

Screening of β -Globin Gene Mutations in Adolescent Schoolgirls in Rural Malang and Sukabumi City, Java Province, Indonesia

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Abstract— Anemia is one of the major health problems in Indonesia. In general, the condition occurs because of iron deficiency. Another major factor that causes anemia in Indonesia is thalassemia. Usually, the thalassemia patient comes with severe anemia for treatment. Since thalassemia is a genetic disorder, it is necessary to know how to prevent and reduce the incidence. The purpose of this study was to determine the prevalence of thalassemia trait, β -globin gene mutants among adolescent schoolgirls in rural Malang area and Sukabumi City. The early detection of thalassemia will encourage early counseling and treatment. The result of this study found that among 180 adolescent schoolgirls from Malang and Sukabumi, 22 were carrying thalassemic β -globin genes with asymptomatic to mild anemic conditions. Only one of the students had symptoms of intermediate thalassemia. The polymorphism encountered was CD26 in Malang and IVS1nt5 in Sukabumi City.

Keywords— *thalassemia; anemia in adolescents; Malang; Sukabumi; Indonesia; CD26, and IVS1nt*

I. INTRODUCTION

Anemia is a major health problem in Indonesia with prevalence of 47.4% among preschool children, 25.4% among school-age children, and 41.8% among pregnant women. The main factor causing anemia in Indonesia is iron deficiency, while other factors are infection, chronic diseases and genetic disorders such as thalassemia [1,2,3]. The prevalence of thalassemia in Indonesia was reported 31% [4]. Other studies showed the prevalence of thalassemia in certain Indonesian provinces, 4.4 % in West Java [5] and about 8 % in East Java [6]. Among 50 students in Jakarta 6% carried β -thalassemia Codon 26 (or hemoglobin variant E, HbE) [7]. Thalassemia has a crucial impact not only on the patients, but also on their families and the Government, particularly in terms of providing cost for the treatment.

Beta-globin mutation data in Iran, India, Thailand, or Malaysia are obtained from premarital and family counseling. The data of β -globin mutation carriers for each country were found to vary, e.g., Iran 0.19%, [8] India 78.17% [9], Thailand 2.4% [10], and Malaysia 81.25% [11]. In Indonesia, the number of thalassemia gene carriers is only acquired from the

data of thalassemia patients who visit hospitals for treatment. Comprehensive data on thalassemia trait among the Indonesian population are absent. Hence, there is urgent need of raising public awareness and governmental action in educating the young generation about the importance to alleviate and control the disease in the future.

In our present study, we chose adolescent schoolgirls because they are the candidates to be part of the successor Healthy Indonesia 2025, the health program of the Indonesian government. This study aims to determine the prevalence of thalassemic β -globin gene carriers among adolescent schoolgirls in rural Malang and Sukabumi City. By providing these data, it is expected that the Government should initiate appropriate programs to better manage thalassemia cases in Indonesia. Furthermore, these data may also encourage the government to educate adolescents with thalassemia minor to prevent the increasing numbers of thalassemia major among the next generation.

II. MATERIALS AND METHODS

A. Subjects

The calculation of sample size to measure the prevalence of thalassemia trait for each district used 80% of power test, 5% level of significance, 10% absolute precision, and 18.8% anemia prevalence among female age 12-18 years [12]. Blood samples from 180 adolescent schoolgirls were collected from Malang area and Sukabumi City, Java Island. Three mL of venous blood were taken from each individual with vacutainer EDTA® for anticoagulant blood collection.

B. Preparing before PCR Sequencing

Complete blood counts (CBC) were performed at Clinical Pathology Laboratory, University Hospital Brawijaya Malang and Bhayangkara Hospital Sukabumi. DNA sequencing was carried out for molecular identification of thalassemia mutations at First Base Molecular Laboratory in Kuala Lumpur, Malaysia.

C. PCR Sequencing

The DNA analysis for β-globin genes was conducted by PCR using the following primers: forward (50-AGT AGC AAT TTG TAC TGA TGG TAT GG-30; reverse, 50-TTT CCC AAG GTT TGA ACT AGC TCT T-30). The experimental condition for PCR was as following: initial denaturation (95°C, 1 min), followed by 35 cycles of denaturation (95°C, 30 seconds), annealing (60°C, 30 seconds), extension (72°C, 30 seconds) and finally (72°C, 10 seconds). The PCR product was isolated by agarose gel electrophoresis, 1200 bp amplicon size excised, and purified by QIAquick gel extraction kit (Qiagen, Tokyo, Japan; www.qiagen.com). After dideoxy reaction using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan), it was subjected to DNA sequencing (ABI PRISM3100 Avant Genetic; Applied Biosystems, Tokyo, Japan; customerservice@lifetech.com) [13].

III. RESULT

Genetic evaluation of 180 samples detected more than one polymorphism of β-globin: CD2, T>C; CD26 or HbE, G>A; IVS1nt5, G>C; IVS2nt16, G>C and IVS2nt74, T>G. One person had double mutation Hb variant and β-thalassemia types were CD26 (HbE) and IVS1nt5. For the screening in Malang, 100 samples were investigated based on the results of the CBC for osmotic fragility and analyzed for HbE. Genetic screening using PCR and sequencing, showed that 72 samples had a mutation in CD2, 15 samples in CD26 or HbE, 67 samples in IVS2nt16, 28 samples in IVS2nt74 and one sample in IVS1nt5. The screening 80 samples in Sukabumi based on the results of the CBC and analysis for hemoglobinopathies showed thalassemia mutations in 35 samples at CD2, in 7 samples at IVS1nt5, in 29 samples at IVS2nt16 and in 14 samples at IVS2nt74.

TABLE 1. DATA OF HEMOGLOBINOPATHY AND GENOTYPE

No	Parameter	Normal Range	Malang (100 samples)		Sukabumi (80 samples)	
			Hb > 12 g/dL	Hb < 12 g/dL	Hb > 12 g/dL	Hb < 12 g/dL
CBC						
1	Hemoglobin	12-16 g/dL	13.62	10.98	13.27	10.4
2	Red Blood Cell	4.2-5.4.10 ⁶ /L	4.88	4.87	4.71	4.92
3	MCV	80-97 ft	83.45	72	85.94	69.35
4	MCH	26.5-33.5 pg	28.04	22.72	28.25	21.5
5	MCHC	31.5-37 g/dL	33.71	31.71	32.89	30.9
6	RDW-CV	15%	13.44	15.34	13.63	17
Thalassemia Calculation test						
1	Mentzer Index	>13	17.24	15	18.33	14.4
2	Ehsani	>15	34.3	23.11	38.43	20.15
3	RDWI	>220	229.36	227.37	250.29	239.56
4	S & L	>1530	1980.25	1224.81	2097.86	1107.5
5	Osmotic Fragile Test	Negative	Negative	1 positive	2 positive	5 positive
6	Hb E Tahlo Test	Negative	7 positive	8 positive	Negative	Negative
Genetic Sequencing β-Globin						
1	CD2, T>C		48	24	26	9
2	HbE or CD26, G>A		7	8	Non	Non
3	IVS1nt5, G>C		Non	1	2	5
4	IVS2nt16, G>C		44	23	25	4
5	IVS2nt74, T>G		19	9	9	4
Genotype Combination						
1	CD2, IVS1nt5, IVS2nt16, IVS2nt74		Non	Non	Non	2
2	CD2, IVS2nt16, IVS2nt74		18	7	9	2
3	CD2, IVS1nt5		23	10	14	3
4	CD2, IVS2nt16		Non	Non	Non	2
5	CD2		4	1	3	Non
6	IVS2nt16		Non	1	2	Non
7	IVS1nt5		Non	Non	2	1
8	CD26		4	1	Non	Non
9	CD2, CD26		Non	1	Non	Non
10	CD26, IVS1nt5		Non	1	Non	Non
11	CD2, CD26, IVS2nt16		2	3	Non	Non
12	CD2, CD26, IVS2nt16, IVS2nt74		1	2	Non	Non
13	Wildtype		16	5	35	5



Fig. 1. PCR Result (A: DNA marker 100 bp; B and C: PCR product 1200 bp)

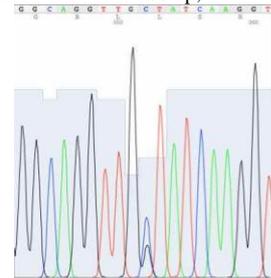


Fig. 2. DNA sequencing for β-globin mutation IVS1nt5

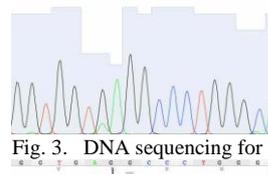


Fig. 3. DNA sequencing for β-globin mutation CD26



Fig. 4. DNA sequencing for double mutation, CD 26 and IVS1nt5

IV. DISCUSSION

This study found five types of β-globin gene polymorphism (codon 2 T>C, Codon 26/HbE G>A, IVS1nt5 G>C, IVS2nt16 G>C, IVS2nt74 T>G) in adolescent schoolgirls in Malang and Sukabumi City. They had never been diagnosed with hemoglobinopathies according to their medical record. The most common polymorphisms were codon 2, IVS2nt16, and IVS2nt74, followed by codon 26/HbE and IVS1nt5.

The polymorphism at codon 2 T>C in both, homo- or heterozygous forms were found more common of all samples. Some samples had a combination of codon 2 (homo- or heterozygous) with other types of polymorphism showing

anemic and non-anemic phenotypes. Polymorphism of codon 2 T>C converts the code CAT to CAC, both coding for histidine. Therefore, this polymorphism will not cause a pathogenic phenotype. Similar condition had been reported [14].

The polymorphism of β -globin gene at codon 26 G>A, or HbE (β^+ -thalassemia) is common in South East Asia. The phenotype of an individual with codon 26 (homo- or heterozygous) is asymptomatic or slightly anemic. This mutation is caused by an alternative-splicing site in the codon and will produce cryptic splicing on mRNA. Cryptic splicing at codon 26 will cause partial truncation of exon 1 and synthesis of unstable of β -globin. The pathogenic state may be affected by the ratio of HbE mRNA and normal mRNA [15-17].

Similar to HbE, the polymorphism of β -globin gene at IVS1nt5 G>C (β^+ -thalassemia) is another common polymorphism in South East Asia. It will produce a new splicing junction site and reduce splicing efficiency, resulting in an aberrant β -globin chain formation. Mutations occurring in the splicing consensus area are type β^+ , phenotype of mild β -thalassemia. We found one sample of Hb level < 9 g/dL with the combination of codon 26 G>A and IVS1nt5. The person did not know about her health condition. The combination of codon 26 G>A (heterozygous) and IVS2nt5 (heterozygous) has a pathogenic effect with phenotype of intermediate thalassemia, because this mutation (occurring in an exon and intron sequence can activate a splicing site that will produce abnormal mRNA [17].

Another polymorphism which was found in our samples was IVS2nt16. This type is the combination of codon 2 with IVS2nt16; IVS2nt74 can be found in patients with β -thalassemia and healthy people [14]. This polymorphism is a framework of the β -globin gene [18,19].

Table 1 shows that 7 out of 100 adolescent schoolgirls in Malang area have polymorphism of CD26 without anemic condition, whereas 8 of them have polymorphism of CD26 with anemia and one suffers intermediate thalassemia with Hb level of 7.5 g/dL and double polymorphism of CD26 and IVS1nt5. This condition may be affected by the total amounts and the ratio between HbE mRNA, IVS1nt5 mRNA, and normal mRNA. In Sukabumi city, we did not find CD26 polymorphism. Two students had polymorphism IVS1nt5 without anemia and 5 students with mild anemia. From Table 1, we can see that the polymorphism commonly found in Malang area is CD26 or HbE variant (also β^+ -thalassemia) and in Sukabumi City it is IVS1nt5 (β^+ -thalassemia). This evidence will assist physicians in establishing the diagnosis of individuals suspected having thalassemia in Malang area or Sukabumi City. The knowledge of the geographic and ethnic background will be valuable for diagnostic, counseling and management of β -thalassemia in Indonesia.

Limitations in our study:

Our research intends to investigate the condition of thalassemia in Indonesia, but due to the vast area of Indonesia and insufficient funding sources, the study must be considered preliminary. Nevertheless, it is hoped that it can contribute to

describing the individual anemic/thalassemic condition in connection with the background of β -globin gene polymorphism.

V. CONCLUSION

The most common polymorphisms found in this study were codon 2, IVS2nt16, and IVS2nt74, followed by codon 26/HbE and IVS1nt5.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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