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The influence of metformin transporter gene *SLC22A1* and *SLC47A1* variants on steady-state pharmacokinetics and glycemic response

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Abstract

Interindividual variation is important in the response to metformin as the first-line therapy for type-2 diabetes mellitus (T2DM). Considering that OCT1 and MATE1 transporters determine the metformin pharmacokinetics, this study aimed to investigate the influence of *SLC22A1* and *SLC47A1* variants on the steady-state pharmacokinetics of metformin and the glycemic response. This research used the prospective-cohort study design for 81 patients with T2DM who received 500 mg metformin twice a day from six primary healthcare centers. *SLC22A1* rs628031 A>G (Met408Val) and Met420del genetic variants in OCT1 as well as *SLC47A1* rs2289669 G>A genetic variant in MATE1 were examined through the PCR-RFLP method. The bioanalysis of plasma metformin was performed in the validated reversed-phase HPLC-UV detector. The metformin steady-state concentration was measured for the trough concentration (Css^{min}) and peak concentration (Css^{max}). The pharmacodynamic parameters of metformin use were the fasting blood glucose (FBG) and glycated albumin (GA). Only *SLC22A1* Met420del alongside estimated-glomerular filtration rate (eGFR) affected both Css^{max} and Css^{min} with an extremely weak correlation. Meanwhile, *SLC47A1* rs2289669 and FBG were correlated. This study also found that there was no correlation between the three SNPs studied and GA, so only eGFR and Css^{max} influenced GA. The average Css^{max} in patients with the G allele of *SLC22A1* Met408Val, reaching 1.35-fold higher than those with the A allele, requires further studies with regard to metformin safe dose in order to avoid exceeding the recommended therapeutic range.

Introduction

The incidence of diabetes mellitus (DM) in Indonesia is getting higher every year, reaching 2.1% increase since 2013 based on the 2018 National Basic Health Research report. Of the total population, 13.1% has a high level of fasting blood glucose [1]. Consequently, to prevent and

profile, and because of national policy. Should such data be needed, a request can be addressed to the Ethics Committee of the Faculty of Medicine of Universitas Gadjah Mada, Radiopoetro Building 2nd floor, Farmako, Sekip Utara St, Yogyakarta 55128, +62811-2666-869, e-mail: mhrec_fmugm@ugm.ac.id.

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decrease DM-induced mortality and morbidity, a good blood glucose management is needed [2].

During the period of 2013–2021, metformin had been listed in the Indonesian National Formulary along with other oral antidiabetic drugs, including glipizide, glimepiride, and glibenclamide, as a drug provided by primary healthcare centers [3]. Compared to other oral antidiabetic drugs, metformin has a better ability to decrease the level of HbA1c by 1.0–2.0% and has less hypoglycemia effects. However, it is known that the glycemic response to metformin is varied because 35–40% patients have not reached the target for fasting blood glucose [4]. Our previous research revealed high variability in metformin plasma steady-state concentration (PSSC), reaching >100x at the trough and 15x at the peak [5]. Genomic variation likely leads to patients' variability in the drug pharmacokinetic and pharmacodynamic variability, including those of metformin [6]. Metformin has renal excretion as the major elimination pathway with >0.6 genetic component (rGC), indicating that genetic factor greatly affects the variability in metformin renal clearance [7]. Genetic variation has an influence on the protein function in metformin bioavailability or therapeutic effects.

With the hydrophilic property as a cationic species (>99.9%) at a physiological pH, the pharmacokinetics of metformin is effective depending the function of the transporters [8]. The main transporters that have a key role in the pharmacokinetics of metformin to date are Organic Cation Transporter1 (OCT1) and Multidrug and Toxin Extrusion1 (MATE1). Mainly expressed in the liver, OCT1 is a protein transporter that carries metformin to hepatocytes, the target of metformin action. The genetic variation in *SLC22A1* as the OCT1 coding gene can change the protein function, leading to a reduced amount of metformin in the receptors and therefore a declined therapeutic response. A number of studies showed that *SLC22A1* genetic variation resulted in varied steady-state concentration of metformin and various glycemic response [9–12]. Furthermore, latest studies found that such genetic variation was associated with metformin intolerance in the gastrointestinal tract [13,14].

In addition, the *SLC47A1* is a MATE1 protein-coding gene mostly located in the apical membrane of renal tubular cells and canalicular membrane of hepatocytes. MATE1 transports metformin from hepatocytes to the bile and excretes metformin through the kidneys. Some research proved that the polymorphisms in *SLC47A1* affect the pharmacokinetic variability as well as the glycemic response [15,16]. To date, however, the majority of metformin pharmacogenetic studies focus on the effects of OCT1 and MATE1 polymorphisms on glycemic control at various doses. Only one study has linked this to the minimum steady-state concentrations but not to the maximum [10], which is likely associated with a predisposition to lactic acidosis. Meanwhile, a large number of studies of the peak concentrations only focus on single administration of metformin to healthy volunteers for bioavailability-bioequivalence studies but not on repeated administration as an actual condition of metformin use among T2DM patients. Both transporters are known to play an important role in metformin bioavailability. In addition, metformin pharmacogenetic studies conducted prospectively in a similar dose with a control on the adherence factors remain extremely limited. Therefore, this prospective study aimed to analyze how the genetic variation in two metformin transporter encoding genes correlates with not only the glycemic response but also with the minimum and maximum steady-state concentrations.

Materials and methods

Recruitment of the subjects

T2DM patients administered metformin 500 mg twice daily for at least 2 weeks from six primary healthcare centers in Yogyakarta Special Province were involved. An explanation of the

research, such as the objectives, the procedures for the participants to follow as well as the risks and benefits of the research were conveyed both orally and in writing directly to the eligible subjects. The subjects were allowed time to decide whether they would participate in the study. When they have verbally expressed their consent, they signed 2 (two) informed consent forms containing the consent to participate in the study (sheet 1) and to permit the research team to store and use their remaining specimens or DNA (sheet 2). The subjects recruited were in the 30–60 age range and literate, thus requiring no parent or guardian involvement in the subject recruitment procedure to indicate their consent to participate in this study. The ethical clearance was approved by the Ethics Committee of the faculty of Medicine of Universitas Gadjah Mada with the approval letter Number KE/FK/648/EC and conducted in accordance with the Declaration of Helsinki.

Analysis of the genotypes

The genotype analysis was done through Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP).

***SLC22A1* (OCT1) rs628031 (Met408Val).** The PCR primer design used 5'-TTT CTT CAG TCT CTG ACT CAT GCC-3' and 5'-AAA AAA CTT TGT AGA CAA AGG TAG CAC C-3'. The analysis of the 397-bp amplification products was done in 1% agarose gel followed by the restriction digestion in *MscI* with 16–18 hours of incubation at 37˚C. The digestion yielded 397-bp fragments for the homozygous variants (val/val) as well as 210-bp and 187-bp fragments for the wild-type (Met/Met). The size of the digestion products (397 bp, 210 bp, and 187 bp) showed a category of heterozygotes (Met/Val). The genotype analysis were confirmed through the sequencing in a previous study [17].

***SLC22A1* Met420del in OCT1.** The PCR primer design used 5'-AGGTTCACGGACTCT GTGCT-3' as the forward primer and 5'-AAGCTGGAGTGTGCGATCT-3' as the reverse primer. The analysis of the 600-bp amplification products was done in 1% agarose gel at 100 Volt for 30 minutes, and the restriction digestion used *BspHI* with ±12 hours of incubation at 37˚C. The T-CATGA sequence was cut by the *BspHI* enzyme at 197th DNA template base. The *BspHI* identified and digested the AA genotype, but this enzyme did not identify the PCR products with a T-CATTT sequence, making such products remain undigested. The digestion produced 600-bp fragments of AA (wild type) genotype, 403-bp and 197-bp fragments of aa (mutant) genotype, and 600-bp, 403-bp, 197-bp of heterozygotes (Aa).

***SLC47A1* (MATE1) rs2289669 (G>A).** The PCR primer design used the forward primer of 5′-TCA GTT TCC ACA GTA GCG TCG-3′ and the reverse primer of 5′-GAC ACT GGA AGC CAC ACT GAA-3′. The *TaqI* restriction endonuclease digested the amplification products (211 bp), which were then analyzed in 2% agarose gel. The restriction digestion used the *TaqI* with 16–18 hours of incubation at 65˚C. The 211-bp amplicons were digested into 21-bp and 190-bp fragments of AA genotype, 211-bp fragments of GG genotype/wild-type as well as 21-bp, 190-bp, and 211-bp fragments of heterozygous genotype [18].

Pharmacokinetics of metformin steady-state concentrations

The patients reported the time of the last metformin administration which was done for uniform doses and intervals. In 12 hours after the last dose administration, they visited the primary healthcare center for blood sampling to measure the same-day trough and peak concentrations. The sampling for the trough PSSC was immediately done before the next-dose administration (pre-dose), while the peak PSSC sample was taken in 3.5–4.0 hours after the metformin administration (post-dose). The samples were then delivered to the Laboratory of Drugs, Food, and Cosmetics of the Pharmacy Department of Universitas Islam Indonesia to

be centrifuged for 10 minutes at 3500g, and the plasma aliquot was stored in a 2-ml polypropylene tube at -20˚C in a maximum of one hour after the sampling. The metformin plasma concentrations were determined through a validated reversed-phase high performance liquid chromatography (HPLC) assay with Sunfire® C-18 column, 4.6 x 150mm x 5μm from Waters, and SM7 injector with an ultraviolet (UV) detector at 234 nm wavelength [19]. The metformin PSSC could estimate the elimination rate, and the metformin half-life was also calculated using the following formula [20].

$$K\ (/hour) = \frac{ln\ \left(\frac{Css^{max}}{Css^{min}}\right)}{8}$$

$$t_{1/2}\ (hour) = \frac{0,693}{K}$$

Measurement of the glycemic response

The FBG and GA of T2DM patients given metformin monotherapy were measured before and after the continuous administration of metformin 500 mg twice daily for six weeks. The UV/VIS spectrophotometry of Hitachi 902® was used to measure FBG with the GOD-PAP method, and the ELISA reader of ADVIA® was employed in the measurement of GA with the KAOD (*Ketoamine oxidase*) method.

Statistical analysis

The metformin PSSC obtained was displayed in mean ± SD values. A comparison of patients' metformin PSSC among the groups of allele types and genetic variants was made using the independent t-test and one-way ANOVA for normally distributed data as well as the Mann-Whitney and Kruskal-Wallis test for non-normal data distribution. To analyze the patient-related factors affecting the pharmacokinetics of metformin steady-state concentrations and glycemic control, the linier regression was employed with a statistically significant p value of $\leq$0.05.

Results and discussion

There have been no prospective studies of the influence of genetic polymorphisms on the pharmacokinetics of steady-state concentrations and glycemic response that involve T2DM patients who adhere to metformin therapy with a similar dose for a minimum of eight weeks. Given that metformin is a long-term antidiabetic drug, the pharmacokinetic variability of repeated administration can give a more accurate description of the concentration variability, while in a single-dose administration it is left unknown.

The discussion on the effects of genetic polymorphisms on the variability of the pharmacokinetics of steady-state concentrations and glycemic control resulted from metformin use should begin with an understanding of the function, physiological role of OCT1 and MATE1 protein transporters, as well as the level of gene expression in various human tissues. The following table describes the predicted pharmacokinetic variability of metformin steady-state concentrations and its glycemic response with regard to SNPs in *SLC22A1* and *SLC47A1* genes.

Meanwhile, the research findings related to the steady-state pharmacokinetic variability in each genetic variant and allele of both target genes are presented in Table 1.

In general, Table 1 shows that the T2DM patients in the Javanese-Indonesian population have a significant difference in the Css^{max} between the Aa and aa variants. As previously described in Table 2, OCT1 is highly expressed in the basolateral membrane of hepatocytes,

Table 1. Variability of metformin steady-state concentrations according to the genetic variants and alleles.

Group of Patients	Frequency (%)	Css^{min} (μg/mL) (P Value)	Css^{max} (μg/mL) (P Value)
SLC22A1 Met408Val			
AA	5 (6.17)	0,358±0.292	0.818±0.445
AG	53 (64.43)	0.365±0.244	1.323±0.854
GG	23 (28.40)	0.347±0.335	1.006±0.654
		0.964	0.144
SLC22A1 Met408Val			
A Allele (AA genotype)	5 (6.17)	0.596±0.486	1.363±0.743
G Allele (AG and GG genotype)	76 (93.83)	0.600±0.453	1.845±0.944
		(0.986)	0.265
SLC22A1 Met420del			
AA	0 (0.00)	-	2.831±0.518
Aa	3 (3.70)	0.549±0.210	1.778±0.928
Aa	78 (96.30)	0.352±0.272	0.015
		0.222	
SLC47A1 rs2289669			
GG	14 (17.28)	0.440±0.259	1.298±0.573
GA	35 (43.21)	0.316±0.314	1.166±1.030
AA	32 (39.51)	0.372±0.221	1.202±580
		0.337	0.303
SLC47A1 rs2289669			
G Allele (GG genotype)	14 (17.28)	0.720±0.435	1.846±0.727
A Allele (GA and AA genotype)	67 (82.72)	0.574±0.455	1.810±0.978
		(0.277)	(0.618)

Css^{max}, maximum steady-state concentration; Css^{min}, minimum steady-state concentration.

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making the polymorphisms able to reduce the protein function of OCT1 in transporting metformin into hepatocytes as the action target. As a result, metformin is retained in the systemic circulation at a higher concentration than in wild-type patients. Since such variant was not found in this study, no further comparative analysis could be performed. Although the

Table 2. Prediction of the steady-state pharmacokinetic variability and glycemic response affected by the genetic polymorphisms in *SLC22A1* and *SLC47A1*.

Location of *SNPs*	Affected stage of metformin pharmacokinetics	Prediction of the effects of SNPs on the glycemic control parameters (FBG and GA) based on metformin Css as opposed to that of the wild-type variant					
		Css^{max}	Css^{min}	Final FBG value[a]	Changed FBG value[b]	Final GA value[a]	Changed GA value[b]
SLC22A1 encoding OCT1 in the basolateral membrane of intestinal cells	Absorption	lower	minimum effect	higher	higher	higher	higher
SLC22A1 encoding OCT1 in the basolateral membrane of hepatocytes*	Influx to the action target in hepatocytes	higher	minimum effect	higher	higher	higher	higher
SLC47A1 encoding MATE1 in the hepatic canalicular membrane*	Efflux to the bile	lower	minimum effect	lower	lower	lower	lower
SLC22A1 encoding OCT1 in the apical membrane of renal tubular cells	Reabsorption in the renal tubules	lower	Lower	higher	higher	higher	higher
SLC47A1 encoding MATE1 in the brush-border membrane of renal tubular cells*	Efflux from the renal cells to be eliminated via urine	higher	higher	lower	lower	lower	lower

Note

*highly expressed [31]; [a]after the administration of metformin 500 mg every 12 hours for 6 weeks.

[b]obtained from the final value of glycemic control (FBG, GA) minus the baseline value.

OCT1, organic cation transporter 1; MATE1, Multidrug and Toxin Extrusion 1; Css^{max}, maximum steady-state concentration; Css^{min}, minimum steady-state concentration; FBG, fasting blood glucose; GA, glycated albumin.

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difference was insignificant, a finding similar to the prediction was indicated by the difference in the mean Css^{max} between the A allele and the G allele in *SLC22A1* Met408Val, which was 1.363±0.743 g/mL and 1.845±0.944 g/mL, respectively. The presence of the rs628031 Met408-Val polymorphisms in *SLC22A1* is known to decrease the concentration of OCT1 mRNA in the human liver [21], resulting in reduced OCT1 function to transport metformin to hepatocytes. Consequently, polymorphisms in the *SLC22A1* gene decrease the function of OCT1 in transporting metformin to hepatocytes, resulting in the highest Css^{max} being found in the variant-type group. In relation to the risk of lactic acidosis, the G allele has the higher potential than the A allele. Given the accumulation of metformin concentration becomes a predisposition to metformin associated lactic acidosis (MALA), the maximum recommended dose of metformin, particularly on the G allele, should be considered. A number of studies have found that metformin accumulation leads to lactatemia either with or without decreased renal function [22,23]. In fact, there is a 6-fold increased risk of lactic acidosis in the initial use of metformin alongside a decreased renal function, and a 12–13 times higher risk is found in patients with cumulative exposure to high-dose metformin in the past year or initial exposure to high-dose metformin [24]. The administration of subtherapeutic dose is not a solution since the glycemic target is by no means achieved [25]. Metformin dose is not correlated with plasma lactate or serum creatinine as shown in a study involving the incidence of MALA for over 30 years of observation [26]. There is no examination of metformin concentration or control of adherence factors, making the accumulation of metformin in plasma remain a predisposing factor for MALA. However, other factors such as BMI [27] and comorbidities, including renal impairment, also clearly become the co-factors of metformin accumulation to induce MALA [28–30]. Not only the dose but also the long-term metformin use become a risk factor for metformin accumulation due to the distribution of metformin into the erythrocyte compartment as previously found in our study [5].

Using an approach of elimination half-life calculation based on the allele type in *SLC22A1* Met408Val, this study found that the mean Css^{max} was 1.35-fold higher in the G allele group (AG+GG) when compared to the wild-type group. A longer $t_{1/2}$ (1.25 times) was also found in the GG homozygous mutant group (Val/Val) when compared to the AG heterozygous group (Met/Val). Therefore, it is recommended that the maximum dose of metformin for patients with the G allele (AG+GG) is lower than that for the wild-type group, and a longer interval of administration is recommended for the GG homozygous mutant group (Val/Val) in order to minimize the incidence of lactic acidosis.

Meanwhile, the *SLC47A1* that encodes MATE1 is highly expressed in the canalicular membrane of hepatocytes in the bile and in the brush-border membrane of renal tubular cells. Each of which plays a role in the efflux to the bile and efflux from the kidney cells to be eliminated through urine, with predicted lower and higher Css^{max} than those of the wild-type variant, respectively with the similar prediction for the glycemic response (Table 2). The mean metformin concentration in both the peak and trough PSSC is lower in the group of patients with the A allele of *SLC47A1* rs2289669 when compared to the wild-type group although there is no significant difference. The likely decreased function of MATE1 in the canalicular membrane of hepatocytes in the bile which is more significant that in the brush-border membrane of renal tubular cells requires further studies.

It is found in our previous study that differences in the regimen of oral antidiabetic drugs and the duration of metformin use have led to significantly different mean of Css^{max} and Css^{min}, respectively. In addition, the linear regression analysis has shown that only the Css^{max}, alongside the glycemic control factors, affect FBG and GA while the Css^{min} has an influence on FBG. Therefore, this study proceeds with a linear regression test to further analyze the patient factors, including the genetic variants in the two target genes that influence the steady-

Table 3. Patient-related factors correlated with glycemic response after the administration of metformin 1000mg/day for 6 weeks.

Dependent variable	Predictor	Coefficient	Coefficient of correlation	P value	ANOVA test result	Adjusted R Square in the Model Summary
Css^{min} (μg/mL)	eGFR	-0.006	-0.246	0.026	0.015	0.093
	Variant genotype of *SLC22A1* Met420del	-0.551	-0.231	0.043		
	BMI	-0.020	-0.200	0.080		
Css^{max} (μg/mL)	eGFR	-0.013	-0.258	0.018	0.009	0.103
	Variant genotype of *SLC22A1* Met420del	-1.430	-0.288	0.011		
	BMI	-0.029	-0.135	0.228		
Metformin elimination half-life	Duration of previous metformin therapy	3.696	0.254	0.022	0.029	0.064
	Allele type of *SLC22A1* Met408Val	-4.542	-0.181	0.101		
Final FBG	Baseline GA	3.093	0.463	0.004	0.001	0.333
	Variant genotype of *SLC47A1* rs2289669	20.460	0.404	0.011		
FBG change	Baseline FBG	3.135	0.347	0.078	0.000	0.621
	Baseline GA	-1.006	-0.968	0.000		
	Variant genotype of *SLC47A1* rs2289669	20.425	0.299	0.014		
Final GA	Baseline GA	1.142	1.274	0.000	0.000	0.727
	Baseline FBG	-0.077	-0.749	0.001		
	eGFR	0.086	0.305	0.020		
	Variant genotype of *SLC47A1* rs2289669	-0.889	-0.131	0.222		
	Css^{min}	-3.622	-0.176	0.244		
	Css^{max}	2.582	0.443	0.011		
GA change	Baseline FBG	-0.793	-0.838	0.000	0.000	0.460
	eGFR	-0.060	0.353	0.039		
	Variant genotype of *SLC22A1* Met408Val	0.068	-0.192	0.182		
	Css^{max}	1.689	0.365	0.026		

Css^{max}, maximum steady-state concentration; Css^{min}, minimum steady-state concentration; FBG, fasting blood glucose; GA, glycated albumin; eGFR, estimated-glomerular filtration rate; BMI, body mass index.

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level pharmacokinetics, glycemic response, and time estimates for metformin elimination to provide an approximation of the effective metformin dose as presented in Table 3.

With regard to the use of glycemic control parameters, such as FBG and GA in this study but not HbA1c which is commonly used in the majority of metformin pharmacogenetic studies, a strong correlation between these three parameters has been demonstrated in some research [32–36]. The use of GA in Indonesia as both a diagnostic function and parameter for monitoring the success of diabetes therapy remains limited and has not become the gold standard of either the Indonesian Society of Endocrinology or the American Diabetic Association. However, GA is preferred for describing a glycemic control as opposed to HbA1c, especially in patients with impaired renal function or decreased life span of erythrocyte such as hemolytic anemia [37]. In addition, even with a shorter life span of albumin compared to that of HbA1c (±15 days), GA can describe the glycemic control in patients with diabetes mellitus for a

minimum of 2–3 weeks [38], making it more appropriate for this study which involves adherent patients taking metformin for eight weeks (including metformin use duration as the inclusion criteria).

Together with eGFR and BMI, the *SLC22A1* Met408del polymorphisms affect the pharmacokinetics of steady-state concentration of metformin with only a low adjusted R Square. This indicates that the two steady-state concentrations of metformin are mostly explained by other variables which are not involved in this study. Similarly, an extremely weak correlation is also shown by the *SLC22A1* Met408del variant type alongside the duration of previous metformin use and the elimination half-life of metformin. Meanwhile, in the glycemic response based on FBG, only *SLC47A1* rs2289669 affects both the decrease in and the final FBG values, particularly the decreased FBG with 0.621 adjusted R Square.

Encoded by the *SLC47A1* gene, the metformin transporter MATE1 is mostly expressed in the apical membrane of renal tubular cells and canalicular membrane of hepatocytes. It has therefore a major role in the final phase of cationic organic compound excretion, including metformin [39]. On the other hand, a number of studies have investigated *SLC47A1* rs2289669 polymorphisms and their effects on the pharmacokinetics, response, and other biochemical parameters for metformin. The rs2289669 polymorphisms interact with *SLC22A1* rs594709, thus decreasing FBG and postprandial insulin as well as increasing HOMA-IR in the AA genotype group that has *SLC22A1* and *SLC47A1* as opposed to the group with a G allele [40]. Therefore, this study confirms the correlation between rs2289669 *SLC47A1* polymorphisms and FBG values.

Meanwhile, when the final GA parameter is employed, none of the genetic variants studied affect it; instead, it is the baseline glycemic concentration, eGFR, and Css^{max} with a good value of adjusted R Square of 0.727 that influence GA. In addition, changes in GA are affected by the baseline glycemic concentration, eGFR, and Css^{max}. Such findings on the effects of rs2289669 *SLC47A1* polymorphisms are different from those of other studies that use another parameter of glycemic response in metformin use. Research on the effects of rs2289669 on glycemic response to metformin using HbA1c reveals that the AA homozygous variant has the best glycemic response. This is probably caused by the reduced function of MATE1, which has an important role in the renal secretion of metformin, marked by a high AUC but low Cl_R among ten patients with such variant as opposed to those with other variants [18]. This result is similar to that of the research on 142 patients in Slovakia in which 20% of those with AA homozygous variant have two-fold reduced HbA1c after using metformin for six months [41]. Another similarity is found among 116 Caucasian patients with T2DM where those with the A allele *SLC47A1* rs2289669 have 0.3% more reduction in HbA1c upon taking metformin [42]. Therefore, along with the baseline glycemic value and eGFR, the pharmacokinetics of maximum steady-state concentration (Cssmax) has a correlation with GA. The results of Css^{max} examination in this study indicate that 64.6% patients have metformin concentrations in the therapeutic range (0.75–5 g/mL), and only 1/10 has Css^{min} that is greater than or equal to 0.75 g/mL. This can possibly cause Css^{max} to be the only parameter associated with GA. Therefore, these findings confirm the importance of adherence to metformin therapy to guarantee the achievement of metformin therapeutic concentrations.

Although the best efforts have been made through multicenter studies in some primary healthcare centers, there is a limitation in this study related to the number of patients involved. It becomes one of the factors in the incomprehensive analysis of the effects of polymorphisms on the pharmacokinetics of metformin steady-state concentrations and glycemic response. The difficulty in involving patients who are adherent to metformin therapy for a minimum of eight weeks is also a challenge for further studies.

Conclusion

In general, this study has found that the three polymorphisms absolutely have no effects on the pharmacokinetics of metformin steady-state concentrations. Although a further analysis involving other variables indicate the influence of *SLC22A1* Met408del polymorphisms on the pharmacokinetics of metformin steady-state concentrations, the variables that are not studied here in fact play a more major role (>95%). Alongside the baseline glycemic value, rs2289669 *SLC47A1* affects FBG while only eGFR and Css^{max} influence GA, but the three SNPs studied do not. These findings lead to a recommendation of further studies involving more subjects for a safe approach of metformin dose, particularly in T2DM patients with the G allele *SLC22A1* Met408del to prevent metformin accumulation beyond the recommended therapeutic range.

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References

1. Ministry of Health of the Republic of Indonesia. Riskesdas (Basic Health Research) 2018 2019.
2. Perkeni (Indonesian Association of Endocrinologists). Pedoman Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia (Guidelines for the Management and Prevention of Type 2 Diabetes Mellitus in Indonesia) 2021.
3. Ministry of Health of the Republic of Indonesia. Formularium Nasional (National Formulary) 2021.

4. Cook MN, Girman CJ, Stein PP, Alexander CM. Initial monotherapy with either metformin or sulphonylureas often fails to achieve or maintain current glycaemic goals in patients with Type 2 diabetes in UK primary care. Diabet Med J Br Diabet Assoc 2007; 24:350–8. https://doi.org/10.1111/j.1464-5491.2007.02078.x PMID: 17335466
5. Ningrum VDA, Ikawati Z, Sadewa AH, Ikhsan MR. Patient-factors associated with metformin steady-state levels in type 2 diabetes mellitus with therapeutic dosage. J Clin Transl Endocrinol 2018; 12:42–7. https://doi.org/10.1016/j.jcte.2018.05.001 PMID: 29892566
6. Holstein A, Seeringer A, Kovacs P. Therapy with oral antidiabetic drugs: applied pharmacogenetics. Br J Diabetes Vasc Dis 2011; 11:10–6. https://doi.org/10.1177/1474651410397583.
7. Leabman MK, Giacomini KM. Estimating the contribution of genes and environment to variation in renal drug clearance. Pharmacogenetics 2003; 13:581–4. https://doi.org/10.1097/00008571-200309000-00007 PMID: 12972957
8. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, et al. Clinical Pharmacokinetics of Metformin: Clin Pharmacokinet 2011; 50:81–98. https://doi.org/10.2165/11534750-000000000-00000 PMID: 21241070
9. Becker ML, Visser LE, van Schaik RHN, Hofman A, Uitterlinden AG, Stricker BHC. Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus. Pharmacogenomics J 2009; 9:242–7. https://doi.org/10.1038/tpj.2009.15 PMID: 19381165
10. Christensen MMH, Brasch-Andersen C, Green H, Nielsen F, Damkier P, Beck-Nielsen H, et al. The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c. Pharmacogenet Genomics 2011; 21:837–50. https://doi.org/10.1097/FPC.0b013e32834c0010 PMID: 21989078
11. Mahrooz A, Parsanasab H, Hashemi-Soteh MB, Kashi Z, Bahar A, Alizadeh A, et al. The role of clinical response to metformin in patients newly diagnosed with type 2 diabetes: a monotherapy study. Clin Exp Med 2015; 15:159–65. https://doi.org/10.1007/s10238-014-0283-8 PMID: 24740684
12. Umamaheswaran G, Praveen RG, Damodaran SE, Das AK, Adithan C. Influence of SLC22A1 rs622342 genetic polymorphism on metformin response in South Indian type 2 diabetes mellitus patients. Clin Exp Med 2015; 15:511–7. https://doi.org/10.1007/s10238-014-0322-5 PMID: 25492374
13. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. Diabetologia 2016; 59:426–35. https://doi.org/10.1007/s00125-015-3844-9 PMID: 26780750
14. Tarasova L, Kalnina I, Geldnere K, Bumbure A, Ritenberga R, Nikitina-Zake L, et al. Association of genetic variation in the organic cation transporters OCT1, OCT2 and multidrug and toxin extrusion 1 transporter protein genes with the gastrointestinal side effects and lower BMI in metformin-treated type 2 diabetes patients. Pharmacogenet Genomics 2012; 22:659–66. https://doi.org/10.1097/FPC.0b013e3283561666 PMID: 22735389
15. He R, Zhang D, Lu W, Zheng T, Wan L, Liu F, et al. SLC47A1 gene rs2289669 G>A variants enhance the glucose-lowering effect of metformin via delaying its excretion in Chinese type 2 diabetes patients. Diabetes Res Clin Pract 2015; 109:57–63. https://doi.org/10.1016/j.diabres.2015.05.003 PMID: 26004431
16. Stocker SL, Morrissey KM, Yee SW, Castro RA, Xu L, Dahlin A, et al. The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin. Clin Pharmacol Ther 2013; 93:186–94. https://doi.org/10.1038/clpt.2012.210 PMID: 23267855
17. Umamaheswaran G, Praveen RG, Arunkumar AS, Das AK, Shewade DG, Adithan C. Genetic analysis of OCT1 gene polymorphisms in an Indian population. Indian J Hum Genet 2011; 17:164–8. https://doi.org/10.4103/0971-6866.92094 PMID: 22345987
18. He R, Zhang D, Lu W, Zheng T, Wan L, Liu F, et al. SLC47A1 gene rs2289669 G>A variants enhance the glucose-lowering effect of metformin via delaying its excretion in Chinese type 2 diabetes patients. Diabetes Res Clin Pract 2015; 109:57–63. https://doi.org/10.1016/j.diabres.2015.05.003 PMID: 26004431
19. Ningrum VDA, Wibowo A, Fuaida I, Ikawati Z, Sadewa AH, Ikhsan MR. Validation of an HPLC-UV Method for the Determination of Metformin Hydrochloride in Spiked-human Plasma for the Application of Therapeutic Drug Monitoring. Res J Pharm Technol 2018; 11:2197–202. https://doi.org/10.5958/0974-360X.2018.00406.7.
20. Shargel L, Wu-Pong S, Yu ABC. Applied Biopharmaceutics and Pharmacokinetics. New York: Appleton & Lange Reviews: Mc Graw Hill; 2005.
21. Koepsell H, Lips K, Volk C. Polyspecific Organic Cation Transporters: Structure, Function, Physiological Roles, and Biopharmaceutical Implications. Pharm Res 2007; 24:1227–51. https://doi.org/10.1007/s11095-007-9254-z PMID: 17473959
22. Richy FF, Sabidó-Espin M, Guedes S, Corvino FA, Gottwald-Hostalek U. Incidence of Lactic Acidosis in Patients With Type 2 Diabetes With and Without Renal Impairment Treated With Metformin: A

Retrospective Cohort Study. Diabetes Care 2014; 37:2291–5. https://doi.org/10.2337/dc14-0464 PMID: 24879835

23. Liu F, Lu J, Tang J, Li L, Lu H, Hou X, et al. Relationship of plasma creatinine and lactic acid in type 2 diabetic patients without renal dysfunction. Chin Med J (Engl) 2009; 122:2547–53. PMID: 19951568
24. Eppenga WL, Lalmohamed A, Geerts AF, Derijks HJ, Wensing M, Egberts A, et al. Risk of lactic acidosis or elevated lactate concentrations in metformin users with renal impairment: a population-based cohort study. Diabetes Care 2014; 37:2218–24. https://doi.org/10.2337/dc13-3023 PMID: 24842984
25. DeFronzo R, Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: Current perspectives on causes and risk. Metabolism 2016; 65:20–9. https://doi.org/10.1016/j.metabol.2015.10.014 PMID: 26773926
26. Huang W, Castelino RL, Peterson GM. Adverse event notifications implicating metformin with lactic acidosis in Australia. J Diabetes Complications 2015; 29:1261–5. https://doi.org/10.1016/j.jdiacomp.2015.06.001 PMID: 26104729
27. Davis TME, Jackson D, Davis WA, Bruce DG, Chubb P. The relationship between metformin therapy and the fasting plasma lactate in type 2 diabetes: The Fremantle Diabetes Study. Br J Clin Pharmacol 2001; 52:137–44. https://doi.org/10.1046/j.0306-5251.2001.01423.x PMID: 11488769
28. van Berlo-van de Laar IRF, Vermeij CG, Doorenbos CJ. Metformin associated lactic acidosis: incidence and clinical correlation with metformin serum concentration measurements. J Clin Pharm Ther 2011; 36:376–82. https://doi.org/10.1111/j.1365-2710.2010.01192.x PMID: 21545617
29. Lazarus B, Wu A, Shin J-I, Sang Y, Alexander GC, Secora A, et al. Association of Metformin Use With Risk of Lactic Acidosis Across the Range of Kidney Function: A Community-Based Cohort Study. JAMA Intern Med 2018; 178:903–10. https://doi.org/10.1001/jamainternmed.2018.0292 PMID: 29868840
30. Bodmer M, Meier C, Krähenbühl S, Jick SS, Meier CR. Metformin, sulfonylureas, or other antidiabetes drugs and the risk of lactic acidosis or hypoglycemia: a nested case-control analysis. Diabetes Care 2008; 31:2086–91. https://doi.org/10.2337/dc08-1171 PMID: 18782901
31. Shikata E, Yamamoto R, Takane H, Shigemasa C, Ikeda T, Otsubo K, et al. Human organic cation transporter (OCT1 and OCT2) gene polymorphisms and therapeutic effects of metformin. J Hum Genet 2006; 52:117–22. https://doi.org/10.1007/s10038-006-0087-0 PMID: 17111267
32. Yang C, Li H, Wang Z, Zhang W, Zhou K, Meng J, et al. Glycated albumin is a potential diagnostic tool for diabetes mellitus. Clin Med 2012; 12:568–71. https://doi.org/10.7861/clinmedicine.12-6-568 PMID: 23342412
33. Nathan DM, McGee P, Steffes MW, Lachin JM. Relationship of Glycated Albumin to Blood Glucose and HbA1c Values and to Retinopathy, Nephropathy, and Cardiovascular Outcomes in the DCCT/EDIC Study. Diabetes 2014; 63:282–90. https://doi.org/10.2337/db13-0782 PMID: 23990364
34. The Challenge of the Use of Glycemic Biomarkers in Diabetes: Reflecting on Hemoglobin A1C, 1,5-Anhydroglucitol, and the Glycated Proteins Fructosamine and Glycated Albumin | Diabetes Spectrum | American Diabetes Association n.d. https://diabetesjournals.org/spectrum/article/25/3/141/32469/The-Challenge-of-the-Use-of-Glycemic-Biomarkers-in (accessed July 13, 2022).
35. Danese E, Montagnana M, Nouvenne A, Lippi G. Advantages and Pitfalls of Fructosamine and Glycated Albumin in the Diagnosis and Treatment of Diabetes. J Diabetes Sci Technol 2015; 9:169–76. https://doi.org/10.1177/1932296814567227 PMID: 25591856
36. Yoshiuchi K, Matsuhisa M, Katakami N, Nakatani Y, Sakamoto K, Matsuoka T, et al. Glycated Albumin is a Better Indicator for Glucose Excursion than Glycated Hemoglobin in Type 1 and Type 2 Diabetes. Endocr J 2008; 55:503–7. https://doi.org/10.1507/endocrj.k07e-089 PMID: 18445997
37. Koga M, Kasayama S. Clinical impact of glycated albumin as another glycemic control marker. Endocr J 2010; 57:751–62. https://doi.org/10.1507/endocrj.k10e-138 PMID: 20724796
38. Kim KJ, Lee B-W. The Roles of Glycated Albumin as Intermediate Glycation Index and Pathogenic Protein. Diabetes Metab J 2012; 36:98–107. https://doi.org/10.4093/dmj.2012.36.2.98 PMID: 22540045
39. Otsuka M, Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y. A human transporter protein that mediates the final excretion step for toxic organic cations. Proc Natl Acad Sci U S A 2005; 102:17923–8. https://doi.org/10.1073/pnas.0506483102 PMID: 16330770
40. Xiao D, Guo Y, Li X, Yin J-Y, Zheng W, Qiu X-W, et al. The Impacts of SLC22A1 rs594709 and SLC47A1 rs2289669 Polymorphisms on Metformin Therapeutic Efficacy in Chinese Type 2 Diabetes Patients. Int J Endocrinol 2016; 2016:e4350712. https://doi.org/10.1155/2016/4350712 PMID: 26977146
41. Tkáč I, Klimčáková L, Javorský M, Fabianová M, Schroner Z, Hermanová H, et al. Pharmacogenomic association between a variant in SLC47A1 gene and therapeutic response to metformin in type 2

diabetes. Diabetes Obes Metab 2013; 15:189–91. https://doi.org/10.1111/j.1463-1326.2012.01691.x PMID: 22882994

42. Becker ML, Visser LE, Schaik RHN van, Hofman A, Uitterlinden AG, Stricker BHC. Genetic Variation in the Multidrug and Toxin Extrusion 1 Transporter Protein Influences the Glucose-Lowering Effect of Metformin in Patients With Diabetes: A Preliminary Study. Diabetes 2009; 58:745–9. https://doi.org/10.2337/db08-1028 PMID: 19228809