Review Articles

Thalassemia syndromes in Saudi Arabia
Meta-analysis of local studies

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ABSTRACT

The geographical distribution of Alpha and Beta-Thalassemias differ markedly. Alpha-Thalassemia being particularly prevalent in Southeast Asia and Beta-Thalassemia in the Mediterranean basin. Thalassemia syndromes are common in Saudi Arabia: the Beta-Thalassemia genes occur with variable frequency in different regions of Saudi Arabia and both B$^+$ and B^o thalassemia have been reported. Alpha-Thalassemia is also highly prevalent here and the interaction with the sickle cell gene is commonly observed. Over the last few years, a great deal of information regarding the clinical, molecular and management of these disorders has accumulated in the literature. In this paper we summarize some of the recent studies on the subject along with our experience and our attempt to highlight a number of questions still awaiting answers.

Keywords: Thalassemias, Saudi Arabian study, literature review.

The normal human hemoglobins are designated by capital letters. HbA (or A1) refers to the predominant hemoglobin (more than 95%) found in normal adults. Hb A2 refers to the normal adult minor component that constitutes 2.5% to 3.5% of the total hemoglobin. HbF, or fetal hemoglobin, is the predominant hemoglobin of the fetus and young infant but is present only in trace amounts (less than 2%) in the adult. These normal human hemoglobins have specific polypeptide chains.

The use of recombinant Deoxyribonucleic acid (DNA) and somatic cell hybridization technologies has permitted the localization of the genes for most of the human hemoglobin polypeptide chains on specific chromosomes. The –genes are located on chromosome 16; –, -, and –genes constitute a tightly linked complex on chromosome 11.[1,2]

Thalassemia syndromes are the hereditary disorders of hemoglobin synthesis in which production of one or more globin chains is either decreased or absent. The most common types of thalassemias are alpha-thalassemia and beta-thalassemia, in which either of the 2 chains are synthesized in reduced quantities. There are isolated reports of absent chain production but this entity is poorly defined.   A related condition which is asymptomatic either in heterozygous or homozygous state is hereditary persistence of fetal hemoglobin (HPFH). Alpha-thalassemia gene is highly prevalent in Saudi Arabia and the interaction with the sickle cell gene is commonly observed.

The -thalassemias are also common in Saudi Arabia along the coastal strip of the Red Sea and in the Eastern province around Jubail, Qateef, Dammam, and Hofuf. Although -thalassemia disease has been known for many years in these areas and many of its manifestations are recognized, the details of actual incidence, the natural history or clinical course of the disease from early childhood to death are unknown.   This is largely because of inadequate facilities for mass population screening, variable severity, and manifestations, and complexity of the interaction of the disease process with other health related events eg. sickle cell disease. There are at least 150 transfusion-dependent homozygous

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-thalassemia patients who are receiving medical care at local hospitals in the Eastern Province, and 33 of these are being followed up in the Department of Pediatrics, King Fahd Hospital of the University at Al-Khobar. Despite the prevalence of thalassemias and other blood genetic disorders in Saudi Arabia, there is little common knowledge in the profession. This review is an attempt to highlight, in a simple way, the basic essentials of thalassemia and its variants.

Incidence. Thalassemia primarily affects people of Mediterranean, African and Asian origin, and there is a well-known thalassemia belt which extends from Africa to Asia. In view of population migration, the distribution of the thalassemia gene appears to be worldwide and to affect every ethnic group. Apart from Italy, Greece and Turkey, cases of -thalassemia have been found in the Middle East, Iran, Pakistan, India and China. The incidence of -thalassemia gene frequency in these countries is in the range of 5-25%. Alpha-thalassemia is common in the Far East, especially Thailand, where nearly one-fourth of the population carry the gene for one or the other type of thalassemia.

-thalassemia major, that occurs mostly in the Mediterranean and Middle Eastern region (and in countries where people from several areas have migrated) is also prevalent, with variable frequency, in different regions of Saudi Arabia. Both B^o and B^+ thalassemias have been reported,[3] and the incidence of sickle cell -thalassemia in the Eastern region is about 10%.[4]

The analysis of our cord blood screening data on 29,246 samples screened over a 6 year period revealed that the incidence of alpha-thalassemia gene in Qateef, Dammam and Al-Khobar areas of the Eastern Province as assisted by the presence of 1-2% Hb Barts in the cord blood (used as a criteria for the diagnosis of alpha-thalassemia-2), was 30%, 11% and 7%.[5] However, this underestimates the presence of alpha-thalassemia gene in many populations. The use of more sophisticated techniques, like restriction enzyme analysis, have demonstrated a much higher prevalence of alpha-thalassemia genes. The finding of nearly 50% of alpha-thalassemia genes in the Saudi population by other workers[6] seems valid since a number of factors may have contributed to such a higher incidence of alpha-thalassemia in the Qateef area: malaria has been hyperendemic in this region and its carriers presumably had a selective advantage over those with normal Hb electrophoretic pattern. We have also observed that in the same area, approximately one-third of the cord bloods, with AFS and FS hemoglobin electrophoretic pattern, were found to have concurrent alpha-thalassemia gene.[7]

The analysis of our data on gene frequency has also shown an excess of cases with FS phenotype compared to that expected from the observed gene frequency at all the 3 participating hospitals namely King Fahd Hospital of the University, Alkhobar, Dammam Maternity and Children's Hospital, Dammam and Qatif Central Hospital, Qatif.[8] Since the hemoglobin FS electrophoretic pattern is the same for conditions like sickle cell anemia, sickle cell - °-thalassemia, sickle cell/HPFH, sickle cell - thalassemia, and sickle cell – thalassemia; the convincing reason for the excess cases could be the interaction of sickle cell gene with other abnormal globin genes which are highly prevalent in the area. The only reliable way to differentiate between the above-named conditions is either globin chain synthesis or preferably by gene mapping of the index cases and immediate family members to ascertain the carrier state.

Globin chain synthesis was determined on 23 cases of our patients with sickle cell disease and by this technique, we were able to differentiate, reliably, between three associated conditions of homozygous sickle cell (SS); sickle cell - ° thalassemia and SS - thalassemia.

Pathophysiology. The pathophysiology of alpha- and beta-thalassemia is quite similar. Anemia is the result of intramedullary erythrocytic destruction, shortened red cell life span, secondary impairment of hemesynthesis and peripheral hemodilution. The clinical severity of each type of thalassemia syndrome, whether alpha or beta, is related to the extent of imbalance of alpha and beta chain production, and in beta-thalassemia, to the capacity of HbF to compensate for deficient beta chain synthesis. This imbalance of alpha or beta chain production leads to premature destruction of the red blood cells in peripheral blood and the precursors in bone marrow, resulting in accumulation of free alpha or beta chains. The continued synthesis of a large excess of alpha chains aggregate to form inclusion bodies. These alpha chain aggregates lead to membrane damage and premature destruction of red cells.[9] In bone marrow, the alpha chain inclusions precipitate in the normoblasts, which are destroyed within the bone marrow before reaching reticulocyte stage, causing ineffective erythropoiesis. The red cells that gain entry into the circulation are small, distorted and filled with inclusions. They also contain a very small amount of hemoglobin that produces the typical microcytic hypochromic appearance of RBCs. These abnormal cells are rapidly removed during passage through the spleen. This process results in marked anemia requiring blood transfusion. Furthermore, there is increased intestinal iron absorption leading to hemosiderosis. Hemosiderosis is probably responsible for damage to the heart, liver, pancreas, endocrine glands and other organs. Due to the presence of a large amount of fetal hemoglobin, less oxygen is released to the tissue, and this produces tissue hypoxia. As a result, erythropoiesis is increased, leading to bone marrow

Table 1 - Deletion and non-deletion of major types mutants that cause -thalassemia.

I.	Type of mutation (Genotype)	Phenotype	Ethnic group
1.	Transcriptional mutants	+	Turkish, Italian, Mediterranean, American Black
2.	RNA processing mutants		
	a. Splice junction	o	Mediterranean, Asian, Indian, Egyptian, Kuwait
	b. Consensus sequence	+	Asian, Indian, Mediterranean, Algerian, Saudi
	c. Cryptic splice site	+	Malaysian, Japanese, Mediterranean
3.	Non-functional MRNA		
	a. Nonsense mutants	o	Asian, Indian, Thai, Saudi, Mediterranean
	b. Frameshift mutants	o	Mediterranean, Asian, Egyptian, Mexican
4.	RNA cleavage and polydenylatin mutants	+	French, Greek, Turkish, Mediterranean
II.	**Gene deletion**		
	° thalassemia		
	( )° thalassemia		
	(o)° thalassemia		
	( )° thalassemia		

expansion and extramedullary hemopoiesis with skeletal and organ changes.

Regarding secondary abnormalities that occur in the thalassemia patients, increased iron absorption is a constant feature. The anemia is also aggravated by folic acid deficiency because of the high folate requirement by to erythroid hyperplasia. Splenomegaly contributes to anemia by acting as a third space, increasing intravascular volume and causing hemodilution.[10]

The molecular basis of thalassemia. The wide geographical distribution and varying clinical severity of thalassemia syndromes have suggested that several lesions exist at the molecular level. Unlike hemoglobinopathies, which result from a change in one or more nucleotides within the structural genes, the imbalance between alpha and non-alpha globin chains in thalassemic disorders involves many stages of gene expression in the erythroid precursor cells. These include defective transcription and metabolism of globin Messinger Ribonucleic acid (MRNA), transitional defects, deletion of globin structural genes, and others. To characterize the lesions in thalassemia, several procedures have been utilized, including globin chain synthesis, molecular hybridization techniques, measurement of globin structural genes and gene mapping. Table 1 lists the major types of mutations that cause -thalassemia. A repository of the mutation causing -thalassemia with the original references is updated regularly and published in Hemoglobin.[11] The molecular mechanism of - thalassemia have been defined in Asian populations. Cell free synthesis studies, as well as the molecular hybridization techniques have been demonstrated that various types of -thalassemias (like -thalassemia) are due to deficient MRNA (Table 2) and the

Table 2 - Deletion and non-deletion mutants that cause alpha-thalassemia

1.	**Deletions**
	a. 3.7 Kb (rightward deleton)
	b. 4.2 Kb (leftward deletion)
	c. o mutations
2.	**Non-deletion mutations**
	a. RNA processing
	b. RNA translation
	c. Post-translational instability
	d. Uncharacterized

concentration of MRNA correlates well with the amount of -globin chain synthesis.[12,13]

Beta-thalassemia. The genetic classification of thalassemia is derived from the name of the globin whose production is affected. In beta-thalassemia syndromes, there is an excess of alpha chain synthesis relative to beta chain synthesis.[14] The mode of inheritance is autosomal and the defects are expressed in both the heterozygous and homozygous condition. There are 2 main types of beta-thalassemia, each with subclasses. The heterozygous state is known as thalassemina minor or trait, whereas the homozygous condition has been called thalassemia major or Cooley's anemia. The severity of these types of thalassemia is influenced by a variety of factors, including race and interaction with other inherited erythrocytic disorders.

Heterozygous beta-thalassemia (beta-thalassemia minor or thalassemia trait). -thalassemia is widely dispersed throughout the Middle East and is well recognized in Saudi Arabia. Worldwide over 40 different mutations have been described in patients

with this condition.[6] These include deletions, point mutation involving promotor boxes, base changes that interfere with the processing of messinger RNA and single base changes or base deletions that produce premature chain termination codon or scrambling of the genetic code due to so called frame shift.

The heterozygous for beta-thalassemia inherit only a single beta-thalassemia gene from either of the parents (whether with minor or major beta-thalassemia) which leads to either decreased or absent beta chain syntesis. There is a 50% chance with each pregnancy that the child born will be a carrier of the disease. The initial diagnosis is carried out on the basis of family studies in which one of the parents had AA_2 electrophoretic pattern which elevated levels of HBA_2 and microcytic red cells in the absence of iron deficiency. Analysis of our data revealed that the incidence of S-B thalassemia in our Cohort babies was the same as reported earlier and 19 out of 199 babies being followed were doubly heterozygotes for this condition.[4]

There are at least 7 distinct varieties of beta-thalassemia trait (Table 3), which are distinguishable on the basis of hemoglobin electrophoresis, hemoglobin pattern and expression of severity. The clinical and hematological presentation of these different types of thalassemia are presented in the following sections.

High HbA2 $\pm$ HbF Beta-thalassemia. This is the most commonly encountered type of beta-thalassemia, prevalent in people of Italian, Greek, African, Asian, and Oriental origin. It is characterized by increased levels of HbA_2 (3.5-8%), normal to slightly elevated levels of HbF (2-5%) and typical microcytosis and hypochromia of the red blood cells. Clinical symptoms are usually absent or minimal; occasionally, splenomegaly may be present. Women with this disorder are usually anemic during pregnancy. However, blood transfusion is not indicated. In almost all cases, the peripheral blood smear is markedly abnormal. There are anisocytosis, poikilocytosis, hypochromia, microcytosis and the presence of target cells.

Beta-thalassemia trait of unusual severity. This disorder is rare but has been described in individuals of Swiss, French, Italian, Irish and English extraction.[15] Patients usually are symptomatic. Symptoms include fatigue, pallor and leg ulcers. Hepatosplenomegaly and gallstones are common findings and require the occasional blood transfusion. The Hb concentration in these patients ranges between 7.7 and 16.6 g/dL. HbA_2 is increased, and HbF is between 1.5 and 12.2%.

Delta-, Beta-thalassemia or high F thalassemia. In a small number of cases, the HbA_2 level is normal and fetal hemoglobin is elevated (5-25%). These patients are heterozygous for so-called delta-beta thalassemia or high F beta-thalassemia. The prognosis in these patients is good, and no treatment is indicated.

Beta-thalassemia trait, normal HbA_2 and HbF levels, hypochromic. This is a form of beta-thalassemia in which normal levels of both HbA_2 and HbF are found, and has been described in persons of Turkish, Greek, and Iranian extraction.[16] These heterozygotes are asymptomatic. The hemoglobin concentration is normal to slightly reduced. There are anisocytosis, poikilocytosis, hypochromia and microcytosis similar to some red cell abnormalities noted in the usual form of beta-thalassemia traits.

Silent carrier beta-thalassemia. This kind of beta-thalassemia was first reported in 3 members of a family in 1969 by Schwartz,[17] by globin chain

Table 3 - Beta-thalassemia minor or thalassemia trait.

	Type	Geographical prevalence	Hb-electrophoresis and frequency	Clinical manifestation
1.	High Hb A2 $\pm$ HbF beta-thalassemia	Italian, Greek, African, Asian	HbA2 elevated, HbF elevated	Usually absent or minimal
2.	Beta-thalassemia of unusual severity	Swiss, French, Italian, Irish, English	HbA2 elevated HbF elevated	Symptomatic fatigue, pallor, hepatosplenomegally
3.	Delta-Beta-thalassemia or high F thalassemia	Yugoslavian, Italian	HbA2 normal HbF highly elevated	Asymptomatic
4.	Beta-thalassemia with normal HbA2 and HbF	Turkish, Greek, Iranian	HbA2 normal HbF normal	Symptomatic Hepatosplenomegaly
5.	Alpha-Beta-thalassemia	Mediterranean, Asian	HbA2 high HbF normal	Asymptomatic
6.	Silent carrier beta-thalassemia	Cypriot, Italian, Black American, Oriental, Saudi	HbA2 normal HbF normal	Mild symptomatic
7.	Hemoglobin lepore trait	Italian, Greek, Yugoslavian	HbA2 normal HbF elevated + Hb before**	Symptomatic
**Lepore				

synthesis technique. These heterozygotes had normal red cell morphology, normal Hb concentration and normal amounts of both HbA_2 and fetal hemoglobin.

Alpha-, Beta-thalassemia. Heterozygotes for the inheritance of both alpha- and beta-thalassemia genes have been reported frequently in those populations in which both types of thalassemia have been encountered, such as Italian, Cypriot, Oriental, Black American, Saudis and others.[18] The hematological data on these double heterozuygotes include hemoglobin levels ranging from 91.6-13.9 g/dL, MCV 67-75 fl, MCH 18.0-24.4 Pg, HbA_2 3.2-5.7% and HbF 2-3% with tred cell morphology similar to high A_2 alpha-thalassemia trait.

Hemoglobin Lepore trait. The hemoglobin Lepore trait is a disorder which mimics thalassemia with the inheritance of a structurally abnormal hemoglobin (Hb Lepore). Hb Lepore has frequently been reported in Italians, Greeks, Yugoslavs and Black Americans.[19] Heterozygotes with Hb Lepore trait are usually asymptomatic, however, splenomegaly has been reported in a few cases.

Differentiation of iron deficiency from thalassemia trait. Thalassemia and iron deficiency may share one common picture, ie. hypochromic microcytic red cells. Distinction between iron deficiency and thalassemic disease is easy because of different clinical features, but discrimination between thalassemia traits and iron deficiency may pose problems. Of course, if serum iron or serum feritin values are known and the HbA_2 estimation available, they will help. Microcytosis due to iron deficiency must of course be excluded. Stockman et al[20] have suggested the value of free erythrocyte protophorphyrine (FEP) measurement in the evaluation with microcytosis. In thalassemia trait (FEP is normal, whereas in iron deficiency FEP is elevated. Function derived from Coulter Counter Indices have also been helpful in rapid differentiation between the microcytosis of thalassemia and iron deficiency. England and Fraser[21] described a derived function (DF = MCV-RBC- (5 x Hb) divided by 3.4). Values of the DF over 2 were associated with iron deficiency, whereas values less than 2 indicated thalassemia trait. Mentzer[22] showed that the ration of MCV/RBC was also predictive. Values of more than 12 usually indicate iron deficiency and those less than 11 usually are associated with thalassemia trait.

Homozygous beta-thalassemia (Cooley's anemia). This clinical syndrome was first described in 1925 by Thomas Cooley and Lee.[23] There are 3 variants of homozygous beta-thalassemia, distinguishable on the basis of the severity of expression. These are °-thalassemia, + thalassemia, and ++ thalassemia. In °-thalassemia homozygotes, no beta chain synthesis occurs. The hypochromic anemia of thalassemia major is severe enough for the patient to become transfusion-dependent at an early age. The diagnosis of thalassemia major is made on the basis of severe erythroblastic hemolytic anemia, typical RBC morphology, greatly elevated levels of HbF, splenomegaly, and marked expansion of the erythroid marrow that produces characteristic roentgenographic changes of the long bones and skull. Both parents usually have beta-thalassemia trait.

Clinical manifestations. The clinical course of homozygous beta-thalassemia in most cases is severe. A brief description of each manifestation along with our observation is given below:

Blood. The anemia of thalassemia major is characterized by severe hypochromia and microcytosis. Affected infants are normal at birth but within a few months develop a severe hypochromic microcytic anemia. When the child becomes symptomatic the hemoglobin level may be as low as 3 to 4 g/dL and regular transfusion becomes essential to maintain hemoglobin at an adequate level. The usual symptoms of anemia like fatigue, lethargy and pallor are present.

Growth endocrine status. The endocrine abnormalities[24] encountered in beta thalassemia usually are evident during the second decade of life and secondary to the chronic iron loading. Growth retardation associated with delayed bone age is seen in inadequately transfused patients. This complication is rarely seen in patients on hypertransfusion programs and if ever it is observed during the second decade of life, there should be a cause other than the low growth hormone levels for growth retardation and the delayed bone age, because the growth hormone levels are within normal range. Nevertheless, many patients with beta-thalassemia have been shown to have low levels of somatomedin,[25] a factor produced by the liver in response to growth hormone and that stimulates cartilage growth.

Liver and gallbladder. Hepatomegaly due to extramedullary hematopoesis is not expected in children on hypertranfusion therapy. Nevertheless these children now develop hepatomegaly as a result of progressive enorgement of hepatic parenchymal and phagocytic cells with hemosiderin deposits. Furthermore, extracellular accumulation of iron induces intralobular fibrosis. Episodes of transfusion related hepatitis also alter the function and play a role in development of fibrosis and cirrhosis. Serum bilirubin rises both due to hemolysis and the liver's decreased ability to conjugate the bilirubin. With the hypertransfusion therapy, the bilirubin rarely rises above 2 mg per dL. The degree of fibrosis correlates well with the iron accumulation and this can improve even by the suboptimal chelatin therapy.

Heart. Most patients with thalassemia die with cardiac complications of iron overload. Recurrent pericarditis with pain, fever and friction rub may be the initial manifestations of myocardial deposition of

iron. This occasionally may require pericardiactomy to relieve constriction. Cardiac arrhythmias may cause death; these include ventricular tachycardia and fibrillation and severe congestive heart failure or both.

Cardiac hemosiderosis follows the iron accumulation in the other tissues.[26] The microscopic evaluations suggest that the order of iron deposition is; first the ventricular myocardium, then the atrial myocardium and lastly the conduction tissue. Gross anatomic changes are dilatation of the atrial and ventricular cavities, gross thickening of the muscle layer resulting in overall weight of the heart to double or triple. Supraventricular arrhythmias correlate well with the extent of iron deposited in the atrial myocardium.

Infection. Thalassemias are classically associated with susceptibility to infections for the following reasons: 1. The patients are exposed to infections transmissible by blood transfusion. 2. Low transfused patients in addition to being anemic are stressed and cachectic. 3. Splenectomy increases susceptibility to some infections. 4. There may be reticuloendothelial blockage because the RE cells are crammed with remains of broken down red cells or with forms of storage iron. 5. Lymphoid hyperplasia may increase the incidence of ear, nose and throat complication and 6. Progressive iron loading may decrease resistance to infection by depleting the leukocytes of ascorbic acid.

The causative organisma are encapsulated pneumococci in two-thirds of cases, and hemophilus influenza type B and meningococcus in the reamining. Clinically, the disease is fulminant and may proceed from mild fever and headache to hyperpyrexia, prostration, shock and death within several hours. As many as a third of children with thalassemia major subjected to splenectomy may develop this complication.

Radiologic changes. The characteristic osseous changes in thalassemia can be shown only in patients who receive infrequent blood transfusions. Regular transfusion to nearly normal hemoglobin levels suppresses the erythropoeisis and prevents bony deformities. Radiological changes usually are not apparent until one year of age.[27] In small bones of the hand and feet, the trabecular pattern is coarse and cystic formation may be seen. Thickening of the cortex and increase of medullary space of long bones may result in increased fragility and fractures. Skull x-rays will show the increase of diploid space and arrangement of trabeculae in vertical rows, causing "hair-on-end" appearances.

Overgrowth of maxilla and delay of pneumotization of sinuses lead to prominence of upper incisors and separation of orbits producing the characteristic thalassemia facies.

Local clinical observation. The hospital record of 33 beta-thalassemia major patients who have been managed and followed in our pediatric hematology clinic during the last 15 years were retrospectively reviewed. Thirty (91%) of our patients had hepatomegaly and splenomegaly was present in 29 (85%) patients. All of them were severely anemic with early growth failure. Iron overload was very high in most of them. In other reports from Qateef area, about 20 patients with beta-thalassemia major[28] and from Hofuf area 42 beta-thalassemia major[29] who are followed regularly in their hospitals showed greater severity of the disease than in ethnic groups elsewhere. This is attributed to lack of awareness regarding public health, delays in seeking medical advice and the absence of functional thalassemia association in the country.

Laboratory findings. The peripheral blood smear shows large hypochromic, microcytic cells, anisocytosis, poikilocytosis with teardrop-shaped red cells, targets, polychromasia and basophilic stippling. Poorly hemoglobinized normoblasts are frequently found in peripheral blood, and their number increases markedly after splenectomy. Reticulocyte counts are not high because of the massive destruction of erythroid precursors in bone marrow. Osmotic fragility is decreased, a phenomenon explained by marked hypochromia. The bone marrow is hypercellular, with marked hyperplasia. The presence of alpha chain inclusions under the phase microscopy or supravital stain is of diagnostic value. HbA_2 is either slightly increased or normal, and fetal hemoglobin ranges from 60-98%. The hemoglobin electrophoresis pattern varies from one patient to another with homozygous beta-thalassemia. In $^+$ homozygous thalassemia, there is some HbA_2 present; HbF ranges from 40-90%. HbA is absent in ^{o} type, and only fetal hemoglobin is present, which is heterogenously distributed in the red cells.

Management. The management of thalassemia major has undergone significant changes over the last two decades.[30] The lifelong management of patients with thalassemia major involves several considerations: 1. Transfusion regimen. Transfusion should use packed RBCs from which WBC has been removed. This prevents the development of WBC antibodies and avoid febrile reactions. The dose of RBCs to be given with each transfusion is based upon the hematocrit (Hct) of the RBC preparation, the frequency of transfusions and the child's weight. There are 2 schools of thought concerning the level of hemoglobin that should be maintained. The "hypertransfusion" regime attempts to keep the hemoglobin above 95 to 100 g/L.[31] The "supertransfusion" regimen attempts to keep the hemoglobin above 120g/L.[32] Due to the supertransfusion regimen possibly requiring frequent transfusion (every 2 to 3 weeks) most centers tend to

follow 'hypertransfusion' regimen. Many studies have been carried out regarding "neocyte" transfusion. Administration of these young red cells[33] with increased survival (neocytes) should theoretically reduce transfusion requirements. However, most of these studies have shown only a modest reduction which did not justify the increased expense. 2. Chelation therapy. Patients receiving regular RBC transfusions are likely to develop iron overload. The repeated transfusion causes hemosiderosis, a pathologic accumulation of iron especially in the liver, the endocrine organs and the heart. This results in organ dysfunction such as diabetes mellitus, congestive heart failure, and cardiac arrhythmias.

This unfavorable prognosis has been improved by iron chelation therapy, the administration of iron-chelating agents that combine with body iron and facilitate excretion in the urine and stool. The iron-chelator, currently available, is desferoxamine (Desferal, DF). DF is an effective chelating agent that is nearly specific for iron. Current DF protocols involve 10 to 12 hour s.c. or IV continuous injections of DF using small battery-driven infusion pumps. The subcutaneous route is generally used. The dose of DF is 30 to 40 mg/kg/day, diluted in 8 to 15 ml of distilled water. Patients are encouraged to use the DF pump 5 to 6 nights per week during sleep.

DF-induced urinary iron excretion can be enhanced by the administration of 100 mg of ascorbic acid given on the days DF is used. Larger doses of ascorbic acid may produce iron toxicity in overloaded patients and should not be given. DF is remarkably safe. Auditory, visual and bony abnormalities have been described, but these have only occurred with DF doses in excess of 60 mg/kg/day. Recently, a number of potential oral iron chelators have been developed in various laboratories.[34] 3. Splenectomy. Most patients with homozygous beta-thalassemia require splenectomy at some time. In the past, before the use of hypertransfusion, the spleen often became massively enlarged in early infancy, and splenectomy was necessary to relieve the mechanical burden caused by its very large size. When splenectomy was carried out before 6 years of age, there was a high risk (10-20%) of severe post-splenectomy infection (PSI), which had a high rate of morbidity and mortality.[35] With the use of hypertransfusion, massive splenomegaly is unusual. Today the usual indication for splenectomy is evidence of increased destruction of transfused RBCs indicated by an increased transfusion requirement. Amounts in excess of 200 ml/kg/year indicate hypersplenism and are a reason to consider splenectomy. In almost every instance, it is possible to defer splenectomy until after 6 years of age, when the risk of PSI decreases greatly.[36] Following splenectomy, transfusion requirements are reduced considerably. Prior to elective splenectomy, immunization with pneumococcal and H. influenza polysaccharide vaccines should be given.

Due to patients with asplenia having an increased risk of severe bacterial infections PSI), prophylactic antibiotics are often advocated. Prophylactic penicillin (penicillin V, 125 mg to 250 mg p.o., b.i.d. or depot penicillin) should always be given to children under 5 years of age. Prophylactic antibiotics in children over 5 years of age are probably not necessary.[30]

Bone Marrow Transplantation (BMT). Bone marrow transplantation (BMT) is the only treatment currently available to cure thalassemia.[37] However, it is not without risk and the complications of graft failure, graft versus heart disease (GVHD), veno-occlusive disease, interstitial pneumonitis and infections together with the toxicity of the conditioning therapy result in a transplant related mortality in children of 10-20%. Indeed, bone marrow transplantation has a low inherent mortality in those patients who were well-chelated and in good condition. Lucarelli et al[38] identified 3 causes of risk using the criteria of degree of hepatomegaly, the degree of portal fibrosis and the quality of the chelation treatment given before the transplant. Patients for whom all 3 criteria were adverse constituted class 3, patients with none of the adverse criteria constituted class 1, and patients with 1 or various associations of 2 of the adverse criteria formed class 2. The results of BMT in children without organ impairment are excellent and BMT must now be considered a real alternative to conventional treatment.[39]

Gene therapy. Theoretically, the replacement of the abnormal thalassemia B gene could cure thalassemia major. Alternatively, strategy for gene therapy could involve the reactivation of Y chain synthesis by reversing the so-called Y-B switch. However, gene therapy for thalassemia major (TM) appears to be distant, through probable.

Screening and prenatal diagnosis. Preventive programs based on heterozygote detection, counselling and fetal diagnosis have been very effective in reducing the birth rate of -thalassemia major.[40] Such programs are ongoing in several at risk areas of the Mediterranean basin, namely, Sardinia, Cyprus, Greece and several region of Continental Italy. In all these programs carrier testing has been carried out voluntarily. In Northern Cyprus, however, engaged couples are required by law to produce a certificate of testing before they can be married.[41] Modern Deoxyribonucleic acid (DNA) technologies have precisely identified many specific thalassemia genes. This has permitted accurate prenatal diagnosis of thalassemia major. In risk pregnancies (both parents have thalassemia tratis) prenatal diagnosis can be performed in the first trimester of pregnancy using fetal DNA obtained by chorionic biopsy. Excessive carrier testing, combined with prenatal diagnosis has been very

successful in reducing the number of thalassemia major birth in Greece and Italy.[42]

Alpha-thalassemia. Thalassemia is the most common genetic disorder the world, and Saudi Arabia is one of the countries with the highest incidence of alpha-thalassemia.[43,44] Approximately 45% of the population in the Eastern Province of Saudi Arabia are heterozygous for a form of α+ thalassemia (-α/αα) and 15% are carriers for a non-deletion form of α thalassemia (α^t/αα). Furthermore, it then appears to be a wide spectrum of α-thalassemia phenotypes resulting from the interaction of these two genotypes.[45] Unlike beta-thalassemia, it is not possible to detect mildly affected carriers of alpha-thalassemia on routine hematological screening since their red cell morphology may be quite normal as is their hemoglobin electrophoresis. Hence it is not possible to gain a true insight into the incidence of alpha-thalassemia in the Eastern oasis population of Saudi Arabia. The normal infants usually reported have about 0.6% Hb Barts (γ4) at birth because they have a normal amount of α chains to bind to both β and γ chains. With decreasing α chain production, Hb Barts increases. By using the presence of elevated levels of Hb Barts (greater than 2%) in the cord blood samples as a marker for α-thalassemia gene the incidence of α-thalassemia in the neonates was determined.

The genetics of alpha-thalassemia is more complex, and the variable levels of hemoglobin Barts in newborns has been used as a marker for the presence of alpha-thalassemia genes in many populations. Recent advances in gene mapping have shown that in most humans the alpha-globin gene loci are duplicated (αα/αα), and the alpha-thalassemias are the result of deletion of one of the 4 structural genes responsible for alpha-globin synthesis. Loss of one alpha gene produces only minimal red cell abnormalities which are not detectable by globin chain synthesis. Deletion of 2 alpha genes, as in homozygous alpha + thalassemia (-α/-α) or heterozygous alpha°-thalassemia (--/αα) produces more severe red cell changes and markedly lower α-chain production, but carriers remain asymptomatic. HbH disease is due to deletion of 3 genes (--/-α) resulting from combined heterozygosity of α-thalassemia 1 and α-thalassemia 2. In Southeast Asia, 3 main haplotypes have been seen which lead to all of the above types of alpha-thalassemia: α/, α/ and --/. However, in Saudi Arabians, it has been shown that HbH disease is produced by the inheritance of 2 genes of equal severity (α^T/α^T) (a non-deletion α thal haplotype) rather than from the combination of α thal 1 and α thal 2 haplotypes. Finally, when all 4 genes are lost (--/--), a physiologically useless condition, hydrops fetalis syndrome is produced HbBarts being the predominant hemoglobin in the fetus. Assuming that the normal condition of α genes is for 2 pairs of locia to be functional, and that the severity of α thalassemia depends upon how many of the α loci are inactive, this model appears to fit well with the present knowledge of α gene loci. In the Saudi population, the major defect in cases with one gene deletion results from the rightward deletion of 3.7 kb fragment.[46] This type of deletion has been reported in Jamaican, Black American, Mediterranean, and Chinese subjects.[47] Leftward deletion is observed in the Saudi population of the Eastern Province, but at a much lower prevalence than the rightward deletion in the same population.[48] In addition, double heterozygote leftward/rightward deletion has been identified in the Saudi population in a few cases.[49]

Heterozygous alpha-thalassemia 2 (silent carrier). This is virtually an asymptomatic condition without anemia or morphologic abnormalities. In fact, the only evidence of the presence of this condition is the elevation of HvBarts (2-5%) at birth, or by *in vitro* studies of synthesis rate for α and β globin chains in reticulocytes, or by using a sophisticated technique of gene mapping.

Alpha-thalassemia trait 1. This condition results when 2 α genes have either been deleted in trans (-α/-α), as seen in Saudis and Blacks, or in cis (--/αα), reported in Southeast Asian populations. In patients with α thalassemia trait, morphologic abnormalities are seen with mild anemia (=12 G/DL). There is microcytosis and hypochromia of the red blood cells with mild degree of anisocytosis and poikilocytosis, α chain synthesis is approximately 25% reduced, with an α/β ratio of 0.7-0.8.

HbH disease. A marked deficiency in a α chain (30-60%) leads to formation of tetramers of β chains, ie. HbH disease is due to intracellular precipitation of unstable HbH with formation of inclusion bodies. Anemia may be severe during pregnancy or after ingestion of oxidant drugs or infection. Splenomegaly is usually present, and hepatomegaly is uncommon.

Patients with HbH have abnormal red cell morphology, including hypochromia, microcytosis, polychromasia, RBC fragmentation, targeting, basophilic stippling and HbH inclusion. The bone marrow shows marked hyperplasia. Hemoglobin H can be detected on electrophoresis as rapidly migrating hemoglobin, and care must be taken to examine fresh blood since a small amount of HbH can be lost if older hemolysates are used.

Homozygous alpha-thalassemia (hydrops fetalis syndrome). This is the most severe form of alpha-thalassemia, in which all of the 4 alpha globin genes are deleted, and has so far not been reported in Saudi Arabian. Hydrops fetalis occurs, resulting in stillbirth or immediate postnatal death, asphyxia being the obvious cause. The hematological characteristics include severe anemia with a large number of

nucleated red cells in the peripheral blood. The major hemoglobin components are HbH Barts and HbH, with some HbPortland.

Other clinical forms. Thalassemia intermedia (mild homozygous state). The combination of alpha-beta thalassemia and high A2 beta-thalassemia produces a form of beta-thalassemia which is intermediate in severity. Also, when a patient with homozygous beta-thalassemia inherits an alpha-thalassemia gene, the disease will be milder because associated alpha-thalassemia gene results in decreased synthesis of alpha chains and therefore, in less accumulation of free alpha chains. This leads to decreased inclusion body formation and less hemolysis. Thalassemia intermedia has commonly been seen in Blacks in whom red cell morphology is abnormal, anemia is moderate, hepatosplenomegaly is present and HbF is predominant. These patients are not transfusion-dependent and usually survive to the third or fourth decade.[50]

Hereditary persistence of fetal hemoglobin (HPFH). This is a type of thalassemia in which normal perinatal F---> A switch fails and the patient continues to synthesize fetal hemoglobin in adult life. This condition has been found primarily in Greeks and Blacks[51] and seems to be inherited as an allele of the chain gene complex. The Greek from has been described only in heterozygous state, but patients with the homozygous form have been identified. Patients with HPFH are clinically asymptomatic. The fetal hemoglobin is homogenously distributed throughout the RBCs in both heterozygous and homuzygous state. Black homozygotes for HPFH synthesize absolutely no or chains, have 100% HbF, and the / chain is balanced. In heterozygotes, the HbF level is 10-30%, and HbA$_2$ is decreased. Studies of globin chain synthesis have revealed balanced / $^+$ synthesis in these subjects. The molecular basis of HPFH in many populations has been define, and it is now known that most types of HPFH involved deletion of the and loci.

In conclusion, thalassemia syndromes are common in Saudi Arabia. The and thalassemia genes occur with variable frequency in different region of Saudi Arabia. The importance of the better understanding of the pathophysiology, clinical manifestation and management is stressed. In the case of thalassemias in Saudi Arabia, however, it still has a long way to go, in establishing the frequency, characteristics and population densities of the disease. An important aspect of the problem that population lacks genetic counselling service within the current state of health services must hold priority.

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