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Molecular Detection of Beta-Globin Gene Mutations of Ethnic Riau Malay Thalassaemia Patients by Polymerase Chain Reaction and Sequens DNA

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Abstract

Beta-thalassaemia major is an autosomal recessive disorder that results in severe microcytic, hypochromic, haemolytic anemia among affected patients. Beta-thalassaemia has emerged as one of the most common public health problems in Indonesia, particularly among Riau Malays. This study aimed to observe the spectrum of mutations found in Riau Malay beta-thalassaemia major patients who attended the Thalassaemia Center, Arifin Achmad Hospital University Riau Pekanbaru, Indonesia. This was a cross-sectional study conducted with 68 Riau Malay beta- thalassaemia major patients. DNA was extracted from the blood collected from the patients and subjected to polymerase chain reaction (PCR) amplification. Pcr product to sequens for the detection of mutations. The results revealed four mutations in the HBB at Riau Malay beta- thalassaemia patients; IVS-1 nt5 (G > C), codon 26/HbE (G > A), IVS-1 nt1 (G > T), IVS-1 nt2 (T> C) the two most common mutations observed were codon 26 (G >A) and IVS-1 nt5 (G > C). Four Riau Malay patients in the present study, however, did not show any mutation in studied.

Key words: Beta-globin gene; thalassaemia; gene mutation; anemia.

1. Introduction

The thalassaemias are a group of anemia that results from a genetic defect which reduces the rate of synthesis of normal globin chains.

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The thalassaemias are among the most common genetic disorders worldwide, occurring frequently in the Indian subcontinent, Southeast Asia and West Africa [1]. Recently, Beta-thalassaemia (BT) has emerged as one of the most common public health problems in Indonesia, particularly among Riau Malays.Thalassaemia is classified according to the chain of the globin molecule that is affected [2]. BT major is an autosomal recessive disorder that results in severe microcytic, hypochromic, haemolytic anemia among affected patients. BT is known to occur due to the mutations in the beta-globin gene (HBB) on chromosome 11 [3]. To date, there are over 200 known mutations in HBB found to be associated with thalassaemia, but it is believed that each population has its own spectrum of mutations [5]. However, this study focused on BT mutations which are believed to occur commonly among the Riau Malay ethnic group. This study aimed to observe the spectrum of mutations found in BT major patients who attended the Thalassaemia Center Arifin Achmad Hospital University Riau Indonesia. Our results added to the existing data on the most common mutations of HBB existing among the Malay ethnic group in Riau.

2. Material and methods

This was a cross-sectional study and was approved by Research and Ethics Committee, School of Medical Sciences, University Riau Indonesia, 68 Riau Malay patients diagnosed to have BT major and requiring a regular blood transfusion were included in this study. Informed consent was obtained from their parents prior to blood collection. All patients were interviewed to complete a questionnaire. Blood samples were later send to laboratories Biomedical, University of Andalas Padang Indonesia, for molecular analysis.

DNA was extracted from the blood using a commercially-available DNA extraction kit, Nitrogen Pure link Genomic DNA Mini Kit . Polymerase chain reactions (PCR) with sets of primers were used to amplify the exon and intron of HBB that created a recognition site of mutation. Primer Forward HBB F sequens 5'-GGTACGGCTGTCATCACTTA-3' with 20 bp and Primer Reserve HBB R sequens 5'-CCCCTTCCTATGACATGAAT-3 with length 21 bp. Primers were desained with software bioinformatics Geneious and sentetised by Integrated DNA Technologies, Singapore. The reaction mixture for PCR amplification consisted of Go Tag Green Master mix PCR, Promega 12,5 µL, Primer F 10 µL , Primer R 10 µL, 25 mM MgCl2 2 µL , ddH_2O 4µL, sample DNA 4 µL and a final volume of 25Ml [6].

The PCR conditions were as follows: 95°C of pre- denaturation for 10 mins, 95°C of denaturation for 45 s, annealing at 66°C for 1 min and 68°C of extension (1 min 30 s) for 35 cycles, followed by 68°C of final extension for 5 mins. In the C1000 ^{TM} Thermal Cycler Single Block Bio-Rad (USA) [7]. Amp icons were then detected by gel electrophoresis in a 1.5 % agarose gel and visualized by GeldocBiorad. Subsequently, the PCR product that was obtained from the PCR amplification sends to Macrogen,Inc Seoul Korea for sequencing

3. Results

PCR amplification successfully generated 697 bp (Fig. 1)

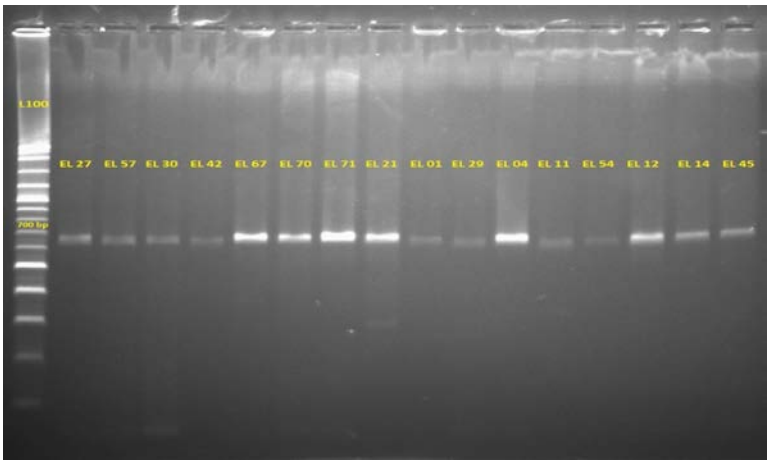

Figure 1: Product of PCR 697 bp.

Amplicon was used for the detection mutation with sequencing. The results revealed four mutations in the HBB in Riau Malay BT patients; IVS-1 nt5 (G > C), codon 26/HbE (G > A), IVS-1 nt1 (G > T), IVS-1 nt2 (T> C). The two most common mutations observed were codon 26 (G >A) and IVS-1 nt5 (G > C). The mutation at codon 26/HbE (G > A) was observed in 43 patients, and other mutation IVS- 1 nt5 (G > C) was observed in 48patients. The remaining mutations, IVS-1 nt1 (G > T) 5 patients,IVS-1 nt2 (T> C) 5 patients. The allele frequencies of mutant allele for the four mutations detected are shown in Table 1. Four Riau Malay patients in the present study, however, did not show any mutation in studied.

Table 1: Polymorphism of β-gene Globin

Type of mutations	Allele	f	%
IVS 1 nt5 (G→C)	CC *(*homozygote*)*	8	11,77
	GC*(*heterozygote*)*	40	58,82
	GG*(*wild type*)*	20	29,41
Cd 26 (GAG→AAG)$^{glu-lys}$	AA*(*homozygote*)*	2	2,94
	GA*(*heterozygote*)*	41	60,29
	GG*(*wild type*)*	25	36,76
IVS 1 nt2 (T→C)	CC*(*homozygote*)*	0	0
	TC*(*heterozygote*)*	5	7,35
	TT(wild type*)*	63	92,65
IVS 1 nt1(G→T)	TT*(*homozygote*)*	0	0
	GT*(*heterozygote*)*	5	7,35
	GG(wild type*)*	63	92,65

BT major is one of the most common single gene disorders in the multiracial population in Indonesia. The heterozygous carriers of BT in Indonesia are about 3-10% among the populations [8], and have clearly emerged as one of the most common public health problems in Indonesia The World Health Organization has highlighted the importance of characterization of the spectrum of BT mutations as one of the ways for community control of BT [9], thus, characterization of the patients in this study is essential for the patient management in this country. This study has enriched the current database. Six common mutations have been identified among individuals originating from East Java, Indonesia, viz. codon 26/HbE (G > A)47,0%, IVS1-nt5 (G > C) 20,6%, codon 35 (-C) 17,6%, codon 41–42 (4 bp del) 2,9%, codon 15 (- T) 5,9 %, IVSII-654 (C> T) 2,9 %, [10]. The most common mutation among Malays, followed by IVS1- nt5 (G > C) 43.1 %, 17.0% respectively [11]. Reported in several studies, revealed that mutation results in IVS-I-5 (G > C) 43.5%, codon 26 /Hb E(G > A) 28.2%, IVS-I-1 (G > A) 5.0%, codon 15 (TGG > TAG) 3.8%, IVS-I-1 (G > T) 3.1% [12]. Hb E is hemoglobin variants which are common in South East Asia. These Hb variants result from amino acid changes at amino acid 26 (glutamate→lysine) these single base substitutions also create an alternate splicing site leading to decreased production of the β-globin chain [13]. IVS1- nt5 (G > C, a large portion of all beta-thalassemic mutations. These mutations affect the splicing process at variable degree, depending on the position in which the mutation occurs [14]. Mutations that affect either of the invariant dinucleotide at the intron-exon junction (the GT motif at the 5' or donor site and the AG motif at the 3' or acceptor site) completely abolish normal splicing and result in beta- + thalassaemia. Mutations occurring in the splicing consensus sequences are instead of beta type, resulting in variable degrees of defective splicing and causing milder types of beta- thalassaemia. Other mutations occurring in exon or intron sequences may activate a cryptic splicing site, thus leading to abnormal mRNA processing. Even in these cases defective splicing occurs at variable degrees, resulting in phenotypes that range from mild to severe [15], The molecular characterization of BT mutations can be considered as a stepping stone in identifying the spectrum of mutations in a population. The results from this study add to the existing data on the spectrum of BT mutations among Riau Malay patients, although a larger number of patients would provide a more accurate representation of the spectrum of mutations.

4. Conclusion

Our results added to the existing data on the common characterization of BT mutations in Riau Malays will help the establishment of a rapid and effective prenatal diagnosis programme, or genetic counselling in this ethnic group in Riau Malay beta-thalassaemia patients.

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