A Metformin Pharmacogenetic Study of Patients with Type 2 Diabetes Mellitus and SLC22A1 Gene Mutation

Elly Usman* C, Yusticia Katar

Department of Pharmacology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia

Abstract

AIM: The purpose of this study was to determine the profiles of patients with Type 2 diabetes mellitus (T2DM) and an SLC22A1 gene mutation to evaluate the effect of metformin pharmacogenetics.

METHODS: To assess the effect of pharmacogenetics, a mutation of the SLC22A1 gene in T2DM patients receiving metformin was investigated. Blood samples were taken from 50 diabetics of Minangkabau ethnicity who met the inclusion criteria and SNP genotyping and blood glucose levels were determined. DNA is extracted and purified from blood samples using DNAzol® Genomic DNA Kits (Thermofischer Scientific) reagents. The Chi-square test and independent sample T test were used to analyze the data. A statistically significant association was defined as p < 0.05. Finally, the GraphPad Prism 7.00 program was used to gather and analyze data.

RESULTS: The adjusted odds ratio for inadequate fasting blood glucose was 1.48 (95% confidence interval [CI] 1.18–1.95) in this study, while the adjusted odds ratio for diet discipline was 1.23 (95% CI 1.18–1.95). The adjusted odds ratio for low physical activity was 1.18 (95% CI 1.05–1.81). According to the sequencing data, the proportion of mutants is high at exon 2 rs683369 (G>C), while the percentage of wildtype and heterozygous mutants is the same at introns rs4646272 (T>G).

CONCLUSION: Obesity, diet discipline, and low physical activity were all found to increase the likelihood of insufficient fasting blood glucose in T2DM patients. Exon 2 rs683369 (G>C) has a high proportion of mutants, but introns rs4646272 (T>G) have the same percentage of wildtype and heterozygous mutants.

Introduction

Metformin, a medicine recommended for its relative absence of adverse effects and great patient tolerance, is the first line of the treatment for Type 2 diabetes mellitus (T2DM) [1], [2]. Metformin, on the other hand, does not operate similarly or ideally in all patients due to variances in individual genetic profiles, resulting in a decrease in the drug’s effectiveness and safety [3], [4]. As a result, determining the genetic component behind metformin response variability is critical, particularly in areas with a high prevalence of T2DM [5], [6]. The previous research has found that the genetically unique Arab, Chechen, and Circassian groups have varied clinical features of diabetes, necessitating specialized diabetes management and treatment strategies for each [7], [8].

Metformin works by lowering hepatic glucose synthesis while boosting glucose absorption in the peripheral tissues [9]. Metformin is unusual in that it does not need to be broken down by the body to effect blood glucose management [10], [11]. Metformin, on the other hand, requires membrane transport proteins produced by solute carrier (SLC) genes to enter cells and reduce hepatic glucose synthesis [12], [13]. The OCT1 and OCT3 proteins, which are predominantly important for hepatic and intestinal metformin absorption, are encoded by the SLC family 22 member 1 (SLC22A1) and 3 (SLC22A3) genes, respectively [14], [15]. Several single-nucleotide polymorphisms (SNPs) in the SLC22A1 gene have been shown to impact metformin pharmacodynamics and pharmacokinetics, and hence, patient responses to the medicine [16], [17].

Despite accounting for a significant amount of Jordan’s disease burden, there have been little investigations on T2DM's hereditary component and the impact of the latter on metformin response. The aim of this study was to determine the profiles of patients with T2DM and an SLC22A1 gene mutation to evaluate the effect of metformin pharmacogenetics.

Materials and Methods

Study design

To assess the influence of pharmacogenetics, profiles of patients with T2DM and SLC22A1 gene mutation were compared between patients using metformin. The Ethics Committee of the Faculty...
A - Basic Sciences Pharmacology

274

50 L for each reaction. DNA samples were amplified using DNAzol® Genomic DNA Kits (Thermofischer Scientific) reagents. The extraction of genomic DNA from whole blood is done according to the provider’s instructions. Chloroform is used to extract DNA from homogenates, resulting in aqueous, interphase, and organic layers. Furthermore, the DNA pellets were washed with 0.1 M sodium citrate in 10% ethanol and organic layer. Furthermore, the DNA pellets were washed with 0.1 M sodium citrate in 10% ethanol and organic layers. With the addition of 100% ethanol and DNA ladder separated the results. Amplicon DNA was extracted and produced in quantities up to 500 ng for sequencing using Illumina’s next generation sequencing technique.

Data analysis

Mean ± SD, median, and percentage were used to capture the quantitative data. The Chi-square test and the independent sample T test were used to analyze the data. Statistical significance was defined as a two-tailed p < 0.05. GraphPad Prism 7.00 was used to gather and analyze data.

Results

Profiles of patients with T2DM (Table 1).

Table 1: Profiles of patients with T2DM

| Variables | Adequate fasting blood glucose (n=25) (%) | Inadequate fasting blood glucose (n=25) | p-value |
|-----------|-----------------------------------------|----------------------------------------|---------|
| Sex       |                                         |                                        | 0.966*  |
| Male      | 7 (28.0)                                | 8 (32.0)                               |         |
| Female    | 18 (72.0)                               | 17 (68.0)                              |         |
| Age (Years) |                                        |                                        | 0.086*  |
| 19–29     | 0                                       | 1 (4.0)                                |         |
| 30–49     | 5 (20.0)                                | 16 (64.0)                              |         |
| 50–64     | 19 (76.0)                               | 17 (68.0)                              |         |
| ≥65       | 2 (8.0)                                 | 3 (12.0)                               |         |
| Ethnicity |                                         |                                        | 0.023** |
| Minangkese| 23 (92.0)                               | 21 (84.0)                              |         |
| Batak/kese| 1 (4.0)                                 | 2 (8.0)                                |         |
| Others    | 1 (4.0)                                 | 2 (8.0)                                |         |
| Occupational |                                       |                                        | 0.783*  |
| Working   | 21 (84.0)                               | 20 (80.0)                              |         |
| Not working|                                       |                                        |         |
| 4 (16.0) | 5 (20.0)                                |                                        |         |
| Physical activity |                                       |                                        | 0.048** |
| Low       | 13 (52.0)                               | 5 (20.0)                               |         |
| High      | 12 (48.0)                               | 20 (80.0)                              |         |
| Diet discipline |                                       |                                        | 0.041** |
| Not good  | 14 (56.0)                               | 11 (44.0)                              |         |
| Good      | 11 (44.0)                               | 14 (56.0)                              |         |
| Regularly check blood sugar |                                       |                                        | 0.871** |
| Regular   | 21 (84.0)                               | 20 (80.0)                              |         |
| Not regular|                                       |                                        |         |
| 4 (16.0) | 5 (20.0)                                |                                        |         |
| Age at diagnosis T2DM (years) | 49.93±8.30                             | 51.12±9.62                             | 0.531   |
| Basal Mass Index (BMI) (kg/m²) | 23.71±6.42                             | 25.42±5.20                             | 0.047** |

*p-value ≤ 0.05 is considered significant; *a, defined as fasting blood glucose level ≥ 126 mg/dL, according to the American diabetic association (ADA) guidelines; *b, Chi-square test; *c, Independent sample T test, T2DM: Type 2 Diabetes Mellitus.

Table 1 shows that ethnicity, physical activity, diet discipline, and body mass index are all associated with fasting blood glucose levels (p < 0.05). However, no significant relationship was seen between sex, age, occupation, and age at diagnosis of T2DM and fasting blood glucose (p > 0.05).

The unadjusted (univariate) and adjusted (multivariate) odds ratios and 95% CIs for inadequate fasting blood glucose (Table 2).

Table 2 shows that obesity was associated with a higher risk of low fasting blood glucose, with an
Table 2: The unadjusted (univariate) and adjusted (multivariate) odds ratios and 95% confidence intervals for inadequate fasting blood glucose

| Variables               | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|-------------------------|------------------------|----------------------|
| Sex                     |                        |                      |
| Male                    | 0.88 (0.34–2.25)       | 0.85 (0.29–2.01)     |
| Female                  | 0.92 (0.36–2.89)       | 0.89 (0.31–2.11)     |
| Age (Years)             |                        |                      |
| 19–29                   | Ref                    | Ref                  |
| 30–49                   | Ref                    | Ref                  |
| 50–64                   | 0.98 (0.31–3.22)       | 0.95 (0.29–3.15)     |
| ≥65                     | 0.93 (0.28–2.99)       | 0.90 (0.26–2.94)     |
| Ethnicity               |                        |                      |
| Minanginese             | 0.93 (0.27–1.43)       | 0.90 (0.21–1.32)     |
| Batakinese              | 0.88 (0.21–1.12)       | 0.79 (0.19–0.98)     |
| Others                  | 0.73 (0.19–1.09)       | 0.69 (0.17–0.88)     |
| Occupational            |                        |                      |
| Working                 | Ref                    | Ref                  |
| Not working             | 1.11 (0.37–3.32)       | 0.98 (0.32–3.01)     |
| Physical activity       |                        |                      |
| High                    | Ref                    | Ref                  |
| Low                     | 1.20 (1.11–1.89)*      | 1.18 (1.05–1.81)*    |
| Diet discipline         |                        |                      |
| Good                    | Ref                    | Ref                  |
| Not good                | 1.25 (1.16–1.91)*      | 1.23 (1.09–1.71)*    |
| Regularly check blood sugar |                  |                      |
| Regular                 | Ref                    | Ref                  |
| Not regular             | 0.71 (0.22–0.85)       | 0.68 (0.21–0.81)     |
| Age at diagnosis T2DM (years) |                |                      |
| <50                     | Ref                    | Ref                  |
| ≥50                     | 0.98 (0.34–0.93)       | 0.93 (0.32–0.89)     |
| Body Mass Index (BMI)   |                        |                      |
| Normal                  | Ref                    | Ref                  |
| Overweight              | 0.57 (0.37–1.92)       | 0.95 (0.31–1.88)     |
| Obesity                 | 1.01 (1.21–2.01)*      | 1.48 (1.18–1.95)*    |

Ref, reference; *p<0.05, significance was considered; OR, Odds ratios; CI, Confidence intervals. T2DM: Type 2 diabetes mellitus.

Table 3: Gene sequencing results in T2DM patients receiving metformin

| Exon/Intron | SNPs | Genotyped | f (%) |
|------------|------|-----------|-------|
| Exon 1     | rs200710420 (G>A) | GG        | 47    |
|            |       | GA        | 3     |
|            |       | AA        | 0     |
|            | rs1867351 (T>C)  | TT        | 28    |
|            |       | TC        | 17    |
|            |       | CC        | 5     |
| Intron     | rs4646272 (T>G)   | TT        | 21    |
|            |       | TG        | 21    |
|            |       | GG        | 8     |
|            | rs74795793 (T>C)  | TT        | 47    |
|            |       | TC        | 2     |
|            |       | CC        | 2     |
|            |       | GG        | 2     |
|            | rs201942835 (G>T) | TT        | 49    |
|            |       | GT        | 1     |
| Intron     | rs4646273 (G>A)   | GG        | 29    |
|            |       | GA        | 18    |
|            |       | AA        | 3     |

T2DM: Type 2 diabetes mellitus.

Table 3 shows that rs200710420 (G>A), which is present in Exon 1, has a greater GG (wildtype) genotype (94.0%) than mutants and no homozygous mutants. Wildtype (TT) was likewise shown to be greater (56.0%) than mutants in rs1867351 (T/C). Heterozygous (TC) mutants were detected in 34% of the cases, whereas homozygous mutants were found in 10.0% of the cases. There was rs4646272 (T>G) in the intron, with the same proportion of wildtype and heterozygous mutants (42.0%). Wildtype (TT) was reported to be greater (94.0%) than mutants in rs74795793 (T>C). Heterozygous mutants accounted for 4.0% of the total, whereas homozygous mutants accounted for 2.0%.

Exon 2 yielded a larger proportion of mutants than wildtype for rs683369 (G>C). Homozygous (CC) mutants accounted for 70.0% of the total, heterozygous (GC) mutants 26.0%, and wildtype 4.0%. Wildtype (GG) mutants were detected in more than 49.0% of rs201942835 (G>T) mutants, heterozygous mutants were found in 2.0%, and homozygous mutants were not found. Wildtype (GG) was detected in a larger percentage (58.0%) than mutants in the intron rs4646273 (G>A). Heterozygous (GA) mutants were detected in 36.0% of the cases, whereas homozygous (AA) mutants were found in 6.0% of the cases.

According to the sequencing data, the proportion of mutants is high at Exon 2 rs683369 (G>C), while the percentage of wildtype and heterozygous mutants is the same at introns rs4646272 (T>G).

**Discussion**

Recent breakthroughs in identifying common T2DM variations highlighted their association with the disease’s pathogenesis, which assists in the assessment of individual risk and treatment effectiveness [18], [19]. Despite its rising prevalence in Indonesia, T2DM has not been adequately investigated pharmacogenically in the Indonesian population. As a result, the present study is extremely important since it gives information on the relationship between metformin metabolism and Indonesian genetic profiles.

This study found that Exon 2 rs683369 (G>C) has a high proportion of mutants, but introns rs4646272 (T>G) have the same percentage of wildtype and heterozygous mutants. The extent to which T2DM-predisposing polymorphisms in the SLC22A1 genes are associated with good glycemic control was investigated in this study. The OCT proteins, which are organic cation transporters that play crucial roles in the regulation of essential metabolic processes, are encoded by the aforementioned genes, which are highly relevant to the field of drug transport [20].

This study revealed that obesity, diet discipline, and low physical activity were all found to increase the likelihood of insufficient fasting blood glucose in T2DM.
patients. Obesity is a cause of diabetes and insulin resistance. Adipose tissue releases more non-esterified fatty acids, glycerol, hormones, and pro-inflammatory cytokines in obese people, which might contribute to the development of insulin resistance [21, 22].

The significant rise in the incidence of diabetes in emerging nations is due to dietary choices and a sedentary lifestyle. Recently, increased HbA1c levels in Type 2 diabetics have been identified as one of the primary risk factors for microvascular and macrovascular problems. Diet management can help patients with their increased HbA1c levels, preventing them from acquiring diabetic complications [23].

The majority of the advantages of physical exercise for diabetes control come from changes in insulin action, which may be achieved with both aerobic and resistance training. Physical training advantages are reviewed, as well as advice for various activities, physical activity-related blood glucose control, diabetes prevention, gestational diabetes mellitus, and safe and effective techniques for physical activity with diabetes-related problems [24].

The genetic connection of these SNPs with fasting blood glucose levels in the management of diabetes is also influenced by the age at which diabetes is diagnosed. When treating diabetic patients, several covariate variables should be taken into account. It is also crucial to understand the influence of these variables on T2DM patients' genetic connections with fasting blood glucose levels. One possible weakness of the present study is that the length of the illness was not taken into account, and people who had the condition for a longer period may have had lower endogenous insulin production, implying that endogenous insulin levels were varied among the subjects.

This research suggests that increased awareness of diabetes complications leads to improvements in dietary knowledge and physical activity. In addition, maintaining a healthy body mass index helps to keep the condition under control. Stakeholders (health-care practitioners, health-care institutions, diabetes-care organizations, and so on) should assist patients to recognize the relevance of nutrition in disease management, adequate self-care, and improved quality of life.

**Conclusion**

Obesity, diet discipline, and low physical activity were all found to increase the likelihood of insufficient fasting blood glucose in T2DM patients. Exon 2 rs683369 (G>C) has a high proportion of mutants, but introns rs4646272 (T>G) have the same percentage of wildtype and heterozygous mutants.

**Acknowledgments**

The authors would like to thank Universitas Andalas for their grant research and all of the participants in this study.

**References**

1. Arimany-Nardi C, Koepsell H, Pastor-Anglada M. Role of SLC22A1 polymorphic variants in drug disposition, therapeutic responses, and drug-drug interactions. Pharmacogenomics J. 2015;15(6):473-87. https://doi.org/10.1038/tjp.2015.78 PMid:26526073
2. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: Association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics. 2005;115(3):e290-6. https://doi.org/10.1542/peds.2004-1808 PMid:15741354
3. Ministry of Health Republic of Indonesia. Basic Health Research. Ministry of Health Republic of Indonesia. Jakarta: Ministry of Health Republic of Indonesia; 2013.
4. Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease?: A meta-analysis of prospective studies. Arch Intern Med. 2004;164(19):2147-55. https://doi.org/10.1001/archinte.164.19.2147 PMid:15505129
5. Shu Y, Brown C, Castro R, Shi R, Lin E, Owen R, et al. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. Clin Pharmacol Ther. 2008;83(2):273-80. https://doi.org/10.1038/sj.cpt.6100275 PMid:17609683
6. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in Type 2 diabetes: A patient-centered approach position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care. 2012;35(6):1364-79. https://doi.org/10.2337/dc12-0413 PMid:22517736
7. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of Type 2 diabetes: Perspectives on the past, present, and future. Lancet. 2014;383(9922):1068-83. https://doi.org/10.1016/S0140-6736(13)62154-6 PMid:24315620
8. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. N Engl J Med. 1995;333(9):541-9. https://doi.org/10.1056/NEJM199508313330902 PMid:7623902
9. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BHC. Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus. Pharmacogenom J. 2009;9:242-7. https://doi.org/10.1007/s11528-009-0011-6 PMid:19381165
10. Ajlouni K, Khader YS, Batehah A, Ajlouni H, El-Khateeb M. An increase in prevalence of diabetes mellitus in Jordan over 10 years. J. Diabetes Complicat. 2009;23(5):317-24. https://doi.org/10.1016/j.jdiacomp.2007.01.004 PMid:18413210
11. Song I, Shin H, Shim E, Jung I, Kim W, Shon J, Shin J. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. Clin Pharmacol Ther. 2008;84:559-62.

12. Wang ZJ, Yin OQ, Tomlinson B, Chow MS. OCT2 polymorphisms and in-vivo renal functional consequences: Studies with metformin and cimetidine. Pharmacogenet Genom. 2008;18(7):637-45. doi:10.1097/FPC.0b013e328302cd41
PMid:18551044

13. Tzvetkov MV, Vormfelde SF, Balen D, Meineke I, Schmidt T, Sehrt D, et al. The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. Clin Pharmacol Ther. 2009;86(3):299-306. doi:10.1038/clpt.2009.92
PMid:19536068

14. Mahrooz A, Alizadeh A, Hashemi-Soteh MB, Ghaffari-Cherati M, Hosseyni-Tale SR. The polymorphic variants rs3088442 and rs2292334 in the organic cation transporter 3 (OCT3) gene and susceptibility against Type 2 diabetes: Role of their interaction. Arch Med Res. 2017;48(2):162-8. doi:10.1016/j.arcmed.2017.03.010
PMid:28625319

15. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop, L. The many faces of diabetes: A disease with increasing heterogeneity. Lancet. 2014;383(9922):1084-94. doi:10.1016/S0140-6736(13)62219-9
PMid:24315621

16. Karalliedde J, Gnudi L. Diabetes mellitus, a complex and heterogeneous disease, and the role of insulin resistance as a determinant of diabetic kidney disease. Nephrol Dial Transplant. 2014;31(2):fu405. doi:10.1093/ndt/gfu405
PMid:25550448

17. Lango H, Palmer CN, Morris AD, Zeggini E, Hattersley AT, McCarthy MI, et al. Assessing the combined impact of 18 common genetic variants of modest effect sizes on Type 2 diabetes risk. Diabetes. 2008;57(11):3129-35. doi:10.2337/db08-0504
PMid:18591388

18. Nigam SK. The SLC22 transporter family: A Paradigm for the impact of drug transporters on metabolic pathways, signaling, and disease. Annu Rev Pharmacol Toxicol. 2018;58:663-87. doi:10.1146/annurev-pharmtox-010617-052713
PMid:29309257

19. Pochini L, Galluccio M, Scalise M, Console L, Indiveri C. OCTN: A small transporter subfamily with great relevance to human pathophysiology, drug discovery, and diagnostics. SLAS Discov. 2019;24(2):89-110. doi:10.1177/2472555218812821
PMid:30523710

20. Djuc T, Zhou K, Yee S, van Leeuwen N, de Keyser C, Javorský M, et al. Variants in pharmacokinetic transporters and glycemic response to metformin: A metgen meta-analysis. Clin Pharmacol Ther. 2017;101(6):763-72. doi:10.1002/cpt.567
PMid:27859023

21. Al-Eitan LN, Amomani BA, Nassar AM, Elsaaqq BZ, Saadeh NA. Metformin pharmacogenetics: Effects of SLC22A1, SLC22A2, and SLC22A3 polymorphisms on glycemic control and Hba1c levels. J Pers Med. 2019;9(1):17. doi:10.3390/jpm9010017
PMid:30934600

22. Wondmkun YT. Obesity, insulin resistance, and Type 2 diabetes: Associations and therapeutic implications. Diabetes Metab Syndr Obes. 2020;13:3611-6. doi:10.2147/DMSO.S275898
PMid:33116712

23. Sami W, Ansari T, Butt NS, Hamid MR. Effect of diet on Type 2 diabetes mellitus: A review. Int J Health Sci (Qassim). 2017;11(2):65-71. doi:10.285386

24. Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, et al. Exercise and Type 2 diabetes: The American college of sports medicine and the American diabetes association: Joint position statement. Diabetes Care. 2010;33(12):e147-67. doi:10.2337/dc10-9990
PMid:21115758