Application of ARMS primers for the molecular characterization of β-thalassemia carrier in Palembang, South Sumatra

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Abstract

Thalassemia. β-thalassemia in particular is commonly found in Indonesia. An appreciable frequency of β-thalassemia carriers were found in some eastern part of Indonesia. Yet, no data are available from the western part of Indonesia. Moreover, studies on its clinical or molecular aspects are also hardly available. Only one study on α-thalassemia has been recorded, but more intensive studies have been carried out on β-thalassemia.

During this study, β-thalassemia screening is carried out in 108 individuals from Palembang of South Sumatra representing population of the western part of Indonesia. This particular region has been acknowledged to have long historical relationship with population in Thailand and other populations of Southeast Asian mainland where thalassemia is prevalent. Hematological examination revealed that 10 individuals were β-thalassemia carriers and seven individuals were HBE heterozygote. Molecular analysis employing ARMS primers technique proved that all HBE heterozygotes were codon 26 (GAG->AAG) mutation, while among 10 suspected β-thalassemia carriers, five, two and one subjects were IVS 1 nt5 (G>C), IVS 1 nt10 (G>A) and codon 41/42 (GCT/TTT) mutation respectively. Only two suspected β-thalassemia carriers were still uncharacterized.

The result provides clear evidence about the simplicity and reliability of the technique. In addition, a set of ARMS primer with at least two common ones (i.e. IVS 1 nt5 (G>C) and Cod 26 (GAG->AAG) seems to be reasonably useful in Indonesia. The use of additional primers for codon 41/42 (GCT/TTT) and IVS1 nt 654 (G>T) should be also considered for any particular population in Indonesia especially with strong Chinese influence.

Keywords: ARMS primers - β-thalassemia carrier - HBE heterozygote - molecular analysis

Introduction

Thalassemia, a genetic disorder due to a single gene defect frequently found in Southeast Asian region is thought to be common in Indonesia (Wong, 1983, 1986). Despite its heterogeneity, β-thalassemia is considered to cause more problems. So far, studies on this disorder in Indonesia have been very limited. At the population level, β-thalassemia trait can be found in some populations from the eastern part of Indonesia e.g. Flores (Sofro et al., 1993), Ambon (Sofro et al., 1994a), Ujong Pandang (Sofro et al., 1994b) which reveals an appreciable frequency of β-thalassemia carriers. Yet, no data are available from the western part of Indonesia. Moreover, studies on its clinical or molecular aspects are also hardly available. Only one study on α-thalassemia has been recorded (Tan et al., 1992), but more intensive studies have been
carried out on β-thalassemia. Lie-Injo et al. (1989) reported a number of mutations underlying β-thalassemia cases in Jakarta. Employing a more simple non-radioactive technique using ARMS primers developed by Varawalla et al. (1991), several common mutations similar to those reported by Lie-Injo et al. (1989) have been also reported from Yogyakarta (Lanni et al., 1992, Sofro et al., 1994c) and Jakarta (Setaningsih et al., 1994). In fact, a small number of cases are still yet to be characterized. However, most of the mutations identified are very similar to those found in Thailand and other Southeast Asian populations.

During this study, β-thalassemia screening is carried out in Palembang of South Sumatra. It should be noted that the Indonesian populations are genetically related, with a clinal pattern observed when one goes from the west to the east or vice versa. The Mongoloid genes are common in the west while Melanesian genes are dominant in the east in accord to the evidence of prehistorical and historical population migration (Sofro, 1982). South Sumatra populations are among those with strong Mongoloid and South Asian genes influence and have been acknowledged to have long historical relationship with population in Thailand and other Southeast Asian populations. Therefore, high prevalence of β-thalassemia carrier might be observed in this particular region. Moreover, molecular analysis would be of beneficial to understand the molecular characteristic of the defective gene. Despite simplicity of the DGGE technique for thalassemia analysis in Surabaya (Notopuro, personal communication), molecular analysis using a more simple, fast, reliable and relatively cheap technique would be ideal for populations where high prevalence of β-thalassemia is found.

Materials and methods

\section*{a. Subjects and hematological examinations}

Subjects during this study were 108 unrelated adult healthy individuals residing in Palembang, South Sumatra (Figure 1). Informed consent was requested for each individual to participate in the study. All subjects were students nurse consisted of only seven male and 101 female between 14 and 21 years of age.

Approximately 10 ml of venous blood was drawn from each individual using EDTA-venoject vacutainer (Terumo). Each blood was examined for red blood cell count (RBC), hemoglobin (Hb) concentration, packed cell volume (PCV) and fetal hemoglobin (HbF) staining. HbA2 concentration was determined by eluting HbA1c fraction following electrophoresis in cellulose acetate membrane. HbA1c level of ≥ 4.0 % is considered to be β-thalassemia carrier.

DNA was isolated from the buffy coat by standard method using phenol extraction followed by purification. DNA preparation was then used for PCR analysis.

\section*{b. Analysis of β-globin gene mutations}

Mutations of β-globin gene were analysed by Polymerase Chain Reaction employing oligonucleotide ARMS primers. Having considered the common mutations of β-thalassemia from previous reports (Lanni et al., 1992, Lie-Injo et al., 1989, Setaningsih et al., 1994, Sofro et al., 1994c) the following set of primers (Table 1) were employed, i.e. codon 26 (Cd26; GAG>AAG), IVS-I nt5 (G>G), IVS-I nt5 (G>G), IVS-I nt5 (G>G), Cd 15 (TGG>TAG), Cd 41/42 (delCTT).

\begin{table}[h]
\centering
\caption{A set of ARMS primer used in this study}
\begin{tabular}{|c|c|}
\hline
\textbf{Fragments/mutations} & \textbf{Primers} & \textbf{bp} \\
\hline
Control & A 5'-CAATGATCACACCCCTTCTCGGACCC- & 861 \\
& B 5'-GACGTAGAAGGCTCCAGAAGGAGGAGA- & \\
& normal: 5'-CCCTTTACACCTTCTGGAACCCAGTAC- & 285 \\
& (G>C) & 285 \\
& mutant: 5'-CCCTTTACACCTTCTGGAACCCAGTAC- & \\
& Common C: 5'-ACCTACACCCCTGGAACCCAGA- & \\
& IVS I nt 5 & normal: 5'-GATGAAAGTTGCTGGTGAGCGTGCTAGGTAG- & 450 \\
& (G>A) & Common D: 5'-CCCTTTACACCTTCTGGAACCCAGA- & \\
& & 281 & \\
& & Common C: 5'-ACCTACACCCCTGGAACCCAGA- & \\
& Cd 15 & normal: 5'-TGAGGAGAGTCTTCCCGATCGGAGGAGGAGG- & 500 \\
& (TGG>TAG) & Common D: 5'-CCCTTTACACCTTCTGGAACCCAGA- & 500 \\
& & & \\
& & Cd 41/42 & normal: 5'-GGAGGACGAGATCCCAAGCAGTTCAAGGA- & 443 \\
& (delCTT) & & & \\
& & & Cd 41/42 & 439 \\
& & & & \\
& & Cd 26 (HbE) & normal: 5'-CCCTTTACACCTTCTGGAACCCAGA- & 267 \\
& (GAG>AAG) & & & 267 \\
& & & Cd 41/42 (delCTT) & Common D: 5'-CCCTTTACACCTTCTGGAACCCAGA- & \\
\hline
\end{tabular}
\end{table}
Amplification was carried out in 25 µl reaction mixture consisted of 2.5 pM dNTPs, 1 U Taq polymerase (Boehringer Mannheim), 100 ng DNA, 10 mM MgCl2, 2.5 pM primers. The Thermoline thermocycler was programmed as follows: denaturation 93°C, 15 min; annealing 32°C, 1.5 min; and extension 72°C, 30 min for 30 cycles with extra extension 72°C for 10 minutes. The PCR product was then electrophoresed in TEB buffer using 3% agarose gel with constant voltage 100 volts for 45 minutes. The gel was stained using ethidium bromide and DNA bands were visualized in the UV transilluminator.

Results and discussion

Routine hematological examination, HbA2 measurement and HbF staining test can be performed in all specimens. However, failure in obtaining DNA occurred in one sample of β-thalassemia carriers. Increased HbA2 level was observed in 18 individuals; seven of them were extremely high for HbA2. Based on abnormal globin distribution in Indonesia (Soerjo, 1985), seven subjects (6%) with very high level of HbA2 were considered to be HbE heterozygotes. Consequently, the other 11 subjects (10%) were diagnosed as β-thalassemia carriers. With the exception of one subject having low blood indices (Hb level 11.8 g/dL, PCV 29% and MCV 50 cu µ), the rest are otherwise normal. However, the mean levels of PCV and MCV for all cases are slightly lower than normal. Figures of hematological parameters are summarized in Table 2.

High frequency of β-thalassemia carrier during this study is probably the highest figure so far reported in the country. This may not be surprising since the historical evidence showed close relationship between South Sumatra and Southeast Asia mainland where β-thalassemia has been prevalent. In fact, easy communication and close relationship either in trade or culture, though not necessarily, may be followed by genetic exchange or biological admixture. High frequency of β-thalassemia carrier in Palembang, therefore, suggest that this assumption might not be false.

Molecular analysis employing the ARMS primers provide satisfying concordance result (Figure 2). As shown in Table 3, all suspected HbE heterozygotes are turned out to be mutation at codon 26 (CAG→TTG) which is HbE, an abnormal hemoglobin commonly found in Southeast Asian region. Among 10 suspected β-thalassemia carriers, IVS-I nt5 (G→C), IVS-I nt15 (G→A) and codon 41/42 (delCTT) mutations were found in five, two and one subjects respectively. Two subjects (20%) were not able to be identified using the available primers. Some other set of primers are still required to identify this mutations or otherwise DNA sequencing need to be carried out. However, if HbE is included in β-thalassemia mutation, then only two out of 17 (12%) mutations should be identified.

Compared to the neighboring countries in Southeast Asian region, Thailand in particular where β-thalassemia is prevalent, slight discrepancy can be observed. In addition to IVS-I nt5 (G→C), the most commonly found mutations are codons 41/42 (delCTT), codon 17 (AAG→TAG), codon 19 (AAC→AAG) and IVS-II nt654 (C→T) (Fuchan et al., 1989; Fukuoka et al., 1992). Further north in southern China where most of the Indonesian Chinese are originated, frameshift mutation in codon 41/42 and C→T substitution in IVS-II nt 654 are among the most commonly found, while in eastern China most of the mutations are frameshift mutation in codon 41/42 and codon 71/72 (Huang et al., 1989). The
The occurrence of codon 41/42 mutation in Palampang is therefore not surprising since Chinese influence can be clearly observed in this particular region as any western part of Indonesia.

The various mutation mentioned above shows that thalassemia mutations are heterogeneous. It can be understood that variation in mutation can be observed especially in diverse populations where the action of gene flow, natural selection and other factors influencing gene distribution in population are taken place. Similar situation, therefore, may also be found in Indonesia. Understanding molecular pathology of β-thalassemia carrier in this region will be of valuable information since it has been previously revealed that Indonesian populations can be grouped genetically into western and eastern cluster (Sofro, 1983). Eventually, information about variation in β-thalassemia mutation in these diverse populations is very important. It is not merely important to explain the severity variation of the disorder, but it is also justifiable when molecular approach will be applied for future preventive measures. In this case, a set of ARMS primer with at least the two common ones, IVS 1 nt5 (G→C) and Cd26 (GAG→AAG) normal and mutant ARMS primer respectively.

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References


