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CD35 (DEL C) frameshift mutation in exon 2 of β-globin gene on β-thalassemia carriers

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A R T I C L E I N F O

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A B S T R A C T

Objective : Our work aimed to determine the type and site of mutations of two β-thalassemia carrier sby using a sequencing method .

Methods: DNA genomes were extracted from both carriers blood samples which, by PCR-SSCP analysis, were suspected of having mutation in exon2 of β-globin gene. The extracted DNA were used as template for amplification of the exon by using specific primers. Amplicon were then read by using the Sanger (dideoxynucleotide chain terminator) method with the reverse and forward primers. Sequencing data were analyzed by using software Chromas Bioedit Lite2.1 and 7.1.9

Result : The results of the analysis showed the presence of C deletion at codon 35 (Cd 35(del C)) which causes a codon frameshift on both carriers.

Conclusion : NLS extract and fractions have high SOD antioxidant value but low DPPH scavenging activity and can be used as potential hypoglycemic agent.

1. Introduction

Thalassemia is an autosomal recessive genetic disorder caused by mutations in hemoglobin-forming genes. Mutations may occur at the α-globin or β-globin gene (Weatherall, 2001; Chebab, 2010). When a mutation occurs in α-globin gene, it is called as α-thalassemia, and when mutation occurs in β-globin gene is called as β-thalassemia. β-globin gene mutation leads to the reduced synthesis of β-globin chains (β^+thalassemia) or absence of β-globin chain synthesis (β^+thalassemia), thus resulting in unbalanced globin chain composition. As a result, the red blood cells become easily damaged. The damaged red blood cells causes anemia, which in the long term may bring about bone deformities and growth retardation. Blood transfusion needs to be done regularly in order to maintain normal growth and development (Weatherall, 1997).

The number of thalassemia patients in Indonesia is increasing constantly, and it is predicted that the thalassemia trait carriers in Indonesia are in range of 6-10% of the population (Timan et al., 2002). Indonesian Association of Parents of Children with Thalassemia (POPTI) Yogyakarta branch, in collaboration with the Faculty of Biology, Universitas Gadjah Mada and the Indonesia Thalassemia Foundation (YTI) hold a thalassemia trait carrier screening in Yogyakarta. Of the 96 participants screened, the results of the hematological analysis indicated that 35 individuals were suspected as thalassemia carriers, and 21 of them wereβ-thalassemia carriers. The results were followed by DNA analysis using *polymerase chain reaction-single stranded conformational polymorphism* (PCR-SSCP) method. The analysis showed the site of β-globin gene mutation, in which two carrier individuals were found to have mutation in exon 2 of β-globin gene.

More than 200 types of β-globin gene mutations have been identified, in which the most of them are substitution or deletion of a single nucleotide, or insertion of oligonucleotides (Cao & Galanello, 2010). There are 10 types of β-globin gene mutations identified in Indonesia (Jakarta) where the highest prevalence is IVS1-5 mutation (G → C) (Lie-Injo et al., 1989; Weatherall, 2001). According to Setianingsih et al. (1998), Taman et al. (2010), and Hernanda et al. (2012), there are three types of mutations commonly found in Indonesia, namely Cd 26 (GAG → AAG) or HBE mutations, IVS1-5 mutations (G → C), and Cd 35 (del) mutations. Determination of the type of mutation becomes important here because knowledge about the types of mutations is necessary to know their association with clinical variations of β-thalassemia carriers. Information about clinical manifestations of β-thalassemia is required to manage β-thalassemia cases. Moreover, determination of types of mutations becomes a basis for genetic treatment, ascarried out by Suwanmanee et al. (2002) and Svasti et al. (2009) to solve problems due to mutated IVS2-654 β-globin gene using antisense oligonucleotides and RNA repair.

2. Materials and Methods

2.1 Subjects

Subjects of this studywere two individuals who based on PCR-SSCP analysis werefound to have mutations inexon2 of β-globin gene.

2.2 DNA extraction

Extraction of DNA samples was done using *Genomic* DNA *mini kit for blood and tissue* (Geneaid) with the procedure according to the kit protocol.

2.3 Amplification of exon 2 of β-globin

Amplification of β-globin gene exon 2 was conducted with Polymerase Chain Reaction (PCR) using the primers: 5'TAGGCACTGACTCTCT CTGCCTATT3' (*forward*) and 5'CCTTCCTAT GACATGAACTTAACATT3' (*reverse*) (Gupta and Agarwal, 2003). PCRmix was made with a total volume of 25mL, consisting of *KAPA2G™ fast ready mix* (12.5 mL), forward and reverse primers (1.25 μL each), DNA template (5 μL) and *nuclease free water*.

Amplification of β-globin gene exon 2 was carried out by 35 cycles with an initial denaturation (95°C for 3 m) and the condition ofthe cycle for each cycle: denaturation (95°C for 45 s), annealing (54°C for 30 s), elongation (72°C for 45 s), and the final elongation (72°C for 5m).

2.3 Electrophoresis

Extracted genomicDNAand theβ-globin gene amplificonwere analyzed byelectrophoresis on0.8%and 1% agarose, respectively.

2.4 DNA Sequencing

DNA sequencing was performed by using the Sanger (dideoxynucleotide chain terminator) method with the help of First Base Laboratory, Singapore.

2.4 Data analysis

Sequencing data were analyzed with software Chromas Lite 2.1 and BioEdit 7.1.9 for comparing sequences of the subjects and of normal β-globin gene exon 2 obtained from GenBank (NCBI: NC_000011.10). Amino acid sequence was obtained by converting the nucleotide sequence by using the software MEGA5, then the amino acid sequence alignment was performed by using ClustalW.

3. Result

Hematological analysis (Table 1) shows that the subjectis a β-thalassemia carrier. The higher erythrocytes and HbA2 levels and the lower MCV and MCH than normal are the characteristics of β-thalassemia carriers (Cao & Galanello, 2010). In addition, peripheral blood profile shows hypochromic and microcytic erythrocyte, and target cells (Rund et al., 1992; Cao & Galanello, 2010).

Amplification of β-globin gene exon 2 produces amplicon size of 339 bp, which covers a part of the intervening sequence region1(intron 1), exon 2, and a part of intervening sequence region 2 (introns 2). Exon 2 has a size of 223 bp, starting from nucleotides 222 to nucleotides 445 of the β-globin gene. Sequencing results with forward primers showed double peaks after nucleotides 239 (Figure 1A). It occurred due to the deletion, which caused a shift in DNA nucleotides (*codon frameshift*) of mutant allele. Therefore, overlapping would occur between the DNA

sequences of normal allele and the DNA sequences of the mutant allele (heterozygote).

Table 1. Hematologicaltest results.

Subject	Age	Sex	E (10^6/µL)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HbA_2 (%)	HbF (%)
P 5	10 year	Female	6,72*	12,1	56,8*	18,0*	31,7*	5,5*	1,2*
P 6	43 year	Male	6,81*	13,1*	59,2*	19,2*	32,5	5,4*	1,2*

Remark : E = erythrocytes, Hb = hemoglobin, MCV = mean corpus cular volume, MCH = mean corpus cular hemoglobin , MCHC = mean corpus cular hemoglobin concentration. An asterisk (*) indicates the results of the test under or above the reference value.

Results of the sequencing with reverse primers shows that a formation of double peaks started on nucleotide 238 (Figure 1B) in contrast to the results of sequencing with the forward primers that started the formation of double peaks at nucleotide 240 (Figure 1A). The sequencing results might be different as the deleted nucleotides were lined with the same two nucleotides, i.e. nucleotides with a base C, to form the same sequence of three nucleotides (CCC). This condition caused the deletion read on the chromatogram can be in nucleotides 238, 239 or 240, and the deletion of the three regions will produce the same chromatogram. Nucleotide 238 is located at codon 35, whereas nucleotide 239 and 240 are located at codon 36(Cd 35/36(del C)). In Indonesia, only Cd35(del C) mutations has been reported (Lie-Injo et al., 1989; Setianingsih et al., 1998; Tamametal., 2010; Hernanda et al., 2012), so that this mutation is called as Cd35(del C) mutation.

Cd35(del C) mutation is a mutation found only in some Asian countries, such as Indonesia, Malaysia, and China(Colah et al., 2010; Huang et al., 2010). Cd35(del C) mutation in Indonesia was first reported by Lie-Injo et al. (1989), but it was also reported in Semarang by Tamam et al. (2010) with a prevalence of 13.2%, and in Surabaya by Hernanda et al. (2012) with a prevalence of 17.6%. The prevalence of this mutation in Indonesia is quite high, the third highest after Hbe and IVS1-5(G→C) mutations (Setianingsih et al., 1998; Hernanda et al., 2012).

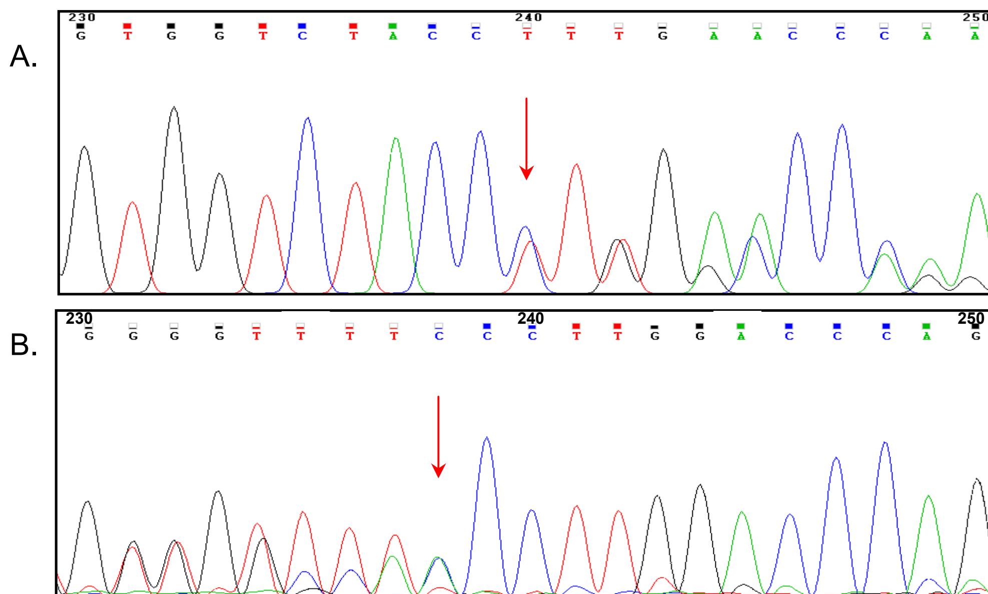

Figure 1. Sequencing chromatogram with theforward primers(A) andreverseprimers(B)

Cd35 (del C) mutation of β-globin genes will change the codon reading frame during translation, causing changes in the sequence of β-globin codon (Figure 2). Mutation may cause the formation of a stop codon or called as *premature translation-termination codon* (PTC), thus also including the non sense mutation. Mutation will result in a formation of PTCat codon 60(Figure 2).

and when it is translated, this will make translational process stop (*premature translation*).

4. Discussion

The presence of non sense codon can trigger the destruction of mRNA, called non sense-mediated mRNA decay (NMD) (Romao et al., 2000), resulting in a decrease in the amount of β-globin mRNA. Mechanism of NMD in the βg globin gene was first described by Changand Kan (1979) in patients with β thalassemia. The mechanism resulted in the absence of β-globin chains; in the heterozygous state, no β-globin protein is synthesized from mutant alleles, and only has half the normal globin β protein, so-called heterozygous β^{+}thalassemia.

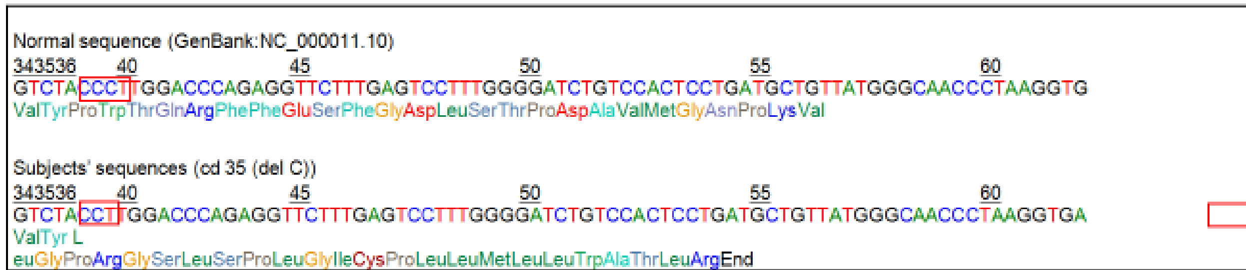

Figure 2. Sequences of β-globin gene exon 2, codon 34-60

Nonsense-mediated mRNA decay (NMD) is one of the mechanisms controlling the quality of mRNA before translation (Peixeiro et al., 2011) to prevent the translation error and the negative effects of the abnormal polypeptide (Thermann et al., 1998). Not all nonsense mutations can investigate the NMD, depending on the site of PTC. Nonsense mRNA mutants which become NMD target are those having PTCat codon 24-87, while the mRNA with PTCat codon s1-23 and codons 80-146 does not become the target of NMD (Figure 3B) (Peixeiro et al., 2011). Messenger RNA (mRNA) which does not become the target will accumulate in erythrocytes, and if it is translated, this will produce short polypeptides. The polypeptide will be hydrolyzed by erythrocyte proteolytic system. Cd35 (del C) mutation generates the PTCat codon 60 (Figure 3A), thus transcribed mRNA will be the target of NMD.

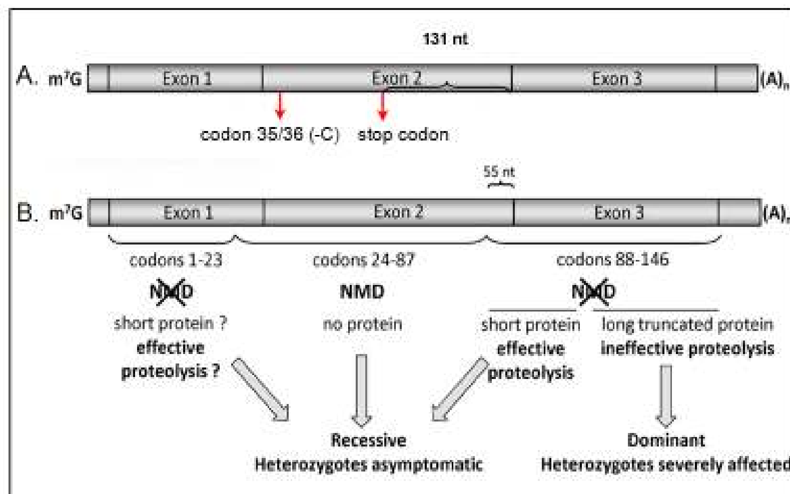

Figure 3. (A) Scheme of mutationsite andstopcodonmutation inβglobinmRNA, (B) Scheme of PTC siteinβglobinmRNAtargeted bynonsense-mediated mRNAdecay (NMD) (Peixeiro etal., 2011)

5. Conclusion

The type of mutationin β-globin gene exon 2 in the β-thalassemia trait carriers represents frameshift or nonsense mutations due to the deletion of a single nucleotide codon 35 (Cd 35 (del C)). Mutations can bring about a change in codon sequences, thus forming PTCat codon 60 and becoming target of NMD.

Acknowledgment

Conflict of Interest

The authors report no conflicts of interest

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