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Scanning SNPs of Diabetes Mellitus related genes; HNF4A, PTPN, KCNJ11, PPAR gamma; among Thalassemia Patients: a Preliminary Study

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Abstract. Thalassemia is one of health problem which is a burden on the Indonesian government. Thalassemia problems include wide-spreading, and clinical conditions, therapy management, and complications. One of the significant difficulties that cause mortality is due to Diabetes Mellitus (DM). This preliminary study aimed to explore the genotyping of SNPs on the genes related with DM in thalassemia patients in Indonesia. The study used a cross-sectional design involving 103 samples from stored DNA patients in Banyumas. There were four genes about DM; HNF4A, PTPN, KCNJ11, and PPAR gamma; which characterized using the PCR-RFLP based technique with appropriate restriction enzymes. The results showed that there was existence of SNP related to diabetes mellitus in thalassemia patients. The T130I (rs1800961) of HNF4A gene has a proportion of genotypes 79% CC, 16% CT, and 5% TT with minor allele frequency (MAF) 13%. The genotype distribution of PTPN gene 1023 C>A were 68% for CC, 30% CA, 2% AA and MAF 17%. PTPN gene of 467 T>C has percentage of CC 90%, CT 10%, and MAF 5%. The KCNJ11 gene at position rs 5219 had a portion of glutamic acid 46% and lysine 54%. Whereas, Pro12Ala (rs18012282) of PPAR gamma; gene has a genotype proportion of CC 94%, CG 5% , GG 1% and MAF 4%. The genotyping of DNA patients revealed the presence of DM-related genes in thalassemia. The coexistence of mutants may relate with the clinical condition. Further study, especially in genotype-phenotype relation is essential to explore the influence of the mutants in worsening the affected people.

Keywords: thalassemia, diabetes mellitus, HNF4A, PTPN,KCNJ11, PPAR gamma

1. Introduction

Thalassemia is a monogenic hematology disease that still the most endemic on a global, regional and national scale. The Southeast Asian population is reported to have a carrier frequency of Hemoglobinopathy and Thalassemia of 45.5% with 1.34 children of 1000 births born with moderate to severe clinical conditions [1]. In Indonesia, the number of the carrier has an average frequency of 3-10% of overall properties [2]. Thalassemia problems are very widely dispersed, ranging from the number of mutations involved in the pathogenesis of the disease, the large number of budgets in management, to the complexity of prevention problems. At present, there are at least more than 800 variations in the globin genes responsible to the pathogenesis of hemoglobinopathy and thalassemia [3], with 300 of them being β thalassemia mutations [4]. Diabetes Mellitus (DM) is the highest hallmark of death due to endocrine abnormalities in thalassemia patients [5]. Efforts to detect prediabetic conditions are an essential part of the management system of thalassemia patients so that the patient's morbidity and mortality can be adequately controlled. The diagnosis of pre-condition is closely related to individual genetic susceptibility. Previous thalassemia studies showed that with knowledge of gene modifier Xmn1 and BCL11A could classify patients who require routine and non-regular transfusions [6]. which have an impact on the better management of thalassemia. The purpose of this study is to identify and characterize genetic markers that underlie the complications of thalassemia patients in endocrine (DM) cases. Currently, the genetic marker and bio panel markers for DM complications in thalassemia are still very limited, so research and data need to be done to reveal more evidence of the genetic potential of these markers. Genetic markers in question are HNF4A; the genes found in the linkage study, Peroxisome proliferator-activated gamma receptor (PPARγ), SNPs in the PTPN1 gene and KCNJ11 [7-10].

2. Materials and Methods

The study used a cross-sectional design with a whole subject of 100 thalassemia major patients. Patient DNA was tested using PCR-RFLP method to determine the genotype of Hnf4a (T130I rs1800961), Ptpn 1023 C> A and PTPN 467 T> C, KCNJ11 E23K (rs5219), PPAR Pro12Ala (rs18012282). Table 1 provides the primers and enzymes used to determine genotyping of these genes.

Table 1. Primers and Enzyms used in PCR-RFLP Experiment for Genotyping of GenesRrelated with Diabetes melitus.

Gene	Primers		enzim restriksi
	Forward 5'-3'	Reverse 5'-3'	
Hnf4a	CCACCCCCTACTCCATCCCTGT	CCCTCCCGTCAGCTGCTCCA	BsmI
Ptpn 1023	TAGCAGAAACCGAGTTTCACC	CCTGGGTAACAGAATCAGACC	BclI
PTPN 467	TTCATTCCTGCAGCACCC AAG	GTTGAGTCACAGAGTGAGTGG	AvaI
KCNJ11	GACTCTGCAGTGAGGCCCTA	ACGTTGCAGTTGCCTTTCTT	BanII
PPAR	CCAATTCAAGCCCAGTCCTTTC	CAGTGAAGGAATCGCTTTCCG	BstU-I

PCR method used serial temperature in the following steps: Initial denaturation 95^0 C for 1 minute, and followed by 30 cycles for denaturation 95^0 C '30 seconds, annealing temperature $53\text{-}57^0$ C 30 seconds, elongation temperature 72^o C for 30 seconds, closed with final elongation temperature 72^o C for 30 2 minutes. Genotypic data was then tabulated to obtain minor allele frequencies and genotype distribution.

3. Results and Discussion

A total of 100 DNA samples from major thalassemia patients underwent a genotyping process which showed in Table 2.

Table 2. Genotyping of The Genes Related Diabetes Melitus ; HNF4A, PTPN, PPAR-γ, KCJN11, among Indonesia patients.

Gene name	SNP locus	Genotype			Allele			HWE (χ^2)*
		base	frequency	Proportion	base	frequency	Proportion	
HNF4A	rs1800961	C/C	81	0.79	C	178	0.87	0.0093
		C/T	18	0.16	T	26	0.13	
		T/T	5	0.05				
PTPN 1023	-1023 C>A SNP	C/C	70	0.69	C	170	0.83	0.73
		A/C	30	0.29	A	34	0.17	
		A/A	2	0.002				
PTPN 467	-467 T>C SNP	C/C	92	0.9	C	194	0.95	1
		C/T	10	0.1	T	10	0.05	
		T/T						
PPAR-γ	rs1801282	C/C	96	0.94	C	197	0.97	0.1
		C/G	5	0.05	G	7	0.03	
		G/G	1	0.01				
KCNJ11	rs5219	K/K	29	0.28	E	93	0.46	0.69
		E/K	53	0.52	K	111	0.54	
		E/E	20	0.2				

Table 1 shows the distribution of minor HNF4A alleles by 13% with Hardy Weinberg Equilibrium (HWE) 0.009. Minor T alleles in HNF4A appear to deviate from allele distribution in other Asian populations. Data on the SNPS database shows that Asian people are in the range of 2-4%, but this population is reaching 13%. Data also confirmed by the HWE value in the non-equilibrium state (<0.05). The fact that some patients were coming from one descendant and Banyumas were in peripheral areas with local marriages, allowed the effect of an isolated gene. It caused the spreading of the alleles proportion is not in Equilibrium state. Percentage of T130I HNF4A polymorphism in thalassemia populations in Banyumas in the form of 16% heterozygotes and 5% homozygous mutants. T130I HNF4A polymorphism study by Zhu and colleagues in Japan obtained as much as 3.5% mutations in subjects with type 2 diabetes mellitus and 0.8% in non-diabetic subjects [11]. There were also 3 people suspected of MODY diabetes mellitus patients in Spain obtained results that the three patients were heterozygous T130I HNF4A polymorphisms and only one patient correlated with MODY [12]. The families of the three patients were measured for parameters of glucose metabolism and clinical symptoms leading to the T130I HNF4A polymorphism. The results showed that 74% of close family patients were heterozygous from the T130I polymorphism. Subjects with heterozygous T130I polymorphisms were diagnosed as diabetes mellitus by 50%, prediabetes by 21% and 29% as normoglycemia.

Based on the results of the study in table 1, the proportion of PTPN1 -1023C> A polymorphisms in the thalassemia population in Banyumas is heterozygous at 29% and homozygous mutants at 2%.

Allele C was obtained at 81% and A allele as much as 19%. The results of this study are in line with the previous study of Meshkani in Iran showed a proportion of heterozygotes in the polymorphism -1023C> A in the group of diabetic patients as much as 13.2% and the proportion of C alleles as much as 93.4% and A alleles as much as 6.6% [13]. Another study reported that the two groups of subjects who got the C allele results were more prevalent in the group of diabetic patients (93%) than the control group (82%). In addition, the study showed that there were significant differences in the parameters of insulin sensitivity, signs of obesity, hypertension and several metabolic parameters such as lipid profiles between groups of diabetic patients and the control group [14].

Population diversity data of PTPN1 -1023C> A polymorphism published by the National Center of Biotechnology Information (2018) states that the global MAF (minor allele frequency) allele A is 0.1288 / 645. The proportion of A allele in 5 large populations is the EAS (East Asian) population of 9%, the SAS (South Asian) population is 22%, the EUR (European) population is 8.5%, the AMR (Ad Mixed American) population is 6.7% and AFR (African) by 16%. The similarity between the proportions of the SAS population (population of Pakistan, India, Bangladesh) and thalassemia patients in Banyumas is possible because of racial similarity in the two populations due to the process of trade and migration from the Mongoloid race to the Indonesian mainland. The polymorphisem in the PTPN gene at both locus 1023 and 467 shows the same value as the Asian population in general. This polymorphisem at the locus 1023 and 467 in the PTPN gene is an important note because this PTPN functions at the formation of the protein tyrosine phosphatase-1B which has negative feedback on insulin release and also has an inverse effect on leptin [15]. The presence of this polymorphism can cause changes in insulin regulation in thalassemia patients, which may result in a decrease in the function of insulin in addition to the result of excessive iron deposits.

PPAR-γ and KCNJ11 gene polymorphism detection showed a positive value in 6 people for PPAR gamma and more than 50 people had a constitution of Proline or Alanine proteins at position 23 or rs5219. The presence of mutants in these genes indicates that thalassemia patients can experience a state of diabetes mellitus because the mutants of these genes can also exacerbate an iron deposit disorder in the pancreas which interferes with insulin action. PPAR-γ and KCNJ11 polymorphisms and previous genes can disrupt and accelerate the incidence of diabetes mellitus in thalassemia patients [16, 17]. Scanning and following up on these genes are, therefore, something necessary for clinical consideration.

4. Conclusion

There is some mutant locus in the Diabetes Mellitus related genes of HNF4A, PTPN, KCNJ11, and PPAR gamma among Thalassemia Patients in Banyumas. Further study must be addressed to elucidate the mechanism of these genes on Insulin metabolism.

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