

# Thalassemia and Abnormal Hemoglobin

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## Abstract

Thalassemia and abnormal hemoglobins are common genetic disorders in Asia. Thalassemia is not only an important public health problem but also a socio-economic problem of many countries in the region. The approach to deal with the thalassaemic problem is to prevent and control birth of new cases. This requires an accurate identification of the couple at high risk for thalassemia. However, the diagnosis of thalassemia carrier states need several tests which are not practical for screening the population at large. Recently we have used two simple laboratory tests to screen for potential thalassemia carriers and hemoglobin E individuals. There is also a new development in using the automatic HPLC to diagnose thalassaemic diseases and the carriers. This system gives both qualitative and quantitative analysis of hemoglobin components in the same run with good precision and reproducibility. The system has been applied to study thalassemia and abnormal Hb in adult and cord blood. This system has enabled us to do both pre-natal and postnatal diagnosis of thalassemia within the few minutes. However, none of these screening tests can accurately give specific diagnosis of the thalassemia genotype. Specific thalassemia mutation can be carried out by DNA analysis. Many DNA techniques have been used for point mutation detection and small deletion. For the last few years there is a development of DNA chip technology that has been applied for thalassemia mutation as well. Clinically, thalassemia is very heterogeneous in the manifestation. In spite of seemingly identical genotypes, severity of beta thalassaemic patients can vary greatly. Heterogeneity in the clinical manifestation of beta thalassaemic diseases may occur from the nature of beta globin gene mutation, alpha thalassemia gene interaction and difference in the amount of Hb F production which is partly associated with a specific beta globin haplotype. However, there is still some beta thalassemia cases that have a mild clinical symptom without those known genetic factors interaction suggesting that there are other additional factors responsible for the mildness of the disease.

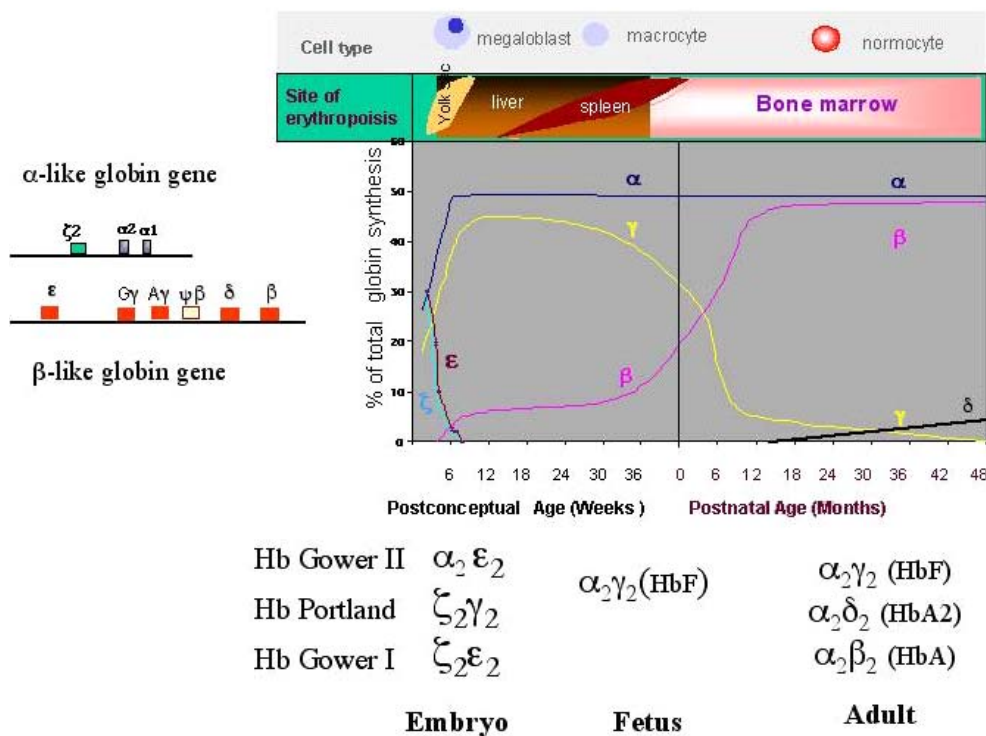
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## 1. Introduction

Hemoglobin is the oxygen carrying protein in the red cell. It composes of two major components: protein, called globin and the heme group that has the iron molecule and porphyrin ring. The globin chains of hemoglobin molecule consists four polypeptide subunits, two identical  $\alpha$ -subunits and two identical non  $\alpha$ -subunits. The  $\alpha$ -like globin chains ( $\alpha$  and  $\zeta$  chain), containing 141 amino acid residues and non  $\alpha$ -like globin chains ( $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$  chain), containing 146 amino acid residues. There are two types of  $\gamma$  chain,  $\gamma^G$  and  $\gamma^A$  globin chain, with the amino acid difference at the position 136 is glycine and alanine, respectively.

There are seven different types of structural genes coding for human globin chain. The structural genes for  $\alpha$ -like globin chains ( $\alpha$  and  $\zeta$  chains) are located on

the short arm of the 16th chromosome, forming 25 kilobases (kb) cluster, with each haploid genome containing two  $\alpha$ -like globin genes. The structural genes for  $\beta$ -like globin chains are found on the short arm of the 11th chromosome, forming a cluster approximately 60 kb, with each haploid genome being composed of only one cluster. The genes in each complex together with several inactive pseudogenes are in the same 5' to 3' orientation and are arranged in the order of expression at different stages of development. The  $\zeta$  and  $\epsilon$ -chains are synthesized in early fetal life, creating embryonic Hb Gower I ( $\zeta_2\epsilon_2$ ) that is subsequently replaced by embryonic Hb Gower II ( $\alpha_2\epsilon_2$ ), Portland ( $\zeta_2\gamma_2$ ). They are found only during the first 10 to 12 weeks of fetal development and presumably synthesized by erythroid cells derived from the yolk sac. In prenatal stage, the predominant molecule is Hb F ( $\alpha_2\gamma_2$ ) with small amount



**Figure 1.** Expression of α- and β-like globin genes at different stage of development.

of adult hemoglobin ( $\alpha_2\beta_2$ ) and the site of erythropoiesis shift from yolk sac to the liver and spleen. In postnatal life fetal hemoglobin is gradually decreased and then replaced by Hb A ( $\alpha_2\beta_2$ ) and Hb A<sub>2</sub> ( $\alpha_2\delta_2$ ) after the first year of life. Hemoglobins A and A<sub>2</sub> are synthesized by the erythroid precursor cells in bone marrow. Hence during development there is a coordinated switching of hemoglobin synthesis, affecting both the site of erythropoiesis, as well as the types of polypeptide chains and hemoglobins synthesized (Figure 1).

**2. Globin Gene Structure and Expression**

All the globin genes have the same general structure. A promotor region is located upstream of each gene and control the rate of gene transcription. The promotor region is the binding site for RNA polymerase II. Each gene contains the exons which are the coding blocks containing the genetic information for amino acid sequences of the globin gene. The three exons are separated two non-coding blocks, called intervening sequences or introns. When DNA is transcribed to messenger RNA (mRNA), both exons and introns are transcribed. Before the mRNA is transported to the cytoplasm there is important processing events take place. The sequences transcribed from the introns are completely excised. The 5' end of the mRNA is capped by the addition of 7-methylguanosine triphosphate residues and methylation of the first nucleotides. Capping serves to increase the efficiency of both mRNA splicing and initiation of translation. A poly A tail is added to the 3' end, which in-

creases the stability of the mRNA. The mature mRNA, with introns excised and both ends modified, is then transported to the cytoplasm, where it directs the synthesis of globin chains on the polyribosomes. Finally, two α-like and two β-like globin chains combine spontaneously with heme to form hemoglobin.

**3. Hemoglobinopathies**

Hemoglobinopathies can be mainly divided into two groups. The first one is abnormal hemoglobins, in which the abnormal globin chains were produced. The second defects, the thalassemias, which are genetic abnormalities causing the reduction or absence in globin chains synthesis.

*3.1. Abnormal Hemoglobins*

Abnormal hemoglobin occurs from genetic mutation including point mutations, deletions or insertions of the globin genes. Over 90% of known variants have arisen by substitution of one amino acid residue in one chain type (one point mutation) and over 60% involve the β globin chain. Because an individual inherits only two β globin genes, a β chain variant usually constitute about half of the total hemoglobin in the red cells and gives rise to significant change in the function of the red cell. In contrast, most individual have four a globin genes therefore α chain variants usually contribute only about 25% of the total hemoglobin and the red cell function consequence to be milder than those produced by β

chain variants. This consideration explains why 50% of  $\beta$  chain variants are associated with clinical manifestations, compare with only 20% of  $\alpha$  chain variants that have been identified to date. Two other types of hemoglobin chain variants, those with  $\gamma$  chain variants and those with  $\delta$  chain variants, are rare cases. The  $\gamma$  chain variants occur during intrauterine life and disappear from the circulation very rapidly after birth, and do not affect the oxygen-carrying function, since the duplicate of the  $\delta$  chain genes is a protective device. The  $\delta$  chain variants modified the structure of Hb A<sub>2</sub> and, since it accounts for only 2-3% of the total hemoglobin, they may often escape detection and do not produce diseases. No variants of  $\epsilon$  and  $\zeta$  chains have been reported.

Hemoglobin variants may cause clinical symptom or not depending on the site where the mutation occurs.

### 3.1.1. Changes in Surface Amino Acids

Nearly all substitutions on the surface of the hemoglobin molecule, except Hb S, are harmless because most of these residues have no specific functional role. For example, Hb E [ $\beta$ 26 (Glu→Lys)] the most common human hemoglobin variant in Southeast Asia, has no clinical manifestation either heterozygotes or homozygotes. About one half of the known hemoglobin mutations are of this type and were only discovered accidentally or through surveys of large populations.

### 3.1.2. Changes in Internally Located Amino Acids

Changing an internal residue often destabilized the hemoglobin molecule. This can occur through the weakening of the heme-globin association or as a consequence of other conformational changes. Some abnormal globin chain that does not form a tetramer may aggregate with each other (known as "Heinz bodies") and adsorb hydrophobically to the erythrocyte cell membrane. The membrane permeability is thereby decreased causing premature cell lysis. Carriers of unstable hemoglobin therefore suffer from "hemolytic anemia" of various degree of severity. Some hemoglobin variants are rapidly degraded by proteolytic enzyme.

### 3.1.3. Changes Active Site

Some defective subunit cannot bind oxygen because of a structural change near the heme group directly affects oxygen binding. For example, substitution of tyrosine for the histidine proximal or distal to heme stabilizes heme in ferric form (Fe<sup>3+</sup>), which can no longer bind oxygen. The tyrosine side chain is ionized in this complex with ferric ion of the heme. Mutant hemoglobin characterized by a permanent ferric state of two of the heme are called "hemoglobin M". The letter M signifies that the altered chains are in the methemoglobin (ferrihemoglobin) form. These patients are usually cyanotic.

### 3.1.4. Changes at the $\alpha_1\beta_2$ Contact

Mutations at the  $\alpha_1\beta_2$  contact often interfere with hemoglobin quaternary structural changes. Some abnormalities at this subunit interfaces lead to the abnormal oxygen affinity. Most such hemoglobins have an increased O<sub>2</sub> affinity so that they release less than normal amount of O<sub>2</sub> in the tissues. Individual with such defects compensate for it by increasing the concentration of erythrocytes in their blood, secondary polycythemia. Some amino acid substitution at the  $\alpha_1\beta_2$  interface results in a decreased oxygen affinity. Individual carrying such hemoglobins are cyanotic.

## 3.2. Thalassemias

The thalassemias are a group of disorder in which synthesis of the  $\alpha$ ,  $\beta$ ,  $\gamma$ , or  $\delta$  globin chains is either reduced or totally absence. This results in erythrocytes with reduced hemoglobin content and morphological abnormalities. The aggregation and precipitation of excess unmatched globin chains within erythrocytes lead to premature destruction within the bone marrow or the circulation. There are two major types of thalassemia, which have clinical significances alpha ( $\alpha$ ) and beta ( $\beta$ ) thalassemia.

### 3.2.1. Alpha thalassemia

The  $\alpha$ -thalassemia ( $\alpha$ -thal) is characterized by a reduced rate of a globin chain production. DNA mapping has revealed that the gene deletion is the major cause of  $\alpha$ -thalassemia and that non-deletion is very rare. In  $\alpha$ -thalassemia 1 ( $\alpha^0$ -thalassemia) there is a deletion that removes about 17.5 kb of DNA including the  $\alpha 1$  and  $\alpha 2$  genes from the  $\alpha$  globin gene cluster. The 5' breakpoint may start within the third exon of the  $\Psi\zeta$  gene and the 3' end may terminate within the hypervariable region located at the 3' end of the  $\alpha$  globin gene complex. In  $\alpha$ -thalassemia 2 ( $\alpha^+$ -thalassemia), one  $\alpha$  gene in a chromosome is functional. There are two types of  $\alpha$ -thalassemia 2, one involving a deletion of 4.2 kb of DNA including the  $\alpha 2$  gene (leftward or 4.2 kb type) and another involving 3.7 kb of DNA between the duplicate  $\alpha$  genes (rightward or 3.7 kb type). The latter is more common in Southeast Asia and the point of crossover which gives rise to it is located within the highly conserved region around the third exon of the  $\alpha$  globin gene, in which the homology is more than 99%.

$\alpha$ -Thalassemia is present throughout Southeast Asia. Its distribution is heterogeneous. In Thailand, the overall frequency of a thalassemia is 20~30%. The frequency of  $\alpha$ -thalassemia 1 is higher in the Northern than in the Southern part; 10% in Chiangmai and 3.5% in Bangkok whereas  $\alpha$ -thalassemia 2 is between 16~20%.

### 3.2.2. Beta Thalassemia

The  $\beta$ -thalassemias are a heterogeneous group of dis-

order characterized by decreased or absent  $\beta$  globin chain synthesis. Point mutations and small deletions or insertions in the nucleotide sequences are mainly responsible for the molecular defects of  $\beta$ -thalassemia around the world and in Thailand. In contrast to  $\alpha$ -thalassemia, gene deletion does not appear to be a common underlying abnormality in  $\beta$ -thalassemia. Two types of  $\beta$ -thalassemias are known;  $\beta^+$ -thalassemia, in which the production of  $\beta$  chains is reduced, and  $\beta^0$ -thalassemia, in which the production of  $\beta$  chain is entirely eliminated.  $\beta^+$ -thalassemia generally involves defects at RNA processing or promoter region of the gene. In some cases it results from a mutation within the introns of the  $\beta$  globin gene. In  $\beta^0$ -thalassemia the absent of  $\beta$  chain synthesis is resulted from several causes such as a complete block at transcription or RNA processing, leading to lacking of the  $\beta$  globin mRNA production. In some case it is caused by point mutation in the DNA sequence, for instance a nonsense mutation providing a production of an incomplete  $\beta$  globin chain.

The frequency of  $\beta$ -thalassemia, in general, varies from 1~9%. It is more common in some islands with high incidence of indigenous breeding such as Cyprus and Maldives.

### 3. Clinical Classification

Thalassemia syndromes are classified into three clinical groups:

- 1. Severe thalassemia (thalassemia major)
- 1. Thalassemia intermedia
- 1. Asymptomatic thalassemia (thalassemia minor)

#### 3.1. Severe Thalassemia

This consists of thalassemic diseases with severe anemia and associated symptoms. Hemoglobin levels of the patients with severe thalassemia are usually 6 g/dl or lower. Untreated, the patients die early, at birth or before in the case of Hb Bart's hydrops fetalis or in the first two decades of life.

Severe thalassemia consists mainly of two categories, i.e. homozygous  $\alpha$ -thal 1 and numerous  $\beta$ -thalassemic diseases.

#### 3.2. Thalassemia Intermedia

This consists of thalassemic diseases with mild to moderate anemia with hemoglobin levels of 7 g/dl or higher at steady state. Generally the patients have very mild or are free of symptoms, not requiring blood transfusions. But complications do occur in these patients. They live a somewhat shortened life span, but a few can attain old age.

Numerous genotypes give rise to thalassemia intermedia phenotype. Six major groups are:

##### 3.2.1. $\beta$ -thalassemia Diseases

Numerous  $\beta^+$ -thal genes either in homozygous state

or in double heterozygosity with one of the  $\beta^0$ -thal genes or other  $\beta^+$ -thal genes. Or the would-be severe  $\beta^0$ -thal in conjunction with an  $\alpha$ -thal or a high Hb F gene which alleviate the degree of imbalanced globin chains.

##### 3.2.2. $\beta^0$ -thalassemia/Hb E disease

About half of  $\beta^0$ -thal/Hb E patients manifest as thalassemia intermedia, while the other half have severe thalassemic disease.

##### 3.2.3. Hb H Disease

Practically all cases of the common Hb H disease, either of the  $\alpha$ -thal 1/ $\alpha$ -thal 2 or  $\alpha$ -thal 1/Hb CS genotypes, manifests as thalassemia intermedia. Exception is that the patients with Hb H disease frequently develop acute hemolysis as a complication of acute infection.

##### 3.2.4. Homozygous Hb CS

Hb CS is prevalent in S.E. Asia. In homozygous state it can manifest as a very mild hemolytic anemia, although some are asymptomatic.

##### 3.2.5. $\alpha$ -thal 1/ $\alpha$ -thal 2 - Hb E Disease

This is a relatively common genotype that manifests as thalassemia intermedia characterized by Hbs A+E +Bart's.

##### 3.2.6. Dominant $\beta$ -thalassemia Trait

All the  $\alpha$ -thal and  $\beta$ -thal heterozygotes are asymptomatic. Some very rare  $\beta$ -chain mutants result in either unstable hemoglobins or undigested shortened versions of  $\beta$ -globin chain that precipitate in the red blood cells leading to premature destruction. Heterozygotes of such mutants then have symptoms of thalassemia intermedia.

#### 3.3. Asymptomatic Thalassemia (thalassemia minor)

All the heterozygotes or thalassemia traits are asymptomatic. But many homozygous and double heterozygous states are also symptoms - free. These are homozygous  $\alpha$ -thal 2, homozygous Hb E, some homozygous Hb CS, double heterozygosity between one of the  $\alpha$ -thal genes with either  $\beta$ -thal or Hb E, for examples.

## 4. Clinical Aspects

#### 4.1. Severe Thalassemia

Homozygous  $\alpha$ -thalassemia 1 (Hb Bart's hydrops fetalis). Homozygosity for the severe form of  $\alpha$ -thal leads to the most severe form of thalassemia. Because of the absence of  $\alpha$ -globin chain synthesis the fetus does not have either Hb F or Hb A, thus incompatible with life. The baby either dies in utero or soon after birth, at the

gestation age of 30~40 weeks. The fetus is characteristically hydropic with enlarged liver, spleen and placenta. Ultrasonography can detect the hydropic picture as early as 18~20 weeks of gestation. Hemoglobin electrophoresis characteristically shows about 70-80 per cent of Hb Bart's, the rest being embryonic hemoglobins with a conspicuous absence of Hb F and Hb A. Over 75 per cent of the mothers carrying Hb Bart's hydropic fetuses develop toxemia of pregnancy. Thus prenatal diagnosis should be performed for at-risk pregnancy with selective abortion to decrease the dangerous burden of useless pregnancies.

#### 4.2. Severe $\beta$ -thalassemic Diseases

The clinical aspect of the severe homozygous  $\beta$ -thalassemia or Cooley's anemia is well known. A severe case of  $\beta^0$ -thal/Hb E disease has the same clinical features.

In S.E. Asia  $\beta^0$ -thal is much more common than  $\beta^+$ -thal and so is  $\beta^0$ -thal/Hb E disease. Hemoglobin levels in a large number of  $\beta^0$ -thal/Hb E patients in steady state range from 3 g/dl to 13 g/dl with an average of 7.7 g/dl, a remarkable variability in severity. Determinants for different degrees of severity have been systematically investigated. Concomitant inheritance of an  $\alpha$ -thal 1 gene or associated homozygosity for an *Xmn* I genotype leading to elevated Hb F are two known factors, unknown ones still remaining. Thus in dealing with  $\beta^0$ -thal/Hb E disease one must bear in mind that more than one clinical identity exist.

In contrast to thalassemic patients in the West, our patients are non - or minimally transfused and iron chelated, due to economic reason.

#### 4.3. General Feature

The clinical aspect of severe  $\beta^0$ -thal/Hb E is that of congenital chronic hemolytic anemia. At birth the baby is asymptomatic because Hb F level is high. As Hb F production waning off replaced by inefficient  $\beta^E$  production, at the age of 3 to 6 months the baby begins to be anemic with hepatosplenomegaly. Full blown picture consists of retardation of physical development, thalassemic or Mongoloid facies, anemia, jaundice, bulged abdomen, absence of secondary sexual development. Chronic leg ulcer is observed in a few cases.

#### 4.4. Massive Erythropoiesis

Due to anemia that stimulates erythropoietin production erythropoiesis is massively increased, 10 to 15 times normal. This leads to bone resorption and extramedullary hemopoiesis. Extramedullary hemopoietic masses around the ribs and at paravertebral sites can cast frightening x-ray pictures although no symptoms. However, such mass in the spinal canal leads to paraplegia and in the skull to convulsion.

#### 4.5. Iron Overload

Iron overload occurs in every case without exception. The skin is darkened. Iron deposition is found in the bone marrow, liver, spleen, heart, pancreas and elsewhere. Arrhythmia is not frequently encountered as in Cooley's anemia. Although liver fibrosis from iron overload is a usual finding, ascites and other signs of cirrhosis are very rare. Diabetes mellitus secondary to iron deposition in the pancreas does develop if the patients live long enough. We have observed terminal wasting stage in some patients who lived into the third and fourth decades. The patients developed more skin pigmentation, poor appetite, weight loss, increasing anemia and died. This is believed to occur from organs failure due to over all increase in body oxidation brought about by chronic and severe iron overload. Chronic hypoxemia to be described later may be contributory.

#### 4.6. Bone

Besides bone disfigure as a result of massive bone marrow expansion, decreased bone density is remarkable, due to osteoporosis and osteomalacia. Bone fracture is common. Bone healing follows blood transfusions.

#### 4.7. Pericarditis

Acute benign pericarditis is frequently encountered, more in splenectomized patients. In many cases this follows upper respiratory tract infection. Either because of complaint of chest pain and pericardial rub is detected or the rub is accidentally detected during physical examination. The pericardial rub lasts a few days to a few weeks. In the majority of cases this is a transient and benign condition, not requiring treatment. But in a few cases intractable pericardial effusion follows, causing cardiac tamponade and failure. This requires aspiration. However, recurrence of pericardial effusion is prompt. Oral prednisone administration causes dramatic disappearance of the effusion. In a further few cases chronic constrictive pericarditis develops, requiring surgical intervention. Clinicians should be aware of this condition in encountering thalassemic patients with intractable cardiac failure. Histological examination of the pericardium shows nonspecific pericarditis. Viral infection has been suspected as the cause of this pericarditis but has not been proved.

#### 4.8. Heart

Cardiac failure is the cause of death of about half of thalassemic patients, particularly of the older ones. The main cause is iron deposition in the heart. Other causes are anemia, constrictive pericarditis as discussed above and pulmonary artery occlusion of the pulmonary arteries to be discussed.

#### 4.9. Gallstone

Gallstone is frequent due to high bile pigment turnover. Ultrasonography reveals gallstone in as high as 50 per cent of our thalassemic patients.<sup>12</sup> In many of these ascending cholangitis in which the patients develop painful fever with obstructive jaundice. Treatment with antibiotics alone is usually not adequate to solve the complication, necessitating removal of the gall bladder.

#### 4.10. Endocrine Function

The most conspicuous endocrine function defect is the absence of secondary sexual development. Diabetes mellitus frequently occur in untreated adult patients. The thyroid function is rarely disturbed. Hypoparathyroidism is associated with bone resorption, both of which are correctible by blood transfusion. Secretion of growth hormone has been reported as reduced or normal.

#### 4.11. Infections

Infections are major complications and causes of death of severe thalassemic patients. Prospective study reveals increased susceptibility to viral, bacterial and fungal infections. They range from minor infections such as upper respiratory tract infection and diarrhea to pneumonia and septicemia. In splenectomized patients septicemia can be very acute and overwhelming leading to death in a short period. Gram' negative bacteria are frequent causes of septicemia. A fungal infection by *Pythium* organism leads to arterial occlusion and gangrene of the legs. The cause of increased susceptibility to infections in thalassemia does not appear to be defective lymphocytes. Investigations have not yet been able to pinpoint the real mechanisms. Iron overload and severe anemia may be involved. However, the underlying mechanism seems to be very complex, involving reactions between thalassemic RBC vesicles, abnormal RBC surface, complement, platelet, coagulation factors and endothelium.

#### 4.12. Suppression of Body Antioxidants

As iron overload is a constant complication of thalassemia and iron is a strong oxidant, suppression of body antioxidants such as vitamins C and E is a usual finding in thalassemic patients. Effects of hyperoxidation and of antioxidant therapy need further investigation.

#### 4.13. Superimposing Auto-immune Hemolytic Anemia

Some thalassemic patients developed autoimmune hemolytic anemia with increased anemia and strongly positive Coombs' test. This further anemia responded to the usual treatment of autoimmune hemolytic anemia. Investigation in a large number of thalassemic patients show that their red cell surface is an active site of complex immune reaction which is likely associated

with many pathophysiologic phenomena.

#### 4.14. Hypertension, Convulsion and Cerebral Haemorrhage (HCC) Post Multiple Blood Transfusions

This is a new syndrome. Some thalassemic patients after receiving two units or more of continuous blood transfusion develop hypertension, convulsion and cerebral hemorrhage, many of which have died. This complication may develop as late as two weeks after the multiple transfusions that seem to argue against blood volume overload as the cause of hypertension in such cases. Monitoring blood pressure during and after blood transfusions with prompt antihypertensive intervention has reduced deaths from HCC. Investigations suggest that there is as yet unidentified substance occurring in HCC that raises the blood pressure.

#### 4.15. Hypoxemia

A great majority of splenectomized  $\beta^0$ -thal/Hb E patient develops hypoxemia demonstrated by low arterial partial oxygen pressure. The number of platelets in splenectomized thalassemic patients is double that of non-splenectomized ones; young and larger platelets are also observed in the absence of the spleen. Platelet micro-aggregates have been detected in the circulation of these splenectomized patients. Our working hypothesis for the pathogenesis of hypoxemia is that when platelets, which are increased in number and in young and more active forms after splenectomy, aggregate in the circulation, and in the pulmonary vasculature substances released from platelet aggregation diffuse to cause constriction of the musculature at the terminal bronchioles leading to decreased oxygenation and hypoxemia. There was an experimental model in dogs showing that induction of platelet aggregation in the circulation caused manifestation like hypoxemia observed in splenectomized thalassemic patients.

Extensive platelet function studies have revealed several interesting aspects including mixed population of platelets with hypo and hyper functions.

Administration of aspirin as platelet aggregation inhibitor reduces the degree of hypoxemia in the majority of cases. Platelet aggregation inhibitors should be routinely given to splenectomized thalassemic patients and the results need to be better evaluated, including pulmonary artery occlusion modification.

#### 4.16. Thromboembolism

Autopsy examination in a large number of thalassemic patients by Sonakul et al revealed a very striking pulmonary occlusion in the pulmonary arteries. Serial cutting of the lungs revealed in some patients there were as many as 24 lesions/cm<sup>2</sup>. The distribution of the occlusive lesions indicated embolism. The cause of thromboembolism in thalassemia seems to be very complex involving platelet, reactive thalassemic red cell surface,

coagulation factors and endothelium. This phenomenon is under investigation as it is very critical to understand it for better management of thalassemic patients.

## 5. Thalassemia Intermedia

Several thalassemia syndromes, as indicated in the classification, have intermediate or moderate clinical picture. Their hemoglobin levels are usually above 7 g/dl, associated with mild jaundice and hepatosplenomegaly with no defective physical development or thalassemic facies. Homozygous Hb Constant Spring is very mild, clinically barely visible, posing a problem of diagnosis. Iron overload is always demonstrated by increased plasma ferritin level. Normally patients with thalassemia intermedia do not require blood transfusions except when they develop infections precipitating further anemia. Iron chelation may not be necessary in very mild cases, otherwise should be considered.

### 5.1. Hb H Disease

Hb H disease is thalassemia intermedia with special feature and should be treated separately. Patients with common Hb H diseases, in steady state, have hemoglobin levels around 8~9 g/dl; in more than 60 per cent of the cases the hemoglobin level is above 8 g/dl. From the abnormal gene frequencies it is calculated that in Thailand there are several hundred thousands patients with Hb H diseases. Thus we see them all around as doctors, nurses, pharmacists, etc. Among each class of medical students there are usually one or two with Hb H disease. One woman hematologist took several years before detecting that her husband had Hb H disease.

However, hemolytic crisis frequently developing in Hb H patients after acute infections is very critical. Hemoglobin levels may drop from 9~10 g/dl to 3 g/dl overnight and the patients go into shock or renal shut down. Almost any acute infections with high fever can cause hemolytic crisis in Hb H disease. This is due to instability of Hb H; high fever can induce its precipitation leading to massive hemolysis. Attempt should be made to bring body temperature down as soon as possible when Hb H patients develop high fever. But it is not known how effective this measure is in preventing severe hemolysis. When hemolytic crisis occurs blood transfusions and intravenous fluid therapy are necessary to restore balance.

Although splenectomy is always followed by significant elevation of hemoglobin levels in Hb H disease, it

is not recommended. For the patients already do well with said hemoglobin levels in steady state.

## 6. Asymptomatic Thalassemia

This includes all the thalassemia heterozygotes (thalassemia traits) and other complex genotypes without symptoms. Their hemoglobin levels are normal or near normal. There are no jaundice or hepatosplenomegaly. There is no iron overload and some can develop iron deficiency like their normal counterparts.

The most common problem encountered with asymptomatic thalassemia persons is when they are diagnosed to have "thalassemia" without appropriate explanation that leads to panic. Thus it is very important to the clinicians to command understanding of implication of different thalassemia conditions to give appropriate explanation.

## 7. Management

Standard management consists of blood transfusion and iron chelation. Both are not readily available in poor countries with too many patients. Hydroxyurea administration has been found to be able to raise hemoglobin levels through Hb F stimulation. Erythropoietin is found to be able to alleviate anemia and cause prompt healing of leg ulcers. Bone marrow transplantation is increasingly successful. Cord blood transplantation has been tried with success. Gene therapy has been under experimentation and seems to be promising particularly with the molecular biology advancement in better understanding of DNA regulatory regions.

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