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Identification of SNP rs1799853 of CYP2C9 Gene and Blood Sugar Levels In Diabetic Patients

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Abstract:

Introduction: The high prevalence of Diabetes Mellitus Type 2 (DMT2) became the burden in Indonesia. Some factors may influence the antidiabetic response including genetics, physiology, pathophysiology and the environment. Around 15–30% of genetic variation results to the differences of metabolism and drug response between individuals. The purpose of this study is to identify the genotype of CYP2C9 gene in rs1799853 and the therapeutic outcome of blood sugar levels in T2DM patients. Methods: We conducted a cross-sectional-prospective research at Moewardi Hospital Surakarta. We collected the blood sample of 10 T2DM patients who received Sulphonylurea monotherapy. The fasting blood sugar (FBS), 2-hour postprandial blood sugar and HbA1c were measured as therapeutic outcomes. Result: We found the dominant result is the wild type (CC) with 90.9% and mutant heterozygous (CT) were 9.1%. Subjects with wild type show the uncontroll of Fasting blood glucose and HbA1C. Conclusion: The SNP mutation of CYP2C9 rs1799853 gene found in T2DM patients at Moewardi Hospital Surakarta, Solo who received sulphonylurea therapy.

Keywords: DMT2, Sulphonylurea, CYP2C9, Outcome therapy

Introduction

The prevalence of DMT2 in Indonesia will reach 21.3 million in Indonesia in 2030 (1). Based on the Indonesian Basic Health Research, the prevalence of DMT2 patients with age more than 15 years old increased, meaning that younger generations were also threatened by DMT2 (1,2). Some factors became the risk factors for DMT2 and also the drug response, such as genetic, physiology, pathophysiology and environmental (3,4).

Sulphonylurea is the most common oral antidiabetic used in the treatment of DMT2. The drugs in the group of sulphonylurea, are the most commonly used in the treatment of DM type 2 (5). Its mechanism is stimulating the insulin secretion by inhibiting the ATP-dependent potassium channel in the beta cells of the pancreas, which can change the resting potential of the cell and cause the calcium influx and stimulation (6).

The *CYP2C9* and *CYP2C19* are the most responsible sub-enzyme in the sulphonylurea metabolism. The individu with variants of CYP2C9*2 and *3 were found as slow metabolizers than individu with wild-type (7,8). Previous studies also found that individu with one variant need the low dose of sulphonylurea, due to the decrease of *CYP2C9* activity (9,10). The low dose of sulphonylurea is needed to avoid the hypoglycemia as the adverse event (11). The genetic variations can cause the different response of individual metabolism and drug response (4).

This study' objective is to identify the genotype of *CYP2C9* gene in rs1799853 and the therapeutic outcome of blood sugar levels in T2DM patients

Materials and Methods

Research subjects

The subjects of this study were 11 T2DM patients at Moewardi Hospital, Solo. The patients aged more than 18 years old and were diagnosed according to the American Diabetes Association criteria (fasting plasma glucose (FPG) more than 126 mg/dl, or plasma glucose more than 200 mg/dl after 2-h (2h-PPG) of oral glucose (1.75 g/kg) or HbA1c ≥ 6.5%). The inclusion criteria in this study included patients who had complete medical record data and received Sulphonylurea monotherapy for treatment. The exclusion criteria included patients with type I diabetes, patients who have received insulin monotherapy or combinations with other oral antidiabetic therapy, and gestational diabetes patients. This study was approved by the ethics committee of the Anhamd Dahlan University (EC No. 011904040), and written informed consent was obtained by all participants. The study was carried out at the laboratories of the biotechnology, Study Program of

Biology, Faculty of Applied Science and Technology at Ahmad Dahlan University in the period from November 2019 to December 2020.

Biochemical assays

Glucose level was measured by an enzyme-based method (Glucose hexokinase method), and HbA1c was assayed by an ion exchange resin method using the Advia® Chemistry kit.

DNA samples

A total of 11 peripheral blood samples were collected from each subject in tubes containing EDTA (0.1 mmol / L) and stored at –20 oC before use. Genomic DNA was isolated using PureLink Genomic DNA kits (Invitrogen). The concentration and purity of genomic DNA were measured by using electrophoresis and UV spectrophotometer (Shimadzu-1800, Europa GmbH). Genomic DNA was used as a template for in-vitro reactions to identify single nucleotide polymorphisms (SNPs) of the *CYP2C9* gene in rs1799853.

PCR amplification

PCR reaction was performed using T100™ Thermal Cycler (Bio-Rad, United States). A specific area of the *CYP2C9* gene was amplified (713 bp) using GoTaq® Green Master Mix (Promega, USA), and the following primers: Forward: (5`-GACCATTGCCTTGAACATCAC-3') Reverse: (5`-GGTCAGTGATATGGAGTAGGG-3'). The amplification was under the following conditions: initial denaturation (5 min at 94 °C) followed by 35 cycles of denaturation (30 sec at 94 °C), annealing (30 sec at 56 °C), and extension (40 sec at 72 °C). A final extension step was carried out for 5 min at 72 °C. The PCR products were analyzed by 2% agarose gel electrophoresis.

Direct sequencing with PCR products

The products of PCR amplification were continued to direct-sequencing analysis using ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA). PCR reaction was carried out in a 30 µL solution, and the PCR products were purified to be the sequencing templates with PCR product purification. The Basic Local Alignment Search Tool (BLAST) analysis was used to determine the validity of the sequencing results. Alignment (Bioedit Software) was used to determine SNP between DNA base sequences as sequenced with SNP rs1799853 *CYP2C9*. Sequencing data and bioinformatics results from CYP2C9 gene variants were analyzed qualitatively.

Data Analysis

Data Description

The *CYP2C9* (rs799853) allele, genotype frequencies, and demographic characteristics of patients were analyzed descriptively, including data of age, gender, education, occupation, and therapeutic outcome (FBS, 2-hour postprandial blood sugar, and HbA1c levels).

Results

A total of 11 patients under sulphonylurea therapy (6 males and 5 females) were enrolled in the present study. The characteristics of patients in this study were shown in Table 1, with the mean age (±SD) of 69.36 (±0.08) years. Furthermore, Table 1 showed fasting plasma glucose, two hours of plasma glucose, and blood HbA1c levels.

Table 1. Data clinical and biological characteristics of the patient

Characteristics	Respondents	N (%)	Average ± SD
Sex	Male Women	6(45,45%) 5(54,54%)	
Education	Basic (Elementary, Junior High) Height (D1,S1,S2)	8 3	
Job	Work Not working	9 2	
Age			69,36±0,08
FPG			174±125.38
2-H PP			217,63±105,95
HbA1c			13,50±18,49

The amplification of the *CYP2C9* gene with a specific primer was presented in figure 1. The results were shown with a 713 bp band on gel electrophoresis.

(S= Sample; M= DNA marker 100bp).

The results of direct DNA sequencing from patients with wild type genotype and mutant genotypes were shown

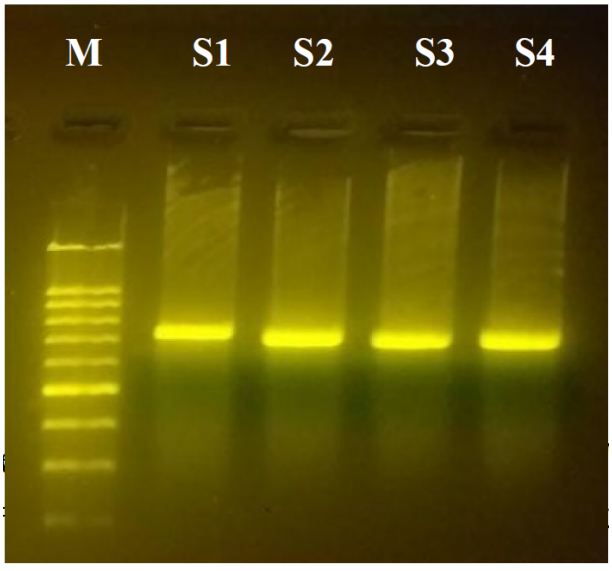

Figure 1. Agarose gel electrophoresis for *CYP2C9* gene on 713 bp

in Figure 2. There were 10 patients with *CYP2C9* gene rs 1799853 wild type (CC-90.9%) and one patient with *CYP2C9* gene rs 1799853 heterozygote mutant (CT-9.09%).

Table 2 presents the proportion of wild-type and mutant heterozygous of CYP2C9*2. Most of the subjects are wild-type (90.9%) and carrying the C allele (97.7%)

Table 2. Genotype and allele frequencies of the *CYP2C9* rs 1799853 polymorphism

	Patients
Genotypes	
Wild type (CC)	10 (90,9%)
Mutant heterozygous (CT)	1 (9,09%)
Allele	
C	0,977 (97,7%)
T	0,022 (2,2%)

Table 3 presents the distribution of wild-type and mutant-heterozygous based on the Fasting Blood Glucose (FBG), 2-H PPG (2 hours post prandial blood sugar level) and HbA1C. The individual with mutant heterozygous shows high level of HbA1C.

Table 3. Association analyses of the rs 1799583 genotype with blood sugar levels of patients.

		Genotypes (n)	
		CC	CT
2H-PPG	Controlled	6	1
	Uncontrolled	4	0
FPG	Controlled	4	1
	Uncontrolled	6	0
HbA1c	Controlled	2	0
	Uncontrolled	8	1

Discussion

Our study finds that the glycemic condition of the subjects are uncontrolled with the high average of FBG, 2-H PP of blood glucose and HbA1C. The number of subjects with high level HbA1C and FBG are higher than the subjects with the lower level of those. All of the subjests in this study were elderly patients.

Many factors can affect the diabetic treatment for elderly patients, such as liver function, kidney function, hyploglycemia effect, psychological stress and other comorbidities. Many comorbidities are resulting the potential of drug-drug interaction, which may influence the blood glucose level. Hypoglycemia due to the drug-drug interaction effect must be avoided in the treatment of DM type elderly patients (13). Thus the diabetic treatment for elderly patients, must be personalized, especially due to the association between allele variations of CYP2C9*2 and CYP2C9*3 and blood ssulphonilurea clearance (14,15)

The *CYP2C9* gene, rs 1799853, is known as the CYP2C9*2, single-nucleotide base substitution from C to T at codon 430 located in exon 3. This SNP causes a change in amino acid residue from arginine to cysteine at codon position 144 (Arg144Cys) on the surface of *CYP2C9* enzyme (qayyyum). The previous studies found the lower frequency of CYP2C9*2 are in Africans, East Asian, and Native Americans (<1 %) and the higher oof those are in Caucasian (16). In the Pakistani and India populations, the frequency of CYP2C9*2 were <5%, and in the Chinese, Japan and Indonesia population, there were CYP2C9*2 (17).

We only find one subjects with mutant heterozygous (CT), where as this subject has high level of HbA 1C. Some og the

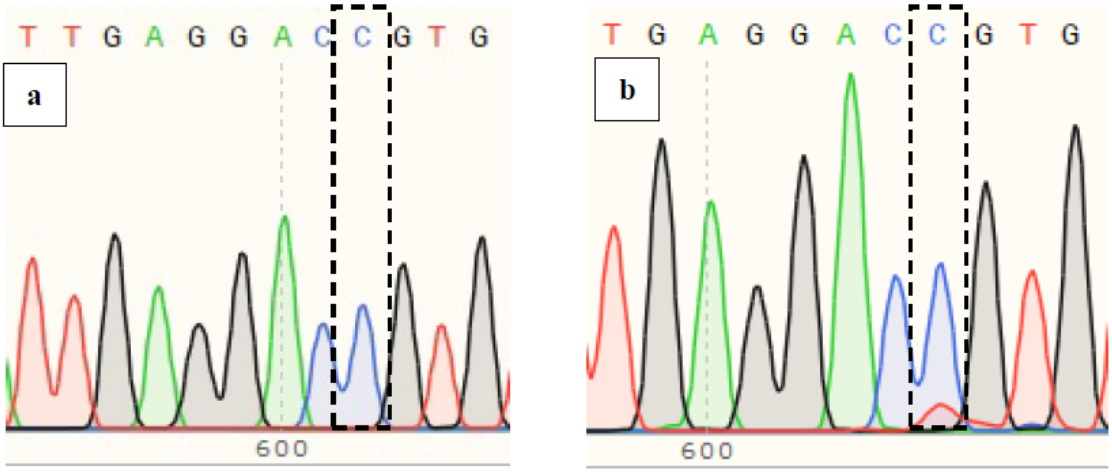

Figure 2. Sequence analysis of DNA samples. (a) Sequence of *CYP2C9 rs 1799853* genotype CC. (b) Sequence of *CYP2C9 rs 1799853* genotype CT.

subjects with wild type (CC) show the high level of FBG and 2-H PP of blood glucose. The previous study with with elderly population in Holland presented that there wa no signifficant differences tolbutamid dose between the wild-type and the mutant heterozygous type. The signifficant difference was found in the CYP2C9*3 in tolbutamide and glimepiride (18,19).

Our study did not confirm the association between CYP2C9*2 and glucose level. However, the future study is suggested to identify the allele variations in CYP2C9*3 in Indonesia variations.

Conclusion

The mutant-heterozygous of CYP2C9*2 is showing the controlled FBG anf 2-H PP of blood glucose level, however the HbA1C level is high.

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