

Mutation spectrum of β -globin gene in thalassemia patients at Hasan Sadikin Hospital - West Java Indonesia

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Abstract: Thalassemia is the most common hereditary haemolytic anemia in Southeast Asia, in which Indonesia is among countries that are at a high risk for thalassemia. It has been reported that mutation in the beta-globin gene is responsible in severe Thalassemia. However, the spectrum of beta-globin gene mutations in Indonesian population varies in different regions. Thus, this study aimed to identify the most prevalent mutation of Thalassemia patients from the Hasan Sadikin Hospital, Bandung, using this as a reference hospital for Thalassemia in West Java. The three most prevalent mutations of beta globin (IVS1nt5, Cd26 (HbE), and IVS1nt1), were conducted in the beginning of this study. Mutations of 291 samples were detected by PCR-RFLP in the Molecular Genetic Laboratory, Faculty of Medicine Universitas Padjadjaran, Bandung. The prevalence of the beta globin gene mutation types were 47.4% IVS1nt5 homozygote, 9.9% compound heterozygote IVS1nt5/HbE, 5.4% compound heterozygote IVS1nt5/IVS1nt1, 1.4% compound heterozygote HbE/IVS1nt1, 1% HbE homozygote, 14.4% Compound heterozygote IVS1nt5/... (no paired mutation), 2.06% compound heterozygote HbE/... (no paired mutation), 1.3% compound heterozygote IVS1nt1/... (no paired mutation), and 7 samples were unidentified. The thalassemia mutation IVS1nt5 homozygote is the most common mutation found in Thalassemia patients at Hasan Sadikin Hospital, Bandung. The samples with unidentified results might carry mutations other than the three that are observed in the present study.

Key words: Thalassemia; Beta globin; Mutation; Hasan Sadikin Hospital.

Introduction

Beta-thalassemia is one of most common autosomal recessive disorders worldwide (1). High prevalence is reported in populations in the Mediterranean, Middle-East, Transcaucasus, Central Asia, Indian subcontinent, and the Far East. It is also relatively common in populations of African descent. The highest incidences are in Cyprus (14%), Sardinia (12%), and Southeast Asia (2, 3). The Southeast Asian region accounts for about 50% of the world's carriers of thalassemia. Indonesia is included in the group of countries that are at a high risk for thalassemia (4).

Beta-thalassemia occurs due to reduced (β^+) or absent (β^0) synthesis of beta globin chains of the hemoglobin (Hb) tetramer, which is composed of two alpha globin and two beta globin chains ($\alpha_2\beta_2$) (2). The heterogeneity of molecular lesions which underlie the failure of erythropoietic cells to synthesize normal haemoglobin in beta thalassaemia (5) is a complicating factor in its molecular diagnosis. More than 100 different mutations have been identified, and most of them are single base substitutions or small deletions and insertions in the beta globin gene. In most of the

populations, a number of beta thalassaemia is caused by a population-specific spectrum of only a small number of prevalent mutations (5). Detection of mutations in patients, therefore, normally employs screening in the first instance for a small number of mutations that are the most common for the population concerned.

A strategy has been designed to rapidly detect nine of the most common mutations in Southeast Asia, which requires the amplification of two segments only of the beta globin gene. The mutations are at positions IVS-1 nt5, IVS-1 nt1, codon 26, codon 15, codon 17, codon 19, codon 30, IVS-1 nt2, and codon 41-42 of the beta globin gene, which together account for around 70-90% of beta thalassemia mutations in most populations of Southeast Asia (6-8). The aim of this study is to explore the specific mutation of Thalassemia patients in the Thalassemia center, Hasan Sadikin Hospital, Bandung, using this as a reference hospital for Thalassemia in the West Java province.

Materials and Methods

DNA isolation

This research involved 291 thalassemia patients.

DNA was isolated from blood samples with the Homebrew method. Mutation detection was conducted with PCR-RFLP in the Molecular Genetic Laboratory, Faculty of Medicine, Universitas Padjadjaran, Bandung. This method refers to the Molecular Biology Laboratory, Eijkman Institute, Jakarta.

PCR-RFLP

PCR amplifications were carried out using primer sets TLF62028-TLR62320 and TLF62392-TLR62703. Primer TLR62320 includes a G at the position equivalent to nt8 of intron 1 instead of the normal A, to create a GCTAGC site for *Cac8I* in the presence of the IVS-1 nt5 G>C mutation.

Primer sequences are TLF62028 (ComC) ACCT-CACCCTGTGGAGCCAC^{SEP}₁₇TLR62320 CTATTG-GTCTCCTTAA ACCTGTCTTGTAACCTTGCTA. PCR product is 293 base pair (bp). To detect IVS1nt5 mutation, HbE mutation, and IVS1nt1 mutation, *Cac8I*, *Bsl1*, *Mnl1* restriction enzymes were used respectively (8).

Results and discussion

The prevalence of the beta globin gene mutation can be seen in Table 1.

The results of the present study showed that the mutation in IVS1nt5 homozygote was the most prevalent among the 291 samples from thalassemia patients at Hasan Sadikin Hospital, Bandung. However, there were 7 samples that remain unidentified. PCR-RFLP products are presented in Figure 1.

The amplification of two segments of the beta globin gene is an approach to allow a rapid detection of mutations in beta thalassemia. The PCR-RFLP method has been confirmed to be more reliable, and gave a definitive identification of the underlying mutation. The mutations are at positions IVS-1 nt5, IVS-1 nt1, codon 26, codon 15, codon 17, codon 19, codon 30, IVS-1 nt2, and codon 41-42 of the beta globin gene, which together account for around 70-90% of beta thalassemia mutations in most populations of Southeast Asia (6-8). In this study, PCR amplifications used primers TLF62028-TLR62320 and TLF62392-TLR62703. Primer TLR62320 includes a G at the position equivalent to nt8 of intron 1 instead of the normal A, to create a GCTAGC site for *Cac8I* in the presence of the IVS-1 nt5 G>C mutation. A *Cac8I* site is also created in the presence of the IVS-1 nt2 T>C mutation, which represents less than 1% of the beta thalassemia alleles in Indonesia. The mutations G to T at IVS-1 nt1, G to A at codon 26, and A to G at codon 19

Table 1. Prevalence of the beta globin gene mutation.

Mutation	Prevalence (%)
IVS1nt5 homozygote	47.4
IVS1nt5/HbE	9.9
IVS1nt5/IVS1nt1	5.4
HbE/IVS1nt1	1.4
HbE homozygote	1
IVS1nt5/-	14.4
HbE/-	2.06
IVS1nt1/-	1.3

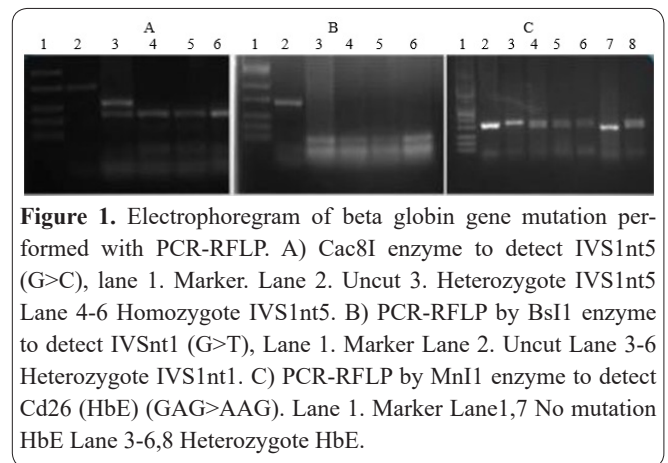


Figure 1. Electrophoregram of beta globin gene mutation performed with PCR-RFLP. A) *Cac8I* enzyme to detect IVS1nt5 (G>C), lane 1. Marker. Lane 2. Uncut. Lane 3. Heterozygote IVS1nt5. Lane 4-6. Homozygote IVS1nt5. B) PCR-RFLP by *Bsl1* enzyme to detect IVS1nt1 (G>T), Lane 1. Marker. Lane 2. Uncut. Lane 3-6. Heterozygote IVS1nt1. C) PCR-RFLP by *Mnl1* enzyme to detect Cd26 (HbE) (GAG>AAG). Lane 1. Marker. Lane 1,7. No mutation HbE. Lane 3-6,8. Heterozygote HbE.

eliminate the natural occurring sites for *BsII*, *MnlI*, and *MaeII*, respectively. The mutations G to A at codon 15, A to T at codon 17, and G to C at codon 30 create sites for *SfcI*, *BfaI*, and *Bsp1286I*, respectively. The detection of the 4 bp deletion of codon 41-42 is essentially as described by Chang *et al* (1992); a C has been introduced at the second position of codon 41 in the sequence of primer TLF62392, which, together with the 4-base deletion, creates a TCGA site for *TaqI* (9).

In this study, thalassemia mutation IVS1nt5 homozygote was the most common in thalassemia patients at the Hasan Sadikin Hospital, Bandung. This variant was recently shown to be very common among the Melanesians from the South West Pacific (10). The same mutant IVS-1 nt5 (G -> C) was reported earlier in high frequencies in Asian Indians (11), and has also been found at very low frequencies in Chinese patients (12-14). Referring to the study done by Chan *et al.* (1987), this variant is not present among 93 β -thalassemic chromosomes observed (15).

The type of thalassemia mutation IVS1nt5 homozygote is the most common mutation found in thalassemia patients at the Hasan Sadikin Hospital, Bandung. There were samples that remain unidentified, indicating they might carry other mutations. Further investigation to detect other types of mutation is therefore needed.

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