child. Important transfusion indices should be calculated yearly. The most useful indicators of appropriate management are:

(i) Annual mean Hb level: Average of the mean pre and mean post transfusion Hb.

(ii) Annual pure red cell consumption (ml/kg/year)

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\text{Total wt (g) of blood given} \times \text{Mean hematocrit (\%)} = \text{Patient's body weight (in mid-year) \times 1.08}^* \\
\]

(*represents density of pure red cells)

Annual pure red cell consumption should not exceed 180 ml or a maximum of 200 ml/kg/yr. An increased value indicates development of hypersplenism or red cell antibodies.

6.3 Iron Chelation

Iron overload occurs from two sources namely transfused blood and enhanced gastro-
intestinal tract iron absorption. Cumulative iron stores are also related to number of transfusions. Each unit of packed red cells provides 200-250 mg of iron and by 10 years of age about 20-30 g iron gets deposited in various parts of the body. Iron overload leads to liver fibrosis, cardiomyopathy and dysfunction of endocrine organs. Without iron chelation, a well-transfused thalassemia patient dies between the age group of 10-25 years, usually secondary to cardiac failure. There are various means by which iron overload can be estimated: (a) Serum ferritin estimation is the easiest and most widely used method of evaluating iron overload. Mean serum ferritin levels directly correlate with the cardiac status of the patient. Six monthly estimation of serum ferritin is recommended for appropriate monitoring of iron stores. However it is now being recognized that reliance on this test alone may lead to errors in management; changes in body iron account for little more than half the variation in serum ferritin concentrations; (b) Hepatic iron content can be assessed from biopsied liver tissue. The measurement of hepatic iron stores, whose concentrations have been shown to correlate highly with total body iron stores, provide the most quantitative, specific, and sensitive method of evaluating iron burden in thalassemic patients. Determinations of hepatic iron concentrations from biopsied liver tissue obtained with ultrasonographic guidance is safe and permits rational adjustments in iron chelating therapy. Being an invasive procedure this is not routinely done; (c) Superconductive Quantum Interference Device (SQUID) biosusceptometry is another reliable noninvasive method of monitoring iron stores(13). However, this facility is not easily available; (d) Magnetic resonance imaging (MRI) can also be used to evaluate iron overload though the results are not very accurate(14).

Out of a variety of iron chelating agents only Inj. desferrioxamine and an oral iron chelator (deferiprone) have been shown to be clinically effective with acceptable toxicity.
Desferrioxamine

Desferrioxamine (DFO), in use for the last 40 years, increases iron excretion both in urine and stool. Chelation treatment, which maintains ferritin <2500 mcg/l and hepatic iron concentration <268 mmol/g of dry weight liver, has been shown to enhance survival rates. Children with serum ferritin <2500 mcg/l have cardiac disease-free survival of 91% after 15 years compared to 20% in those with higher serum ferritin values. One gram of desferrioxamine binds with 85 mg of iron. A daily excretion of more than 30-40 mg of iron can be achieved provided the drug is given 5 days a week. The optimal age at which chelation should be started is uncertain. While hepatic fibrosis sets in within two years of starting transfusion therapy, metaphyseal dysplasia has been reported before the age of three years. To be most effective, chelation therapy should be initiated only after iron accumulation is established, which is usually within 18 months of starting regular transfusions (16). By this time child would have received about 20-30 transfusions. This is usually associated with ferritin levels in the range of 800-1000 mcg/l. The daily dose of desferrioxamine is 40-60 mg/kg given subcutaneously over 8-10 hours. Vitamin C in a dose of 100 to 200 mg given during infusion helps in increasing iron excretion and achieves negative iron balance relatively early. The infusion is usually given at home with a portable battery operated pump at night for 5-7 days per week. However, infusion pumps are costly and unaffordable by many patients in developing countries. A recent study evaluated the effectiveness of twice daily subcutaneous injections in place of prolonged infusion. Though this approach might be useful for developing countries further validation is necessary. Continuous intravenous infusions using surgical implants (portacath) is indicated for patients who are heavily iron loaded with adverse effects, for example, cardiac problems. These implants can
also be used for regular transfusions thus avoiding repeated venepunctures. In general, the goal is to keep the serum ferritin level below 1000 mcg/l.

1. Levels above 7000 mcg/l interfere with growth and prolonged survival. Side effects of desferrioxamine are mostly local irritation at injection sites and febrile reactions, which respond well to antihistaminics and antipyretics. The most feared side effect is infection with *Yersinia enterocolitica* and severe mucormycosis. Uncommon long-term toxicities include oto-toxicity and ocular toxicity. These patients should be regularly monitored for retinal changes, cataract and hearing problems.

**Orof Iron Chelators**

Regular subcutaneous desferrioxamine therapy is very costly and being cumbersome is available to less than 10% patients worldwide and less than 3% patients in India. Thus there is definite need for an effective oral iron chelator. Over last 30 years, hundreds of iron chelators have been tested *in vitro* or in animals. Out of these, only deferasprone was found to be reasonably effective to justify its licensing for general use in few countries. India is the first country, which has started licensing deferasprone for use (Kelser-Cipla). Deferasprone is rapidly absorbed when ingested orally and when given in two or three doses daily, it causes iron excretion comparable to that caused by an equivalent daily dose of desferrioxamine over 8-10 hours. Effectivity of deferasprone over long term has also been shown in recent trials. A dose of 75 mg/kg/day divided into three subdoses, each given one hour before food is usually satisfactory. Serious side effects include agranulocytosis (1.8%) and arthropathy (20%) which necessitate discontinuation of the therapy. Gastrointestinal intolerance, zinc deficiency and fluctuations of liver functions are other side effects, which are reversible and transient in nature (20-22). Patients taking deferasprone should be monitored by blood counts 3-4 weekly and the drug should be
discontinued if counts fall or severe joint pains and swelling occur with no relief to NSAIDs. However, data from two different centers showed that during long-term therapy in approximately 40% of patients, body iron burden stabilizes at or increases to concentrations associated with increased risk of cardiac disease, hepatic fibrosis and early death suggesting that long term deferiprone may not provide adequate control of body iron in a substantial number of patients.

6.4 Splenectomy

Splenectomy is usually not needed if regular transfusion therapy is followed. If the child already has a big spleen, his transfusion requirement increases to more than 1.5 times of the normal or more than 200 ml packed red cells or over 400 ml of whole blood per kg per year, splenectomy is indicated. Other indications are development of leukopenia, thrombo-cytopenia related to hypersplenism or massive splenomegaly causing discomfort. After splenectomy there may be transitory or persistent thrombocytosis. As a rule this carries no risk to the patient, possibly because it is simultaneously balanced by reduction in platelet aggregation. However, aspirin 50 mg/day may be recommended for patients whose platelet count exceeds 800,000/ cu mm. All patients should be vaccinated against Pneumococcus, Haemophilus influenzae b and Meningococcus before splenectomy is done.

Chemoprophylaxis in form of oral penicillin V or intramuscular benzathine penicillin should be given for at least 2 years after splenectomy. However, it is desirable that penicillin pro-phylaxis should continue life-long. Parents should be educated to be aware of rapid onset of serious infections in such patients.
6.5 Bone Marrow Transplantation

Bone marrow transplantation (BMT) is at present the only cure for thalassemia major and is an accepted therapeutic alternative to lifelong regimen of blood transfusion and chelation. In BMT, the patients' bone marrow is first destroyed by drugs in a process called 'conditioning'. Marrow is then harvested from a histocompatible donor and infused intravenously into the patient. The stem cells from the donor find their way into the marrow cavity and start growing there. As these stem cells have come from a normal person the red cells that are produced will also be normal. Since 1982, when first marrow transplant for thalassemia major was performed in 1981 at Seattle, USA, more than 1000 patients have been transplanted, the largest experience being at Pesaro, Italy. The cure rate of BMT is about 70%. In India, at present BMT for thalassemia is being done only at Vellore and the results are comparable with western centers. For BMT, patients are now-a-days categorized into three prognostically useful classes with respect to three risk factors, namely, poor quality of chelation, presence of hepato-megaly and the presence of portal fibrosis. Class I patient has no risk factor, Class II has one or two and Class III has all. In countries, where it is available, marrow transplantation should be considered for all patients with a suitable donor who falls into Class I or II. However, since the patients in Classes I and II already have a good prognosis with modern conventional management, the patients should be explained the risks and benefits of BMT versus conventional management. The results of marrow transplantation are improving steadily, with major progress in the management of transplant related complications. This is because of the use of cyclosporine, effective treatment for cytomegalovirus infection, better aseptic techniques and evolution of systemic antibiotic therapy. At the moment a patient in Class I has only a 5% probability of mortality.
The best results in BMT can only be obtained with a matched sibling donor. The probability of finding an HLA identical sibling is 30%. The chance of finding an unrelated HLA identical donor is extremely rare. However, use of unrelated donor marrow registries has made the procedure somewhat less difficult. An Asian Indian Donor Marrow Registry has recently been established at AIIMS, New Delhi(43).

Peripheral Blood Stem Cell Transplantation

In this technique, stem cells are collected from peripheral blood of the donor by stimulating the release of stem cells from marrow to blood by use of certain drugs and growth factors. The advantages are easier processing, less expensive, faster marrow recovery and lower risk of graft versus host disease (GVHD), rejections and infections. However, there is always a concern for the adverse effects of cytokines and growth factors, which need to be administered to the healthy donor.

Cord Blood Stem Cell Transplantation

Cord blood contains hematopoietic stem cells that can be used for transplantation. Attempts to capitalize this source have been fruitful, as evidenced by the high success rate. Advantages are easy availability, less stringent requirement for HLA matching and lesser incidence of GVHD. However, most of these transplants have been carried in patients of leukemia and data regarding its utility in thalassemia is scarce.

In utero Transplantation of Thalassemia

This is based on the logic that any disease that can be prenatally diagnosed and is treated by BMT after birth could be treated by in utero transplantation. As in early fetal life new sites for hematopoiesis are constantly forming and are being occupied by migrating hematopoietic stem cell (HSC), engraftment of donor HSC is also possible on a competitive basis. Immuno-logical tolerance is another major advantage
at this stage. Even with a low level of engraftment it is possible to clinically ameliorate the disease considerably. Experience in treating hemoglobinopathies with in utero transplantation is at present extremely limited.

6.6 Gene Therapy

Since, thalassemias are characterized by defective synthesis of globin subunits caused by mutations affecting gene regulation or expression, gene therapy remains the ultimate treatment for providing a lifelong cure. In this mode of therapy, patient's bone marrow is harvested and beta globin gene is incorporated into stem cell and reinfused in the body system. The critical factor in gene therapy is an appropriate level of gene expression. Substantial progress has been made in recent years to improve the gene expression by using retroviruses as vectors. These viruses have ability to multiply and encode foreign nucleic acid and contain mechanism for chromosomal integration. Non viral vectors like bacterial artificial chromosome, yeast artificial chromosome, and human artificial chromosome are exciting prospects for the future. It is difficult to foresee the time when an efficient gene therapy will be available in reducing the burden of both the disease and its current treatment!

6.7 Stimulation of Fetal Hemoglobin Synthesis

Increased gamma globin chain synthesis will reduce the imbalance of alpha chains and hemolysis. Several drugs like hydroxyurea, butyrates, 5-azaacytidine, cytosine arabinoside, vinblastine, and busulphan have been shown to increase Hb F synthesis. Therapy with hydroxy-urea and butyric acid compounds seems to be promising as it neutralizes the noxious excess of alpha chains and allows a better survival of the erythroid precursors in the marrow and of the red cells in the peripheral blood. Hydroxy-urea has been used in daily dose of 10-20 mg/kg varying from 4-30 weeks.
The drug is well tolerated and its main toxicity, i.e., leukopenia and thrombocytopenia is fully reversible. However, other studies, have reported only small increases in fetal and total hemoglobin concentrations during the administration of these compounds. Heme arginate, a novel stable form of hemin has shown to increase levels in few thalassemic patients. Recombinant erythropoietin increases recruitment of progenitor cells, some of which produce more gamma globin chains. However, it has been mainly tried in thalassemia intermedia cases. Despite the increase in the design and implementation of therapies aimed at the augmentation of fetal hemoglobin synthesis in beta thalassemia, their use in thalassemia major is still in its infancy. It is likely that combination therapies may prove useful in the augmentation of fetal hemoglobin sufficiently enough to decrease transfusion requirement. Studies to evaluate the effectiveness of combination therapies in patients with thalassemia are continuing.

6.8 Genetic counseling

Genetic counseling is a process of communicating medical aspects about a genetic disorder, especially the information regarding risk of occurrence as recurrence of the disease in the family and preventive options. Accurate diagnosis of the affected member is a paramount importance for genetic counseling. Special genetic investigations like DNA analysis and chromosomal analysis are essential for pediatricians to identify cases with thalassemia or possible thalassemia disorder. These cases should be adequately worked up to identify accurate etiology as far as possible. The investigations should be done even if they are not going to make any difference in the outcome of the child. The thalassemic disorders present not only in neonates, but also in children, stillbirths and fetuses. Hence, autopsy of stillbirth and fetuses termi-
REGISTRATION

SOCIAL WORKER
- Family background information.
- Orientation to the center.
- School and Career orientation.
- Integration in recreational activities.

HEMATOLOGIST
- Medical consultation
- Medical prescription
- Interpretation of Lab. result
- Prevention of complications
- Genetic counselling

LABORATORY BLOOD BANK
- Patient blood testing.
- Testing of donated blood units.

CARDIOLOGIST
- Prevention and treatment of complication.

DENTIST
- Check up.

DIETITIAN
- Dietary counselling upon need
- Nutrition education.

NURSE EDUCATOR
- Nursing consultation
- Demonstration: administration of Desferal and utilization of infusion pump
- Education sessions

CASHIER
- Contribution to the medication and material cost, varying from 2-15% according to family socio-economic status.

PHARMACY
- Dispensing of prescribed medicines and material.

EXIT

TRANSFUSION UNIT
- Transfusion under supervision.
- IV. Desferal administration
- Vaccination

ENDOCRINOLOGIST
- Medical consultation
- Prevention and treatment of complications

OPHTHALMOLOGIST
- Fundoscopy
- Angiography

PSYCHOLOGIST
- Patient evaluation
- Family dynamics evaluation
- Follow up of better adaptation to the disease
- Therapy for needy cases
nated after prenatal diagnosis is essential for genetic counseling. The importance of genetic counseling as an integral part of management of thalassemic disorder has to be realized by all clinicians. Pediatricians with short training can take over the responsibility of providing counseling for common genetic disorders including thalassemia and may need to refer others to a genetic center for counseling and prenatal diagnosis.

Key Messages

- Recognize thalassemia early and differentiate between major and intermediate cases to decide about transfusion therapy.
- Start transfusion therapy soon after diagnosis and maintain a pre-transfusion Hb level of 9.5-10 g/dl.
- Hepatitis B vaccination should be given before start of transfusion therapy.
- Regular monitoring of transfused patients for development of infections, iron overload, growth and endocrinological problems, strict maintenance of clinical record for early identification of any need to alter therapy, e.g., splenectomy.
- Iron chelation therapy in well-transfused children to maintain serum ferritin below 1000 mcg/l permits long-term survival.
- Offer bone marrow transplantation to all patients with suitable donor falling into class I or II only after explaining all risk factors and benefits.