rs841853 of SLC2A1 Gene Polymorphism and Dyslipidemia Among Javanese Type 2 Diabetes Mellitus Patients

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Abstract. Type 2 Diabetes Mellitus (T2DM) is a chronic disease indicated by blood sugar level disorder and also influenced by genetic and environmental factors. Many diabetes patients experience dyslipidemia, which is known as diabetic dyslipidemia. In the latest research, SLC2A1 gene polymorphism is strongly related to T2DM. This research aimed to investigate the involvement of rs841853 of SLC2A1 gene polymorphism in Javanese diabetes patients with dyslipidemia.This cross-sectional study involved 107 T2DM patients as the sample to verify polymorphism. The occurrence of polymorphism was done using the PCR-RFLP method. The mean of the laboratory characteristic among groups was tested using an independent t-test with a significance of $p < 0.05$. In both groups, G allele (wild type) and GG genotype were mostly found. There was no difference in allele frequency ($p=0.185$) and genotype frequency ($p=0.238$). The differences of laboratory characteristic between the GG genotype and TG + TT genotype, either in dyslipidemia or non-dyslipidemia, showed no significance ($p>0.05$). Rs841853 of SLC2A1 gene polymorphism was not a significant risk factor for dyslipidemia in Javanese T2DM patients.

Keywords: Polymorphism, SLC2A1 gene, Dyslipidemia, Type 2 Diabetes Mellitus, Javanese.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disease indicated by blood sugar level disorder and also influenced by genetic and environmental factors. Genome-wide association studies (GWAS), which is a wide-scale genome study association, succeed in identifying genetic locus related to T2DM. Some genetic variations studied in Indonesia, especially in the Javanese ethnicity, which is the largest ethnicity in Indonesia, such as the relation of CAPN10 SNP-19, MTNR1B rs10830963, and SLC2A1 rs841853 polymorphism [1][2][3]. The polymorphism of MTNR1B rs10830963 and SLC2A1 rs841853 gene related to the role of melatonin in the pathophysiology of diabetes mellitus. MTNR1B gene is related to its function as a melatonin receptor, a significant hormone for carbohydrate

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metabolism, while the SLC2A1 gene codes member one protein glucose transporter (GLUT1). It facilitates the transportation of protein glucose through the plasma membrane of mammal cells. In some studies, it was proven that the SLC2A1 polymorphism is correlated to Type 2 Diabetes Mellitus (T2DM), diabetic nephropathy and retinopathy, renal cell carcinoma, and the latest study found that it is correlated with breast cancer and age-related macular degeneration [4][5][6][8].

Many diabetes patients experience dyslipidemia, which is known as diabetic dyslipidemia, due to a metabolism disorder of simultaneous carbohydrate and fat as the result of insulin production defects or insulin action. Dyslipidemia is caused by a low level of high-density lipoprotein cholesterol (HDL-C), the increase of total cholesterol level, along with low-density lipoprotein cholesterol (LDL-C) and triglyceride, which contribute to cardiovascular diseases. Based on the survey by Jakarta Primary Non-communicable Disease Risk Factor Surveillance 2006, the proportion of Dyslipidemia in T2DM patients reached 67.7% (the high level of total cholesterol), 36.8% (the low level of HDL), 91.7% (high level of LDL), and 54.9% (high level of triglyceride) [9]. Hypertriglyceridemia in diabetes is caused by the excess production of Very Low-Density Lipoprotein (VLDL) by the liver [10]. A study by Yanuarita et al., 2023 shows that rs10830963 of MTNR1B.

2. Methods

2.1. Design and Subject of The Study

This study was analytic observational research using a cross-sectional approach with 107 T2DM patients from three community health centers in Semarang as a research sample. The sample has met the inclusion criteria, such as Javanese T2DM patients aged 30 – 70, and signed the informed consent. The research was also granted ethical permission by the Research Ethics Committee at the Faculty of Medicine Universitas Muhammadiyah Semarang, Number 040/EC/FK/2019.

2.2. Genotyping of SLC2A1 Gene Polymorphism

The identification of polymorphism occurrence was done using the PCR-RFLP method with the amplification of SLC2A1 using PCR Mix 21 µl (2,5 µl PCR buffer with MgCl2; 0,25µl dNTP; 1,5 µl Primer forward 5'-TGC AAC CCA TGA GCT AAC AA-3' and reverse 5'-GAA CCC AGC ACT CTG TAG CC-3'; 0,25µl DNA Taq polymerase; 1 µl DNA; 14 µl H2O and 1 µl DNA). PCR is conditioned at 950C predenaturation for five minutes in 33 cycles, 950C denaturation for 30 seconds, 600C annealing for three seconds, 720C elongation for one minute, and 720C final extension for three minutes. 100µl RFLP (10µl PCR product; 5µl XbaI; 10µl enzyme buffer (10×); 75µl H2O, 10µl BSA), then digest PCR product with enzyme mix (10µl: 10µl), then incubated at 370C for an hour, visualized in

2% agarose gel, 120 Volt for 30 minutes. The polymorphism was observed using electrophoresis, which showed T allele (mutant type) (305bp) and G allele (wild type) (232 bp and 73 bp) [2].

2.3. Chemical Clinic Testing

Triglyceride, cholesterol, HDL-C, and LDL-C level was tested by taking a venous blood sample after 8 hours of fasting. Dyslipidemia diagnoses were made based on the criteria of total cholesterol >200mg/dL, LDL cholesterol levels >130mg/dL, or triglyceride levels >200mg/dL [3][11][12].

2.4. Data Analysis

Allele frequency and SLC2A1 rs841853 genotype polymorphism were analyzed using a chi-square test. Then, it was compared with the result of chemical clinic testing among groups using the independent t-test with a significance value of $p < 0.05$.

3. Result

The frequency analysis from 107 T2DM patients found that 46 patients were diagnosed with dyslipidemia and 61 patients were non dyslipidemia. G allele and GG genotype were mainly found in both groups. Statistically, there was no significant difference between the allele frequency (p=0.185) and genotype frequency (p=0.238) either in dyslipidemia or non-dyslipidemia groups.

Based on Table 2, there was no difference in the average level of laboratory characteristic between the GG genotype and TG+TT genotype, either in dyslipidemia or non-dyslipidemia groups. Nevertheless, the higher average parameter was found in the TG+TT group at BMI, FPG, HbA1C, and microalbuminuria parameters of the dyslipidemia group

Table 1. Allele distribution of rs841853 SLC2A1 polymorphisms.

		Subjects (n=107)		
		Dyslipidemia N=46	Non-Dyslipidemia N=61	p*
rs841853 SLC2A1				
Allele	G	61 (66.3%)	70 (57.4)	0.185
	T	31(33.7%)	52 (42.6%)	
Genotype	GG	21 (45.7%)	25 (41%)	0.238
	TG	19 (41.3%)	20 (32.8%)	
	TT	6 (13%)	16 (26.2%)	

* Chi Square test, p <0.05 as significant result

Table 2. The comparison of laboratory characteristic based on the Genotype of SLC2A1 rs841853 Polymorphism.

Variable	Dyslipidemia (N= 46)		p	Non Dyslipidemia (N= 61)		P*
	GG	(TG) + (TT)		GG	(TG) + (TT)	
BMI	23.36±1.98	24.40±3.64	0.651	24.87±3.52	24.43±3.55	0.618
FPG (mg/dl)	170.43±94.63	186.36±84.39	0.384	152.24±70.01	142.08±59.82	0.681
SBP (mmHg)	153.29±26.78	138±14.23	0.947	133.64±11.17	140.47±20.94	0.223
DBP (mmHg)	78.62±10.69	78.52±7.58	0.965	81.04±8.22	82.47±8.93	0.576
HbA1c	7.95±1.69	8.72±2.26	0.44	7.66±2.14	7.34±1.63	0.509
Ureum	32.43±14.68	25.40±8.12	0.085	28.68±9.99	31.97±11.95	0.294
Creatinin	0.95±0.61	0.79±0.22	0.383	0.88±0.39	1.05±0.53	0.348
Microalbumin	226.61±382.08	273.28±389.74	0.221	133.85±187.95	371.86±1348.28	0.634

4. Discussion

The finding of the study shows the most alleles found in pada SLC2A1 rs841853 polymorphism were the G allele (wild type), and the most found was the GG genotype. The previous study by Yanuarita et al., 2021 showed the same finding. However, the classification based on obesity was different from this study, which classified the group based on the case of dyslipidemia. In the mentioned study, the most found allele was also the wild-type allele (G allele) [2]. rs841853 polymorphism is a single nucleotide variant in the SLC2A1 gene on chromosome 1 at 43401438 position. A meta-analysis study reviewing 14 articles and 19 individual studies identified the correlation between polymorphism and Type 2 DM. It was shown that in the control group, the frequency of TT genotype, TG genotype, and GG genotype SNP rs841853 was 30.9%, 12.9%, 38.4%, and 49.5%, while in the case group, the distribution of genotype frequency was 32.9%, 10.2%, 47.2%, and 42.6%. It can be concluded that the finding of the study was similar to the study where the GG genotype was the most found allele, except in the case group in which the TG genotype was the most found [5]. This polymorphism also increases the risk of Type 2 DM in Asians from 1.83 to 2.24 [5].

The respondents of this research were classified into patients with dyslipidemia and non-dyslipidemia, as the case of T2DM is often accompanied by lipid metabolism disorder. The metabolism disorder in T2DM patients is in the form of the increase of triglyceride, LDL, and VLDL levels, as well as a low level of HDL[10]. The level of LDL-C typically was not different in the control group but was different at the small dense LDL, a pro-atherogenic lipoprotein particle. Although no significant difference was found between GG and TG+TT

genotypes in the dyslipidemia group, this research found higher FPG and HbA1c levels in the TG+TT dyslipidemia group. It shows that the dyslipidemia group has poor glycemic control on genotype with mutant allele (T). it is in line with research that shows that in patients with type 1 and type 2 DM, poor glycemic control could increase triglyceride serum, LDL, and VLDL levels, as well as reduce the level of HDL-C.[13] A reasonable glycemic control could lower triglyceride levels and increase HDL serum, in contrast to patients with poor glycemic control. Therefore, diabetes therapy also aims to optimize the glycemic control in diabetes patients.

In the dyslipidemia group, a higher microalbuminuria level was found in TG+TT. The level of lipoprotein a / Lp (a) is not significant in DM patients. However, research mentions that the increase of Lp (a) level is correlated to the case of microalbuminuria and renal disorder.[14], [15] It shows the possibility that the higher level of microalbuminuria in the TG +TT genotype could be attributed to the change in lipoprotein (a) level. Therefore, one of the weaknesses of this research is the absence of lipoprotein measurement to correlate with the increase of microalbuminuria. Besides, this study did not consider the use of lipid-lowering medicine such as statin. DM patients often use statin, although the use badly impacts the Glucose Homeostasis. In non-DM patients, the higher dosage of statin could increase the risk by 10%, compared to the moderate use of statin [16].

5. Conclusion

In both groups, the most found allele was the G allele (wild type), and the most found genotype was the GG genotype. There was no difference in the average level of laboratory characteristic between the GG genotype and TG+TT genotype either in dyslipidemia and or-dyslipidemia groups. Nevertheless, the higher average parameter was found in the TG+TT group at BMI, FPG, HbA1C, and microalbuminuria parameters of the dyslipidemia group. rs841853 of SLC2A1 gene polymorphism was not a significant risk factor for dyslipidemia in Javanese T2DM patients.

Authors' Contributions. Author YT developed the analytical techniques of study, performed data analysis, interpretation, drafting of the article and prepared the final version for publication; AY performed interpretation of data and helped review the data analysis and reviewed the article. All authors are responsible for the content of manuscript and the submission process.

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