

**Thesis Title** Prenatal and Postnatal Diagnosis of Thalassemia in Thailand

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**ABSTRACT**

Hemoglobinopathics are the most common genetic disorders in Thailand with  $\alpha$ -thalassemia,  $\beta$ -thalassemia, hemoglobin (Hb)E and Hb Constant Spring (CS) being prevalent. The molecular defects of thalassemias are heterogeneous  $\alpha$ -thalassemia in Thailand is most often due to gene deletion. The severe form of  $\alpha$ -Thalassemia,  $\alpha$ -thalassemia 1, involves a deletion of the duplicated  $\alpha$ -globin genes whereas the milder form,  $\alpha$ -thalassemia 2, has one  $\alpha$ -globin gene left functioning on the chromosome. Hb CS is a variant with elongated  $\alpha$ -globin chain due to mutation at the termination codon of the  $\alpha_2$ -globin gene, however its mRNA is unstable and thus only small amounts of Hb CS are produced, resulting in an  $\alpha$ -thalassemia 2-like effect. In contrast the majority of molecular

defects in  $\beta$ -thalassemia resulted from point mutations and small deletions or insertions of nucleotides in the  $\beta$ -globin gene.

The two major  $\alpha$ -thalassemia disease are Hb Bart's hydrops fetalis (homozygous  $\alpha$ -thalassemia 1) and Hb H disease ( $\alpha$ -thalassemia 1/ $\alpha$ -thalassemia 2 or  $\alpha$ -thalassemia 1/Hb CS). Homozygous  $\beta$ -thalassemia and  $\beta$ -thalassemia /Hb E are major  $\beta$ -thalassemic syndromes in Thailand.

Since hemoglobinopathies are prevalent in Thailand. Genetic counseling, detection of the high risk couples and prenatal diagnosis should be performed to prevent and control of the severe thalassemic diseases. In this study detection of  $\beta$ -thalassemia heterozygote was examined by the ELISA technique. Quantitation of Hb A<sub>2</sub> and Hb F was performed in 30 cases of  $\beta$ -thalassemia heterozygote and the percentage of Hb A<sub>2</sub> was found to be  $5.5 \pm 0.88\%$  and Hb F was  $2.2 \pm 0.7\%$ . The results were correlated with those from cellulose acetate electrophoresis(CAE) and elution.

Identification of molecular defects causing thalassemia in a particular geographic area is essential so that a prenatal diagnosis program based on the use of DNA analysis can be implemented.

The PCR technique has been applied to investigate the molecular defect causing thalassemia in Thai population in this study. It was used in combination with a direct detection of the amplified DNA product on gel electrophoresis and with non-radioactively labeled ASO-probe hybridization. A total of 35  $\beta$ -thalassemic genes from 23 couples at risk of having fetuses with  $\beta$ -thalassemia/hemoglobin E disease and 5 couples risk for homozygous  $\beta$ -thalassemic disease were analysed. Three common mutations observed were 4-bp deletion at codon 41/42, nonsense mutation at codon 17, nucleotide substitution at the position 5 of IVS 1 of the  $\beta$ -globin gene. Their frequencies were found to be 36 %, 28 % and 16 % in the first group of patients and were 30%, 10%, 12% in the latter, respectively. Other mutations including frameshift mutation at codons 71/72,

nucleotide substitution at the position 654 of IVS 2 were also detected at a low frequency. About 12-20% of the  $\beta$ -thalassemia genes were unknown which need to be further analysed.

The PCR technique in combination with the simple and direct detection methods of the amplified DNA product by gel electrophoresis was also applied for prenatal diagnosis of 33 fetuses at risk of having either  $\beta$ -thalassemic/Hb E or having homozygous  $\beta$ -thalassemic diseases seven were found to be affected. In the fetuses that the diagnoses were unsuccessful by DNA analysis because of the unknown mutations, the diagnosis was subsequently performed by the conventional *in vitro* globin chain synthesis. In 14 fetuses at risk of having homozygous  $\alpha$ -thalassemia 1 the diagnoses could successfully be made in all of 14 fetuses and in 2 out of 14 fetuses were Hb Bart's hydrop fetalis by PCR and hemoglobin electrophoresis after postnatal. The diagnosis of the affected fetuses by the PCR and gel-electrophoresis technique and by Hb electrophoresis were in good agreement. In conclusion, the PCR technique is simple, fast, and powerful, which provides a great help for gene analysis and for prenatal detection of the thalassemic diseases in the country.