FREQUENCY OF α-THALASSEMIA-1 OF THE SOUTHEAST ASIAN-TYPE AMONG PREGNANT WOMEN IN NORTHERN THAILAND DETERMINED BY PCR TECHNIQUE

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Abstract. Five hundred pregnant women were analyzed for the presence of α-thalassemia-1 of the Southeast Asian (SEA)-type by polymerase chain reaction (PCR) technique at the Maharaj Nakhon Chiang Mai University Hospital in Chiang Mai during the period from April to June 1995. Forty-four of them (8.8%) were recognized as carriers, corresponding to a frequency of 0.044. Homozygous α-thalassemia-1 of the SEA-type, the fatal condition of hemoglobin Bart's hydrops fetalis, has an expected frequency of 0.00194, or about 2 hydrops fetalis cases per 1,000 births in this population.

INTRODUCTION

In α-thalassemia-1 of the Southeast Asian (SEA) type both α-globin genes are removed from the α-globin gene-like cluster by a 20 kilobase deletion. Heterozygous persons are clinically normal, but fetuses with homozygous α-thalassemia-1 of the SEA-type develop hemoglobin Bart's hydrops fetalis and are stillborn or die shortly after birth. Moreover, hydrops fetalis poses a serious risk for pregnant women and determining the frequency of α-thalassemia-1 of the SEA-type in pregnant women is important for the proper genetic management of hydrops fetalis. This paper reports on the molecular screening for α-thalassemia-1 of the SEA-type in pregnant women in Chiang Mai using PCR.

MATERIALS AND METHODS

Blood samples were collected from 500 pregnant women who presented for antenatal care at the Maharaj Nakon Chiang Mai University Hospital. DNA was extracted from whole blood and prepared for PCR as described previously (Steger *et al*, 1994). *In vitro* amplification was done with the primers of Chang *et al* (1991), but different PCR conditions were used. The reactions had a volume of 15 μl and contained 20 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mg/ml BSA, 0.05% Tween 20, 200 μM of each dATP, dCTP, dGTP, dTTP, 0.2 μM of each primer, 1.5 μl DNA solution and 0.02 U/μl of *Taq* DNA polymerase. Hot start PCR technique was performed. All reagents of the PCR were combined with the exception of *Taq* DNA polymerase, two beads of paraffin wax (approximate bead volume 15 μl) were added and the mixture heated for three minutes at 94°C. Then the reactions were allowed to cool down to room temperature and *Taq* DNA polymerase was added on top of the hardened wax layer. The samples were then kept in a thermocycler at 85°C for 2 minutes followed by 40 cycles of amplification: annealing at 57°C for 1 minute, extension at 72°C for 1 minute and denaturation at 94°C for 1 minute. In the last cycle extension time was 5 minutes and the denaturation step was omitted. The PCRs were analyzed by electrophoresis on 3% agarose gels and the DNA bands were detected with ethidium bromide and UV light and photographed. Carriers of α-thalassemia-1 of the SEA-type showed a specific 194 basepair (bp) amplicon in addition to a 314 bp amplicon obtained from the normal DNA sequence (Fig 1).

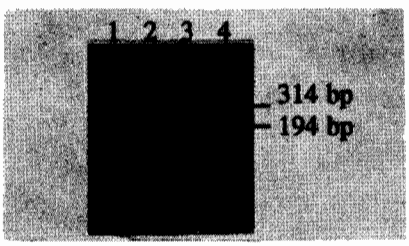

Fig 1–Detection of α-thalassemia-1 of the SEA-type by PCR technique. Lane 1 normal person; lanes 2, 3 : carriers of α-thalassemia-1 of the SEA-type; lane 4 : no template-DNA added to PCR (negative control).

RESULTS AND DISCUSSION

A PCR based test was used to detect the gene deletion underlying α-thalassemia-1 of the SEA-type in a population of the northern Thai city of Chiang Mai. Five hundred pregnant women were screened and the deletion was found in 44 women or 8.8%, supporting the assumed Hardy-Weinberg equilibrium prediction of 42 carriers of α-thalassemia-1 of the SEA-type. The calculated frequency of α-thalassemia-1 of the SEA-type is 0.044; and the expected frequency of homozygous α-thalassemia-1 of the SEA-type, the cause of fatal hemoglobin Bart's hydrops fetalis, is therefore 0.00194. Approximately 2 hemoglobin Bart's hydrops fetalis cases can be predicted in 1,000 births. Of the 3,782 births recorded during September 1994 to August 1995 at the university hospital there were 8 hemoglobin Bart's hydrops fetalis cases (~2/1,000). This study demonstrates that the PCR technique is very helpful in detecting carriers of α-thalassemia-1 of the SEA-type, the first step of the prenatal diagnosis of hemoglobin Bart's hydrops fetalis. Moreover, the PCR technique is rapid since DNA extraction, *in vitro* amplification and subsequent analysis can be done in one working day.

In a previous study on α-thalassemia in northern Thailand, 106 adults from the rural Chiang Mai area were analyzed with the Southern blot technique to detect α-globin gene deletions. In that population sample only five carriers of α-thalassemia-1 of the SEA-type were found (Hundrieser *et al*, 1988), roughly half the figure we would expect from our data. This apparent discrepancy may have been caused by the relatively small sample size that was analyzed. On the other hand, however, the differing results may reflect some differences in the genetic makeup of the northern Thai population.

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