# PCR Detection of α-thalassemia 1 (Southeast Asian Type) Carriers in the South Northern Thailand

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**Abstract**

The alpha thalassemia is the inherited disorder commonly found in Thailand and is characterized by a decrease or lack of synthesis of the α globin chain. One of the severe form of α-thalassemia is α-thalassemia 1 (α-thal 1,--) which is caused by the deletion of both duplicated alpha globin genes. The most common mutation of α-thal 1 found in Thai population is the Southeast Asian type gene deletion (---$^{SEA}$), which is caused by the deletion of 17.5 kb α globin gene. The objective of this study was to survey the α-thal 1 carriers (SEA type) in people who lived in the south northern Thailand. One hundred pregnancies attending Antenatal Clinic unit (ANC) during 16-25 August 1999 at Buddhachinaraj Hospital, Phitsanulok, were studied using the polymerase chain reaction (PCR) technique. Three primers were used, one (A1B) was in the deletion area of α-thal 1, the other two (A4 and A9) were adjacent to the 5' and 3' breakpoint of deletion. PCR products were 570 bp and 194 bp DNA fragment. The 570 bp PCR product was specific to the α-thal 1 determinant and the 194 bp fragment was amplified from either α-thal 2 (-α) or normal alpha globin (αα) determinants. The results showed that 95 samples obtained the 194 bp DNA fragment where 5 samples out of 100 samples obtained both 194 bp and 570 bp DNA fragment. The study demonstrated five percentage of α-thal 1 carriers (SEA type) found in the pregnancies attending ANC unit at Buddhachinaraj Hospital.

**Keywords**: α-thalassemia 1, Southeast Asian type (---$^{SEA}$), PCR

## Introduction

The alpha-thalassemias are the most common genetic disorders and are characterized by the decrease or complete suppression of alpha globin polypeptide chains. It was found to be the most prevalent and widely distributed haemoglobin synthesis defects (Beutler *et al*., 1995). A reduced rate of synthesis of one or more of the globin chains leads to imbalanced globin chain synthesis, defective hemoglobin production, and damage to the red cells or their precursors. Since alpha chains are present in both fetal and adult hemoglobins, a deficience of alpha chain production will affect hemoglobin synthesis in fetal as well as in adult. A reduced rate of alpha chain synthesis in fetal results in an excess of γ chains, which form γ4 tetramers, or hemoglobin

Bart's. In adult life, a deficience of alpha chain results in an excess of β chains which form β4 tetramers or hemoglobin H (Rodger, 1998). The gene cluster, which codes for and controls the reduction of these polypeptides, maps near the telomere of the short arm of chromosome 16. The normal human α-globin cluster includes an embryonic gene (ζ), the duplicated α genes (α2 and α1), three pseudogenes (ψζ1, ψα2 and ψα1) and a gene of undetermined function (θ1) and arranged in the order of telomere-ζ2-ψζ1-ψα2-ψα1-α2-α1-θ1 - centromere (Fucharoen *et al.*, 1995). The genes expressed during the embryonic (ζ) or fetal and adult stage (α2 and α1) can be modified by mutation. Much more frequent are the deletions of variable size from the normal chromosome (αα). The condition in which no α-globin produced from the α-gene complex is called α-thal 1. The deletions that cause α-thal 1 usually remove both α1- and α2-genes from the α-globin gene cluster. The most common α-thal 1 found in Thailand is Southeast Asian type (Fucharoen and Winichagoon, 1992) which occurs from a deletion of 17.5 kb of DNA in which both α-globin genes are removed (Winichagoon *et al.*, 1992) (Figure 1). Compound heterozygosity for α-thal 1 and α-thal 2 results in Hb H disease while homozygosity for α-thal 1 leads to Hb Bart's hydrops fetalis, the most severe form of thalassemic disease (Rodger, 1998). In Hb Bart's, the fetus cannot produce HbF and HbA which leads to lethality, either in utero or soon after birth. The cause of death of hydrops is due to the physiological dysfunction of Hb Bart's. The infants are oedematous or hydropic because of the congestive heart failure due to the profound anemia; considerable enlargement of the liver and spleen. Furthermore, over 75% of mothers bearing a fetus with such hydrops can develop toxemia during pregnancy resulting in death in some cases (Fucharoen and Winichagoon, 1992). Both parents of infants born with hemoglobin Bart's hydrops fetalis generally have the α-thal 1 trait. In the case of hemoglobin H disease one parent has the α-thal 1 trait, and another has α-thal 2 trait. In Northern Thailand, 8.8% pregnant women were analyzed for the presence of α-thal 1; Southeast Asian type (Kitsirisakul *et al.*, 1996). More than 2% of the children in Northern Thailand are expected to be born with Hb H disease or thalassemic hydrops fetalis (Lemmens-Sygulska *et al.*, 1996). In Southern Thailand, the incidence of α-thal 1 traits were 4.3% and Hb Bart's was detected 15.5% from new born (Sriroongrueng *et al.*, 1997).

The technique used was polymerase chain reaction using three oligonucleotide primers bridging the common deletion breakpoint (Bowden *et al.*, 1992, Eisenstein, 1990). One of which was adjacent to the breakpoint of the α-thal 1 allele, and was used to amplify the 570 and 194 bp DNA fragments. The 570 bp product was specific to the α-thal 1 determinant and the 194 bp fragment was amplified from either the α-thal 2 (-α) or normal globin gene (αα) determinants. Both 570 and 194 bp fragments were detected in α-thal 1

trait (--/αα) and HbH patients (--/-α) (Winichagoon *et al.*, 1995) (Figure 1).

The main purpose of this study was to screen for α-thal 1 carriers in pregnancies lived in southern area of Northern Thailand and attended ANC unit at Buddhachinaraj Hospital.

## Materials and Methods

Blood samples and Primers

The blood samples of 116 subjects were studied; 2 positive control of Hb H disease that was from Thalassemic Center, Mahidol University, 14 negative controls of healthy subjects (6 males and 8 females) and 100 pregnancies. Negative subjects resided in south northern Thailand, composing of 4 provinces (Nan, Uttaradit, Pichit and Phitsanulok) and had no history of blood disease. All pregnancie subjects resided in South Northern Thailand and attended ANC unit at Buddhachinaraj Hospital during 16 August to 25 August 1999. The average hematocrit value among these subjects was 35.92 (range of 30-42). Five mls of peripheral blood was drawn from each subject, then was added into EDTA tube. The tubes were placed on ice until DNA extraction was performed.

Three oligonucleotide primers (A4, A1B and A9) were synthesized by Thaican Biotechnology, USA. A4 was 5'-GGGGGCGGCCTTGGGAG GTTC-3', adjacent to the 5'-breakpoint of the α-thal 1 allele. A9 was 5'-ATATATGGGTCTGGAATGTATC-3', located at nucleotide 550 of the 5' to the breakpoint of α-thal 1 and A1B was 5'-GTTCCCTGAGCCCC GACACG -3', corresponding to nucleotides 194 of the normal DNA sequence 3' to the first base of the primer A4 as shown in Figure 1.

DNA isolation

Nuclear DNA was isolated from peripheral leukocytes using Guanidine-HCl extraction method (Karlinsey *et al.*, 1989). Five ml of peripheral blood were lysed in 40 ml of cell lysis buffer. Nuclei were collected and digested with proteinase K. After digestion, the solution was mixed gently with 10% SDS by rocking tube back and forth. 7.5 M Guanidine-HCl were then added to disrupted cells to isolate protein. The solution was incubated at 68-70 °C for 10 min. After centrifugation, the cell pellets and other debris were discarded. The supernatant was collected and absolute ethanol was added. DNA was collected and washed with 80% ethanol. Genomic DNA was then suspended in deionized water and were measured using spectrophotometer at 260 nm.

Amplification of PCR product was performed in a 50 μl mixture of 50-100 ng genomic DNA, 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 0.2 mM of dNTP, 1.25 unit of Taq Polymerase (QIAGEN™) and 0.4 pmol of each primer. The amplification reaction was carried out using OMNIGENE

Instrument thermal cycler. A 35 cycle protocol was used with denaturation at 95 °C for 1 minute, annealing at 63 °C for 2 minutes and extension at 72 °C for 2 minutes.

Determination of PCR product

Following amplification 5 μl of PCR product was electrophoresed in a 1.5% agarose gel at 70 volts for 1 hr. The gel was stained with ethidium bromide and visualized on a UV transilluminator.

**Results**

With the PCR amplification technique using 3 primers, the result showed in Figure 2 and 3 that positive controls showed two bands of 570 bp with primers A4 and A9 and 194 bp with primers A4 and A1B (Figure 2, lane 2), where one band of 194 bp DNA fragment was amplified from negative controls (Figure 3, lane 2) with primer A4 and A1B. In our studies, 5 pregnancies showed both 194 bp and 570 bp DNA fragments as shown in Figure 2, lane 3-5, whereas another 95 samples showed only one band of 194 bp (Figure 3, lane 3-6). The result of 5 pregnancies were recognized as carriers from 100 cases studied, corresponding to 50 in 1000 cases. Since both two alpha thalassemia genes (α1 and α2) are missed in α-thal 1 (Southeast Asian type), the frequency of α-thal 1 gene was found to be 50x0.5 equal to 25 in 1000 cases or 0.025 in pregnancies who live in south northern Thailand. Homozygous α-thal 1 of the Southeast Asian type, the fatal condition of hemoglobin Bart's hydrops fetalis, has an expected frequency of 0.025x0.025 equal to 0.00063, or approximately 6 hydrops fetalis cases per 10,000 new births in this population.

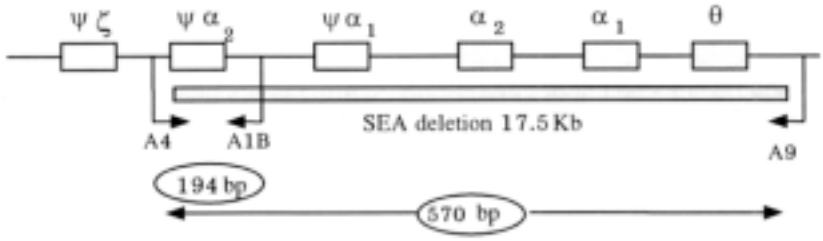


**Figure 1.** Schematic representation of normal and α-thal 1 of Southeast Asia type. Primer A4 and A1B amplify normal area to produce 194 bp PCR product. Primer A4 and A9 amplify the breakpoint in α-thal 1 alleles to produce 570 bp PCR product.

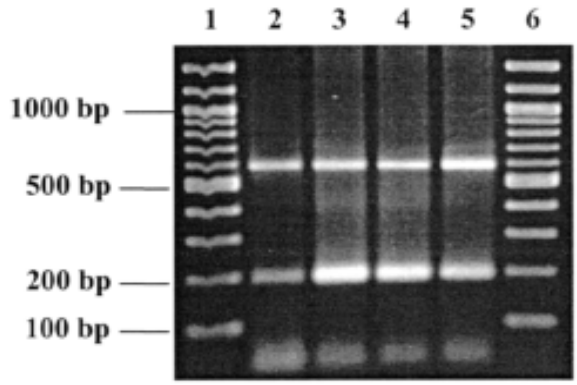


**Figure 2.** Agarose gel electrophoresis of amplified α-thal 1 DNA fragments. The two bands of 194 bp obtained from primers A4 and A1B and 570 bp from primers A4 and A9 were shown in Hb H disease in lane 2 and from 5 pregnancies as represented in lane 3-5. Lane 1 and 6 were 100 bp DNA marker.

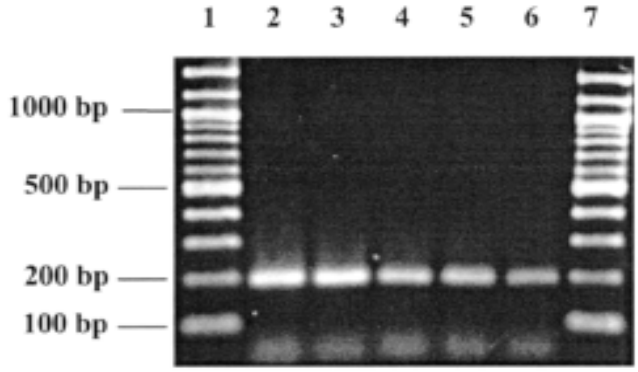


**Figure 3.** Agarose gel electrophoresis of amplified α-thal 1 DNA fragments. The only one band of 194 bp was shown in healthy subject (Lane 2). The other 95 pregnancie samples were also shown only one band of 194 bp DNA fragment as representative in Lanes 5-6. Lane 1 and 7 were 100 bp DNA marker.

## Discussion and Conclusion

The major public health problem in the Southeast Asian countries including Thailand was found to be genetically disorders. One of which was thalassemia which is the genetic disorder of blood disease. Hb Bart's hydrops fetalis is one of thalassemia that caused by the lack of α-globin chain in homozygous thalassemia. Twenty five percent of the fetuses die in utero during 28 and 38 weeks of gestation and the rest at delivery or soon after (Fucharoen *et al.*, 1992). The affected fetuses are hydropic with severe morbidity that makes the condition incompatible with life. Maternal complications such as toxemia of pregnancy have been observed in almost all pregnancies. It is important to set a strategy to prevent and control the further thalassemic offspring which

includes heterozygosity population screening (Galanello *et al.*, 1998), genetic counseling and prenatal diagnosis. Prevention of new births of thalassemias can be carried out by genetic counseling in combination with prenatal diagnosis (Pravatmuang *et al.*, 1995). The high risk couples should be identified at the primary health care level and be referred to a regional, well equipped center for proper counseling and prenatal diagnosis. Therefore, accurate diagnosis and counseling should be provided to the carriers.

This study showed moderate risk in having a new hydrops fetalis fetus of pregnancies in this area. The carriers found was as 5% compared to 30-40% carriers reported in 1996 in the north of Thailand (Lemmens-Zygulska *et al.*, 1996). However, it should be considered that the studies were based on healthy pregnancies with no hematological clinical signs or symptoms. The hematocrit values of 5 positive carriers were 36, 35, 33, 33 and 40, respectively which were in the normal values. Furthermore the duration for collecting samples was in the short period, only in August. The beneficial of the result is not only helping individual or the family planning, but also giving the basis data about α-thalassemia genetic disease for the people in southern part of northern Thailand. These data will support the preventing and controlling program to eliminate α-thalassemia disease from this area.

Further study is needed to establish the family tree of the carrier to avoid new births with α-thalassemic diseases. Thalassemia screening using simple and economical approaches has to be established. Genetic counseling must be offered to those who are carriers or those who are at high risk of having the thalassemic diseases. In addition, PCR has provided tools for accurately determining the various genotypes of α-thalassemia and thus enabling appropriate prenatal testing to be carried out (Fucharoen *et al.*, 1991; Fucharoen *et al.*, 1989).

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