Thalassemia is a Preventable Genetic Disease

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Thalassemia is the most common genetic disease in Thailand and around the world. It is an autosomal recessive disorder. The genetic defect occurs in globin gene(s) which control production of globin chains which are important polypeptides of hemoglobin molecules. There are two globin gene clusters, alpha globin gene cluster on chromosome 16 and beta globin gene cluster on chromosome 11. Normal alpha globin gene cluster consists of one ζ (Zeta) globin gene and two α (alpha) globin genes (αα) on each chromosome 16. The zeta globin gene is active during embryonic life and alpha globin genes are active from fetal life onwards. Normal beta globin gene cluster consist of ε (epsilon), γ (gamma), δ (delta), and β (beta) globin genes on each chromosome 11. The epsilon globin gene is active during embryonic life and all the rest are active from fetal life onwards, with the gamma globin gene being more active in fetal life than the beta globin gene. From fetal life onwards, α chains are components of normal hemoglobin types as listed in the followings:

Hb A (adult Hb) = αβ (two alpha chains and two beta chains)
Hb A2 = αδ (two alpha chains and two delta chains)
Hb F (fetal Hb) = αγ (two alpha chains and two gamma chains)

A defect in the gene(s) causes reduction in alpha or beta globin chain production or production of alpha or beta chains with alterations in structure and causes alpha or beta thalassemia. If one alpha gene is deleted from one chromosome, the haplotype is named alpha thal 2 (αα). If both alpha genes are deleted from one chromosome, the haplotype is named alpha thal 1 (αα). Besides, there are point mutations in the alpha globin gene resulting in new kinds of alpha chain which give rise to new Hb such as Hb Constant Spring (Hb CS). The defect afflicting the beta globin gene is usually a point mutation, leading to reduction of beta chain production. In addition, there are mutations on the beta gene leading to a structurally altered beta globin chain which, for example when associated with alpha globin chains, gives rise to Hb E instead of Hb A. This type of beta gene might be symbolized as betaε gene.

Individuals with a thalassemia gene or genes have a wide range of clinical manifestations from asymptomatic to severe anemia. In the most severe form, namely, Hb Bart’s hydrops fetalis, affected individuals do not survive long after birth and in some cases even succumb in utero. In addition, women carrying an affected fetus are likely to suffer from severe complications such as severe preclampsia and/or placenta previa. This condition is caused by a defect of the alpha globin gene, the genotype being alpha thal 1 / alpha thal 1. Other severe forms of thalassemia include beta thalassemia homozygous or beta thalassemia major (beta thal / beta thal) and beta thalassemia / Hb E disease (beta thal / betaε gene). Affected individuals are severely anemic, and without treatment will develop several clinical features resulting from severe anemia. The striking features include retarded physical growth, extramedullary erythropoiesis evident by bone changes and hepatosplenomegaly, hemolysis resulting in jaundice and gall stone, low body immunity leading to several infections and finally, iron overload in several organs leading to their malfunction such as diabetes from pancreatic iron overload or, more importantly, heart failure which is the most common cause of death of patients. Clinical findings in these patients are not manifested right after birth but usually begin to be evident after 6 months of age or older. The mentioned manifestations of the disease can be prevented by hypertransfusion and iron chelation which are costly and need frequent hospital visits. Also, at the moment, effective iron chelation has to be administered by the subcutaneous injection of iron chelator almost continuously. All these supportive treatments of affected individuals are a life long process. Alternatively, thalassemia can be cured by bone marrow transplantation which is costly, carries risks and also carries a small failure rate. The burden and cost for proper management of each case of severe thalassemia patient are high. On a national scale, the number of thalassemia patients is approximately 1% of the population and the carrier rate for the thalassemic gene approaches 40%. It is estimated that 12,125 new cases of thalassemia diseases result each year from all pregnancies. Therefore, prevention of the disease is preferable.

Severe forms of thalassemia to be prevented
At present, targeted thalassemia syndromes to be prevented include Hb Bart’s hydrops fetalis, beta thalassemia homozygous and beta thalassemia / Hb E disease. Other thalassemia syndromes are considered less severe and usually are not included in thalassemia prevention and control programs. With the knowledge of the targeted diseases, prevention and control of severe thalassemia is now possible. Targeted thalassemia syndromes:

1. Hb Bart’s hydrops fetalis. The genotype of this condition is alpha thal 1 / alpha thal 1 (αα/αα).
2. Beta thalassemia homozygous or beta thalassemia major. The genotype of this condition is beta thal / beta thal.
3. Beta thalassemia / Hb E disease. The genotype of this condition is beta thal / betaε.
Thalassemia screening in pregnancy

In obstetrical practice, prevention of these diseases can be carried out by screening for couples at risk of having affected offspring. According to the genotypes of targeted syndromes mentioned earlier, targeted genes to be screened for in parental genetic makeup are listed as follow:

1. Alpha thal I
2. Beta thal
3. Betaα gene

Individuals with one or more of these genes generally have smaller red cells than normal. Therefore, screening for these individuals can be performed by looking at the mean corpuscle volume (MCV) obtained from an automated CBC or a test called osmotic fragility (OF), which will be positive in microcytic red cells. However, a Hb E heterozygote (a carrier for betaα gene) may have normal size red blood cells, and this necessitates another screening test for this group of carriers. A dichlorophenol indophenol precipitation (DCIP) test can help detect these carriers as a screening test. Therefore, a screening protocol to detect possible carriers of interested genes consists of MCV with DCIP or OF with DCIP.

Thalassemia screening can be performed on pregnant women first then another screening is carried out in the husbands of those women who are positive for MCV (or OF test) and / or DCIP. In some institutes, thalassemia screening is carried out simultaneously in pregnant women and their husbands at the first antenatal visit.

In those who are positive for thalassemia screening, a confirmatory test is performed to see if the couple is at risk of severe thalassemia in the offspring. The first confirmatory test is hemoglobin typing. In some cases, DNA analysis is needed.

Examples of simple Hb typing interpretation

**Alpha thal-1 trait**

MCV < 80 fl with normal hemoglobin typing (AA, A2 being < 3.5%) (also not distinguishable from alpha thal-2 homozygous or iron deficiency which may coexist).

<table>
<thead>
<tr>
<th>Hb H disease</th>
<th>Hemoglobin typing: AA, Bart's H (α - / - -)</th>
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</thead>
<tbody>
<tr>
<td>Hb H disease with CS</td>
<td>Hemoglobin typing: AA, Bart's H CS (α CS - / - -)</td>
</tr>
</tbody>
</table>

**AE Bart's disease**

Hemoglobin typing: A E Bart's (α - / - -; βα hem / βα+)

**EF Bart's disease**

Hemoglobin typing: E F Bart's (α - / - -; βα / βα+) or (α - / - -; βα / βα+)

**Beta thal trait**

Hemoglobin typing: AA, A2 being > 3.5% or > 4%. Genotype βα hem / βα+

**Beta thal homozygous**

Hemoglobin typing: A,F(A) (βthal / βthal) (a coexisting alpha thal-1 gene cannot be excluded by Hb typing only. DNA analysis is needed to exclude alpha thal-1 gene).

**Beta thal / Hb E disease**

Hemoglobin typing: EF(A) (βthal / βE); a coexisting alpha thal-1 gene cannot be excluded by Hb typing only. DNA analysis is needed to exclude alpha thal-1 gene)

**Hb E trait**

Hemoglobin typing: AE with Hb E 25-35%. If Hb E 18-25% a coexisting alpha thal-1 gene has to be considered.

**Hb E homozygote**

Hemoglobin typing consists mainly of Hb E 85-100% (a coexisting alpha thal-1 gene cannot be excluded by Hb typing only. DNA analysis is needed to exclude alpha thal-1 gene)

When a couple at risk of severe thalassemia in the offspring is identified, counseling is carried out to offer prenatal diagnosis to see if the fetus is affected.

Prenatal diagnosis of thalassemia

The first case of prenatal diagnosis of thalassemia was reported from Siriraj Hospital in 1987 by Kanokponsakdi et al. Several cases followed by the same group. Now it is being performed in several centers nationwide. The prenatal diagnosis process involves:

1. Fetal sampling
2. Laboratory diagnosis
3. Proper counseling

Fetal sampling

Fetal sampling involves collecting fetal cells or representatives of fetal cells to be analyzed to see if the fetus is affected with the disease of interest. Techniques for fetal sampling can be described as follow:

1. Chorionic villus sampling (CVS). This procedure can be performed at a gestational age of 10-14 weeks. Chorion frondosum is visualized using ultrasound and collection of a tiny a mount of chorion is carried out using biopsy forceps or negative pressure created by a media-containing syringe connected with a spinal needle under a sterile technique. Chorionic cells are derived from the zygote therefore can represent fetal cells in terms of genetic makeup. The obtained sample is washed in more media to remove blood and maternal decidua before submitting to laboratory testing. Regarding thalassemia testing, DNA study is performed on chorionic cells. Information of parental DNA characteristics or mutation should be available for this sampling procedure. CVS carries a risk of 0.5-1% fetal loss.

2. Amniocentesis. This procedure can be performed at a gestational age of 16-20 weeks. An appropriate amniotic fluid pocket is identified via ultrasound scan and collection of amniotic fluid using a syringe connected with a spinal needle under sterile technique is carried out. Amniotic fluid contains amniocytes which are cells desquamated from fetal skin or from fetal respiratory tract, GI tract, genitourinary tract and fetal membranes (amniotic membrane). DNA extraction can be performed on amniocytes and DNA testing can be carried out to see if the fetus is affected. Amniocentesis carries a risk of 0.5% fetal loss.

3. Fetal blood sampling. This sampling technique is also termed cordocentesis or percutaneous umbilical blood sampling (PUBS). It can be performed at a gestational age of 18-22 weeks. The umbilical cord is located under ultrasound visualization and 1-2 ml of fetal blood is collected via a heparin-containing syringe connected with a spinal needle under a sterile technique. The blood circulating in the umbilical cord is fetal blood and it is possible to perform both hemoglobin typing and DNA analysis (provided that parental DNA data are available). The procedure has more advantages than CVS and amniocentesis in that hemoglobin typing takes less time and results are available more quickly. However, there are some conditions that results can be equivocal. Cordocentesis carries a higher risk with 2-3% fetal loss rate.
A decision of which procedure to perform depends on the gestational age and the available parental information. Also, the familiarity of the operator with the procedure and available laboratory techniques in each institute have to be taken into account. In every procedure, there are always chances of sampling failure and laboratory failure and this has to be counseled to the couple.

In addition, a couple at risk for Hb Bart’s hydrops fetalis in the fetus may opt for ultrasound scanning to avoid risk of the fetal sampling technique. It has been found that fetal cardiomegaly is a very sensitive marker and can be detected early but measurement of cardiothoracic ratio needs to be done in a correct technique. After hydropic features are found, cordocentesis is still advised for correct diagnosis. It has been shown that in some cases, hydropic signs may develop late and termination of pregnancy has to be performed in later gestation. In the majority of cases, however, if serial ultrasound scanning up to 24-28 weeks fails to demonstrate fetal hydrops, then it is less likely that the fetus will be affected with Hb Bart’s hydrops fetalis.

### Laboratory Diagnosis on Fetal Samples

1. Hb Typing. Hb typing can be carried out on fetal blood only. It is the most appropriate method for diagnosis of Hb Bart’s hydrops fetalis. It may be less accurate in diagnosis of beta thalassemia in some cases as in a normal situation, the beta globin gene is not very active in fetal life and Hb A determination may be problematic. If information of parental beta mutation is available, it is prudent to perform this alongside DNA analysis.

2. DNA analysis. DNA analysis can be performed on all types of fetal sample. Cells from the sample are extracted for DNA and amplification of interested gene (s) is carried out. The amplified segments of gene of interest are analyzed to see if the fetus inherited both parental thalassemia genes. Therefore, parental mutations must be characterized prior to diagnosis. Various methods for DNA analysis are available based on each institute, for examples, reversed dot blots (RDB), denaturating gradient gel electrophoresis (DGGE), or DNA sequencing.

### Proper Counseling

Proper counseling must be performed along the way for thalassemia prevention and control.

The first step is usually pretest counseling for thalassemia screening either for the pregnant women alone or pregnant women with their spouses. Posttest counseling involves informing the results and the implications which, in cases where the screening tests are positive, will be the pretest counseling of the next step.

The second step is usually pretest counseling for confirmatory tests in cases which, from screening results, are possible to be a couple at risk for severe thalassemia in the offspring. Posttest counseling involves informing whether the couple is at risk. If so, prenatal diagnosis is offered, informing the benefit, risk and possible failure of the process. Also, options after the results have been obtained are to be pointed out for the couple to consider in advance.

The third step is usually posttest counseling for prenatal diagnosis. If the fetus is affected, options for termination of pregnancy or continuation of pregnancy are discussed. If termination of pregnancy is contemplated, the procedure and risks are also discussed.

**Thalassemia is a preventable genetic disease**

With all the mentioned strategies, thalassemia can be prevented. In addition, research for preimplantation genetic diagnosis and noninvasive prenatal diagnosis for thalassemia is actively carried out around the world and may help prevent thalassemia further.

### REFERENCES

1. Of the following items, which is not the targeted thalassemia disease to be prevented by prenatal diagnosis strategy?
   a) Hb Bart’s hydrops fetalis
   b) Beta thalassemia homozygous
   c) Beta thalassemia major
   d) Beta thalassemia / Hb E disease
   e) Hb H disease

2. The proper prenatal diagnosis procedure for the couple at risk for beta thalassemia homozygous in the offspring with only mutation of one parent known is
   a) Chorionic villus sampling
   b) Amniocentesis
   c) Fetal blood sampling
   d) Ultrasound scanning
   e) All techniques can be used properly

3. A pregnant woman is found to be positive for OF test. Her Hb typing reveals Hb AA2, the amount of Hb A2 being 4.6%. Her husband is also positive for OF test and has Hb typing of EA, Hb E being 21%. From these basic data, the risk of offspring may include
   a) Beta thalassemia / Hb E disease
   b) Hb Bart’s hydrops fetalis
   c) Hb H disease
   d) AE Bart’s disease
   e) All of the above

4. A couple at risk for Hb Bart’s hydrops fetalis comes to see you for prenatal diagnosis at a gestational age of 32 weeks. On ultrasound scan, no signs of fetal hydrops are detected. The next step of action should be
   a) Perform chorionic villus biopsy
   b) Perform amniocentesis
   c) Perform cordocentesis
   d) Continue pregnancy without any invasive procedure
   e) Terminate pregnancy

5. A pregnant woman whose previous child is affected with beta thal / Hb E disease seeks prenatal diagnosis for her current pregnancy at a gestational age of 13 weeks. She has had blood tests revealing a known beta thalassemia mutation with normal alpha globin genes. Her husband also has had blood tests which reveal him to be Hb E trait with Hb E of 28%. The couple would like prenatal diagnosis to be performed as soon as possible and accept the risk of prenatal diagnosis of not more than 3%. Proper fetal sampling procedure in this case is
   a) Chorionic villus sampling
   b) Amniocentesis
   c) Cordocentesis
   d) Ultrasound scan
   e) All techniques can be used properly