Kronologis Proses Pemasukan (Submission) Artikel hingga Terbit (Published)

Judul artikel	:	A Metformin Pharmacogenetic Study of Patients with Type 2 Diabetes Mellitus and SLC22A1 Gene Mutation
Jurnal	:	Open Access Macedonian Journal of Medical Sciences
SJR	:	0,29 (Quartil Q3 sejak 2017 bidang medicine hingga sekarang) https://www.scimagojr.com/journalsearch.php?q=21100824403&tip=sid&clean=0
Submitted	:	01 Januari 2022
Review report	:	05 Feburari 2022
Revise version	:	06 Februari 2022
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Article in press	:	27 Februari 2022
Published	:	01 Maret 2022
Similarity index	:	7% (link hasil pemeriksaan turnitin https://drive.google.com/file/d/1IXqQWq_qL6nakXd09XbQn0b3xUJ_VLxq/view?usp=sharing)

Kronologis sebagai berikut:

1. **Manuskrip di submit pada tanggal 01 Januari 2022 dan mendapatkan email pemberitahuan dari editor Open Access Macedonia Journal of Medical Sciences bahwa artikel sudah dikirim (submitted) (*Gambar 1*). Original paper terlampir (*Lampiran A*)**

[OAMJMS] Submission Acknowledgement

P

Prof. Dr Mirko Spiroski via SFS - Journals (Scientific Foundation SPIROSKI - Journals), Skopje, Republic of Macedonia <noreply@publicknowledgeproject.org>
Sun 1/2/2022 11:27 AM
To: Elly Usman

Elly Usman:

Thank you for submitting the manuscript, "A Metformin Pharmacogenetic Study of Patients with Type 2 Diabetes Mellitus and SLC22A1 Gene Mutation: Patients with Type 2 Diabetes Mellitus and a Mutation in the SLC22A1 Gene" to Open Access Macedonian Journal of Medical Sciences. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL:
https://oamjms.eu/index.php/mjms/authorDashboard/submission/8473

Gambar 1. Pemberitahuan editor bahwa manuskrip submitted

2. **Email pemberitahuan oleh editor terkait hasil review dari 2 orang reviewer pada tanggal 05 Februari 2022**

[OAMJMS] Editor Decision

M

Mirko Zhivko Spiroski via SFS - Journals (Scientific Foundation SPIROSKI - Journals), Skopje, Republic of Macedonia <noreply@publicknowledgeproject.org>
Sat 2/5/2022 11:01 PM
To: Elly Usman

Elly Usman (Author):

We have reached a decision regarding your submission to Open Access Macedonian Journal of Medical Sciences, "A Metformin Pharmacogenetic Study of Patients with Type 2 Diabetes Mellitus and SLC22A1 Gene Mutation: Patients with Type 2 Diabetes Mellitus and a Mutation in the SLC22A1 Gene", Manuscript ID = OJS8473.

Our decision is: Revise your manuscript until February 25, 2022 and submit on the OAMJMS website.

Reviewer A:

...

It is an important article, but it should be improved.
Here are my comments:
1. Add novelty in the last section of the introduction
2. Add further analysis the unadjusted and adjusted odds ratio based on inadequate fasting blood glucose

Reviewer B:
Recommendation: Revisions Required

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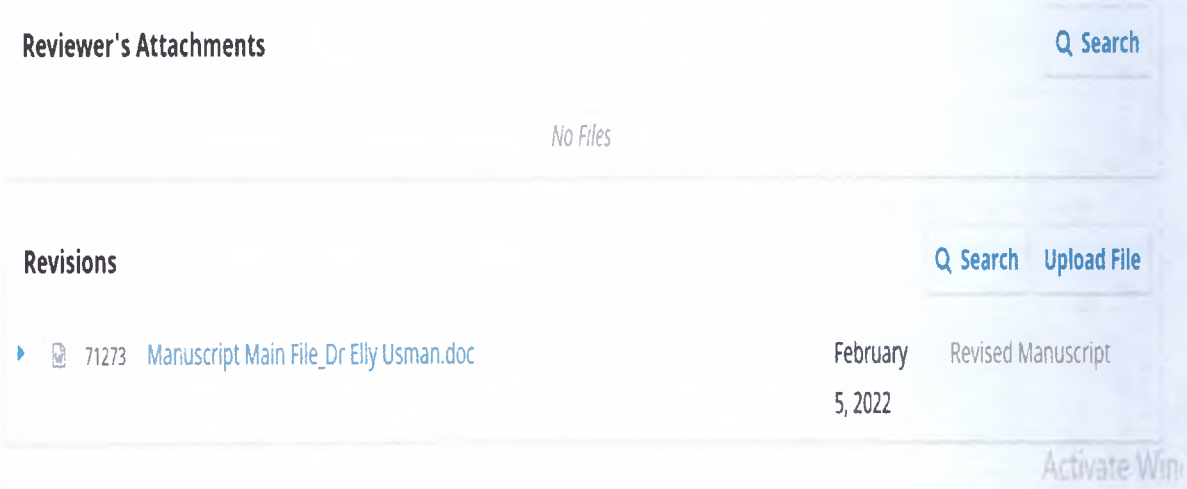
1. Please elaborate more authors analysis on the discussion based the recent related publications.
2. Add research suggestion in the last paragraph of discussion section

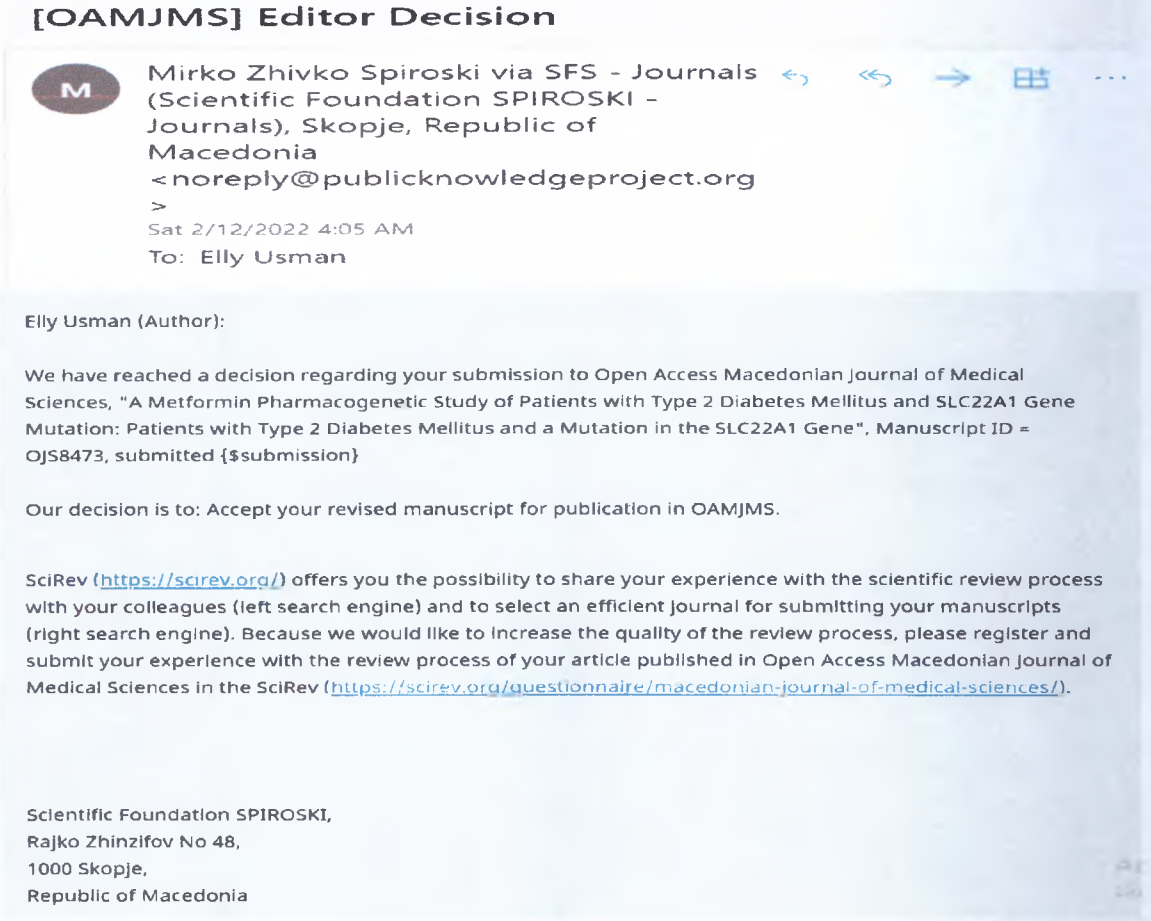
Gambar 2. Pemberitahuan editor terkait hasil review dari 2 orang reviewer

3. **Author mengirimkan perbaikan manuskrip berdasarkan hasil review dari reviewer dan melakukan resubmission ke jurnal system pada tanggal 6 Februari 2022. Perubahan dan penambahan pada manuskrip penelitian berdasarkan hasil review ditandai dengan tulisan bewarna highlight kuning (*Lampiran B*).**



Gambar 3. Penulis mengirimkan hasil revisi berdasarkan review oleh reviewer

4. **Pemberitahuan editor bahwa manuskrip diterima setelah dilakukan revisi pada tanggal 12 Feburari 2022**



Gambar 4. Pemberitahuan editor bahwa manuskrip diterima setelah dilakukan revisi

5. Pemberitahuan editor Proofreading Request pada tanggal 15 Feburari 2022

[OAMJMS] Proofreading Request (Author)

From: Mirko Zhivko Spiroski via SFS - Journals (Scientific Foundation SPIROSKI - Journals), Skopje, Republic of Macedonia <noreply@publicknowledgeproject.org>
Sent: Saturday, February 15, 2022 4:06 AM
To: Elly Usman <ellyusman@med.unand.ac.id>
Subject: [OAMJMS] Proofreading Request (Author)

Dear Elly Usman:

Your submission "A Metformin Pharmacogenetic Study of Patients with Type 2 Diabetes Mellitus and SLC22A1 Gene Mutation: Patients with Type 2 Diabetes Mellitus and a Mutation in the SLC22A1 Gene" to Open Access Macedonian Journal of Medical Sciences now needs to be proofread by folllowing these steps.

1. Click on the Submission URL below.
2. Log into the journal and view PROOFING INSTRUCTIONS
3. Click on VIEW PROOF in Layout and proof the galley in the one or more formats used.
4. Enter corrections (typographical and format) in Proofreading Corrections using "Mark up text with edits" or "Use annotation and drawing markup tools to add comments in PDFs".
5. Save corrected Galley Proof with the addition "Corrected" to the file name (i.e., OAMJMS-9A-4528.pdf should be saved as OAMJMS-9A-4528-Corrected.pdf).

Gambar 5. Pemberitahuan editor proofreading request

6. **Author langsung mengirimkan proofreading ke journal system pada tanggal 15 Feburari 2022**

OAMJMS_10A-8473_20220211_V0.pdf OAMJMS_Copyright.docx

Dear editor Open Access Macedonian Journal of Medical Sciences

Here I attached a file of my Corrected Galley Proof oamjms.2021.8473.

I hope this manuscript could be published soon. Thank you for your kind help.

Best regards,

Dr. Elly Usman

oamjms-10a-8473-20220211-v0-Corrected.pdf

oamjms-copyright-signed.docx

Gambar 6. Author mengirimkan proofreading ke journal system

7. **Pemberitahuan editor bahwa artikel sudah diterbitkan pada jurnal Open Access Macedonian Journal of Medical Sciences. 2022 Feb 16; 10(A):273-277.**

[OAMJMS] Your Article was Published ×

Participants

MSc, Eng Ivo Spiroski (ivos)

Elly Usman (elly)

Messages

Note From

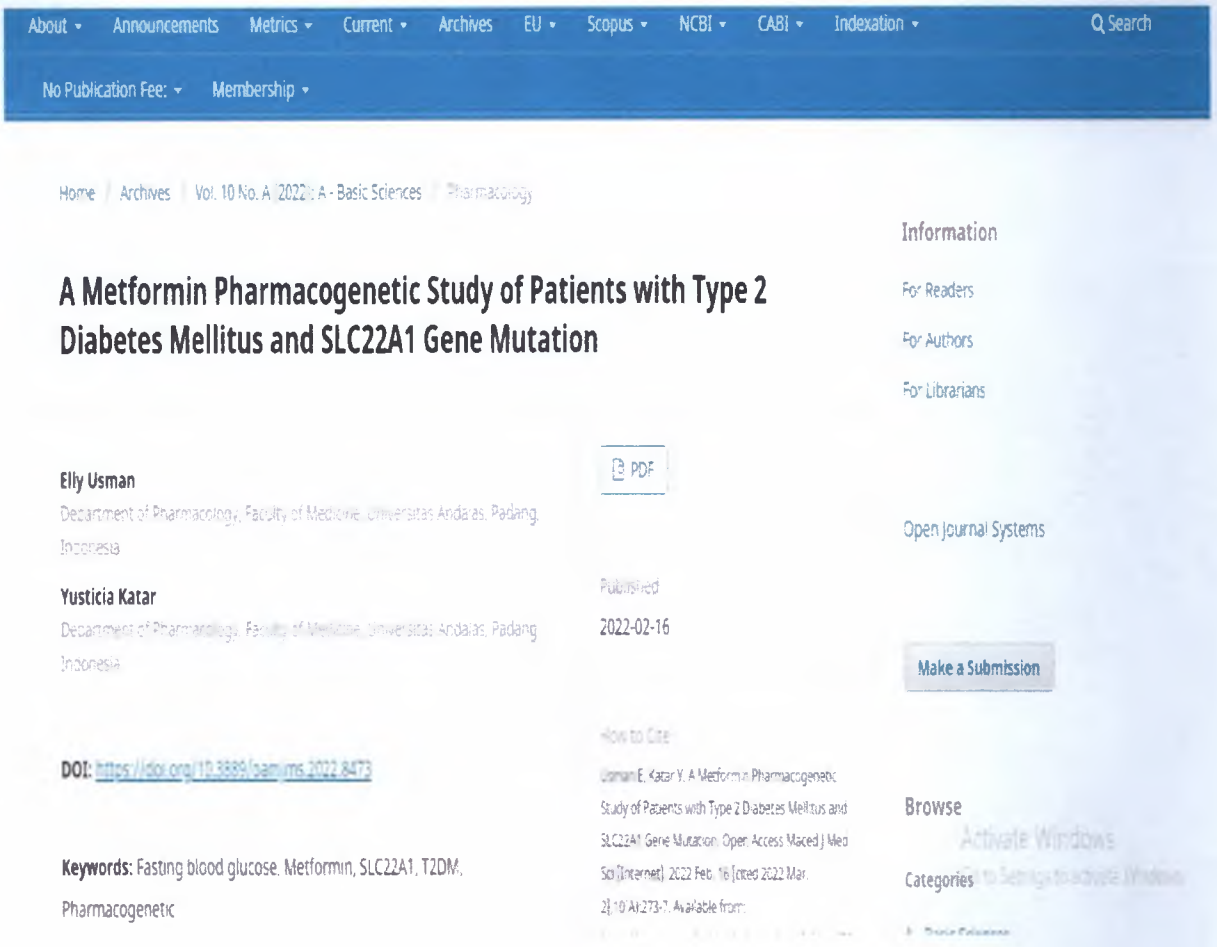
Dear Elly Usman, ivos 2022-02-27 04:28 AM

Please note that your paper "A Metformin Pharmacogenetic Study of Patients with Type 2 Diabetes Mellitus and SLC22A1 Gene Mutation", was published in Open Access Maced J Med Sci (OAMJMS).

DOI: https://doi.org/10.3889/oamjms.2022.8473

Username: Elly Usman

Thank you for your fine contribution. On behalf of the Editors of the Open Access Macedonian Journal of Medical Sciences, we look forward to your continued contributions to the Journal.



Gambar 7. Pemberitahuan editor bahwa artikel sudah diterbitkan

LAMPIRAN A

PAPER DENGAN VERSI PERTAMA KALI DIKIRIM (*ORIGINAL VERSION*)

1. PAPER DENGAN VERSI PERTAMA KALI DIKIRIM (*ORIGINAL VERSION*)

Research article

Manuscript Title:

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

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Running title

Patients with Type 2 Diabetes Mellitus and a Mutation in the SLC22A1 Gene

Abstract

Background: The purpose of this study was to determine the profiles of patients with type 2 diabetes (T2DM) and an SLC22A1 gene mutation in order 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 a p-value < 0.05. Finally, the GraphPad Prism 7.00 program was used to gather and analyze data.

Results: The results of this study found that ethnicity, physical activity, diet discipline, and BMI 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 ($p>0.05$). 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.

Keywords: Fasting Blood Glucose, Metformin, SLC22A1, T2DM, Pharmacogenetic

Introduction

Metformin, a medicine recommended for its relative absence of adverse effects and great patient tolerance, is the first line of 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]. 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 in order to effect blood glucose management [10], [11]. Metformin, on the other hand, requires membrane transport proteins produced by solute carrier (SLC) genes in order 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 solute carrier 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 response to the medicine [16], [17].

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 of Medicine, Universitas Andalas, Padang, West Sumatera, Indonesia, gave the study ethical permission (No: 95/KEP/FK/2018). The Declaration of Helsinki was followed in all of the procedures used in this study. All recruited individuals provided written informed permission after being told of the study's aim and assured of patient confidentiality.

Patients recruitment

Patients with T2DM in Padang, West Sumatra, Indonesia. The population sampled satisfies the following criteria: age 25-50 years, fasting blood sugar levels less than 100 mg/dL, and blood sugar levels two hours after providing glucose 75 grams less than 140 mg/dL. If a sample's liver or kidney function is affected, it will be discarded. A total of 5 mL of blood is collected from the sample and kept at -80°C until needed.

Data collection

Direct interviews were used to obtain information in Padang City in 2018. Clinical data was gathered using a study-specific questionnaire that was filled out by healthcare professionals interviewing the individuals. Demographic information was obtained as part of the clinical data. During the interview, a phlebotomist took blood samples to determine fasting blood glucose levels using ethylenediamine tetraacetic acid (EDTA) collection tubes.

Isolation and Purification of Genomic DNA

DNA is extracted and purified from blood samples 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. With the addition of 100% ethanol and Trizol reagent, DNA was deposited from the interphase and organic layer. Furthermore, the DNA pellets were washed with 0.1 M sodium citrate in 10% ethanol and 75% ethanol in a sequential order. After resensing the dry pellets in 8 mM NaOH, DNA can be kept at -20°C in HEPES buffer pH 7-8 with 1 mM EDTA.

PCR and SLC22A1 gene sequencing

Primer Blast (NCBI) software was used to create PCR primers and sequencing. HPLC was used to purify the produced primer. The PCR technique was used to reproduce the DNA fragments. The PCR procedure was carried out with the Gotaq TM PCR Core System kit (Promega) and a total volume of 50 L for each reaction. DNA samples were amplified for 35 cycles, and the amplicon was kept at 4°C once the procedure was done. Electrophoresis of the application's results in a 2% agarose gel with Gelred 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-value < 0.05. GraphPad Prism 7.00 was used to gather and analyze data.

Results

Profiles of patients with type 2 diabetes mellitus (Table 1).

Table 1. Profiles of patients with T2DM

Variables	Adequate Fasting Blood Glucose (n=25)	Inadequate Fasting Blood Glucose[a] (n=25)	p-value
Sex			0.986[b]
Male	7 (28.0%)	8 (32.0%)	
Female	18 (72.0%)	17 (68.0%)	
Age (Years)			0.086[b]
19-29	0	1 (4.0%)	
30-49	5 (20.0%)	4 (16.0%)	
50-64	19 (76.0%)	17 (68.0%)	
≥65	2 (8.0%)	3 (12.0%)	
Ethinicity			0.023*[b]
Minangnese	23 (92.0%)	21 (84.0%)	
Bataknese	1 (4.0%)	2 (8.0%)	

Others	1 (4.0%)	2 (8.0%)	
Occupational			0.783[b]
Working	21 (84.0%)	20 (80.0%)	
Not working	4 (16.0%)	5 (20.0%)	
Physical activity			0.048*[b]
Low	13 (52.0%)	5 (20.0%)	
High	12 (48.0%)	20 (80.0%	
Diet discipline			
Not good	14 (56.0%)	11 (44.0%)	0.041*[b]
Good	11 (44.0%)	14 (56.0%)	
Regularly check blood sugar			
Regular	21 (84.0%)	20 (80.0%)	0.871*[b]
Not regular	4 (16.0%)	5 (20.0%)	
Age at diagnosis T2DM (years)	49.93±8.30	51.12±9.62	0.531[c]
Body Mass Index (BMI) (kg/m^2)	23.71±6.42	25.42±5.20	0.047*[c]

*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.

Table 1 shows that ethnicity, physical activity, diet discipline, and BMI 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 ($p>0.05$).

Gene sequencing results in T2DM patients receiving metformin (Table 2).

Table 2. Gene sequencing results in T2DM patients receiving metformin

Exon/ Intron	SNPs	Genotyped	f	%
Exon 1	rs200710420 (G>A)	GG	47	94.0
		GA	3	6.0
		AA	0	0
	rs1867351 (T/C)	TT	28	56.0
		TC	17	34.0
		CC	5	10.0
Intron	rs4646272 (T>G)	TT	21	42.0
		TG	21	42.0
		GG	8	16.0
	rs74795793 (T>C)	TT	47	94.0
		TC	2	4.0
		CC	1	2.0
Exon 2	rs683369 (G>C)	GG	2	4.0
		GC	13	26.0
		CC	35	70.0
	rs201942835 (G>T)	GG	49	98.0

		GT	1	2.0
		TT	0	0
Intron	rs4646273 (G>A)	GG	29	58.0
		GA	18	36.0
		AA	3	6.0

Table 2 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 current study is extremely important since it gives information on the relationship between metformin metabolism and Indonesian genetic profiles.

This study found 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 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].

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.

Acknowledgements

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

Conflict of interest statement

There were no potential conflicts of interest stated by the authors.

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2. PAPER DENGAN VERSI KEDUA DIKIRIM [*REVISIED MANUSCRIPT VERSION*]

Research article

Manuscript Title:

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

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Affiliations:

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Running title

Patients with Type 2 Diabetes Mellitus and a Mutation in the SLC22A1 Gene

Abstract

Background: The purpose of this study was to determine the profiles of patients with type 2 diabetes (T2DM) and an SLC22A1 gene mutation in order 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 a p-value < 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% 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.

Keywords: Fasting Blood Glucose, Metformin, SLC22A1, T2DM, Pharmacogenetic

Introduction

Metformin, a medicine recommended for its relative absence of adverse effects and great patient tolerance, is the first line of 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]. 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 in order to effect blood glucose management [10], [11]. Metformin, on the other hand, requires membrane transport proteins produced by solute carrier (SLC) genes in order 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 solute carrier 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 response 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 type 2 diabetes (T2DM) and an SLC22A1 gene mutation in order 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 of Medicine, Universitas Andalas, Padang, West Sumatera, Indonesia, gave the study ethical permission (No: 95/KEP/FK/2018). The Declaration of Helsinki was followed in all of the procedures used in this study. All recruited individuals provided written informed permission after being told of the study's aim and assured of patient confidentiality.

Patients recruitment

Patients with T2DM in Padang, West Sumatra, Indonesia. The population sampled satisfies the following criteria: age 25-50 years, fasting blood sugar levels less than 100 mg/dL, and blood sugar levels two hours after providing glucose 75 grams less than 140 mg/dL. If a sample's liver or kidney function is affected, it will be discarded. A total of 5 mL of blood is collected from the sample and kept at -80°C until needed.

Data collection

Direct interviews were used to obtain information in Padang City in 2018. Clinical data was gathered using a study-specific questionnaire that was filled out by healthcare professionals interviewing the individuals. Demographic information was obtained as part of the clinical data. During the interview, a phlebotomist took blood samples to determine fasting blood glucose levels using ethylenediamine tetraacetic acid (EDTA) collection tubes.

Isolation and Purification of Genomic DNA

DNA is extracted and purified from blood samples 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. With the addition of 100%

ethanol and Trizol reagent, DNA was deposited from the interphase and organic layer. Furthermore, the DNA pellets were washed with 0.1 M sodium citrate in 10% ethanol and 75% ethanol in a sequential order. After resensing the dry pellets in 8 mM NaOH, DNA can be kept at -20°C in HEPES buffer pH 7-8 with 1 mM EDTA.

PCR and SLC22A1 gene sequencing

Primer Blast (NCBI) software was used to create PCR primers and sequencing. HPLC was used to purify the produced primer. The PCR technique was used to reproduce the DNA fragments. The PCR procedure was carried out with the Gotaq TM PCR Core System kit (Promega) and a total volume of 50 L for each reaction. DNA samples were amplified for 35 cycles, and the amplicon was kept at 4°C once the procedure was done. Electrophoresis of the application's results in a 2% agarose gel with Gelred 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-value < 0.05. GraphPad Prism 7.00 was used to gather and analyze data.

Results

Profiles of patients with type 2 diabetes mellitus (Table 1).

Table 1. Profiles of patients with T2DM

Variables	Adequate Fasting Blood Glucose (n=25)	Inadequate Fasting Blood Glucose[a] (n=25)	p-value
Sex			0.986[b]
Male	7 (28.0%)	8 (32.0%)	
Female	18 (72.0%)	17 (68.0%)	
Age (Years)			0.086[b]
19-29	0	1 (4.0%)	
30-49	5 (20.0%)	4 (16.0%)	
50-64	19 (76.0%)	17 (68.0%)	
≥65	2 (8.0%)	3 (12.0%)	
Ethinicity			0.023*[b]
Minangnese	23 (92.0%)	21 (84.0%)	
Bataknese	1 (4.0%)	2 (8.0%)	
Others	1 (4.0%)	2 (8.0%)	
Occupational			0.783[b]
Working	21 (84.0%)	20 (80.0%)	
Not working	4 (16.0%)	5 (20.0%)	
Physical activity			0.048*[b]
Low	13 (52.0%)	5 (20.0%)	
High	12 (48.0%)	20 (80.0%	
Diet discipline			
Not good	14 (56.0%)	11 (44.0%)	0.041*[b]
Good	11 (44.0%)	14 (56.0%)	
Regularly check blood sugar			
Regular	21 (84.0%)	20 (80.0%)	0.871*[b]
Not regular	4 (16.0%)	5 (20.0%)	
Age at diagnosis T2DM (years)	49.93±8.30	51.12±9.62	0.531[c]
Body Mass Index (BMI) (kg/m^2)	23.71±6.42	25.42±5.20	0.047*[c]

*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.

Table 1 shows that ethnicity, physical activity, diet discipline, and BMI 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% confidence intervals for inadequate fasting blood glucose (Table 2).

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)	0.89 (0.26-2.94)
Ethinicity		
Minangnese	0.93 (0.27-1.43)	0.90 (0.21-1.32)
Bataknese	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	0.97 (0.37-1.92)	0.95 (0.31-1.88)
Obesity	1.51 (1.21-2.01)*	1.48 (1.18-1.95)*

Ref, reference; *p<0,05, significance was considered

Table 2 shows that obesity was associated with a higher risk of low fasting blood glucose, with an unadjusted odds ratio of 1.51 (95% CI 1.21–2.01) and an adjusted odds ratio of 1.48 (95% CI 1.18-1.95). Diet discipline was also significantly risk of inadequate fasting blood glucose the unadjusted odds ratio was 1.25 (95% CI 1.16–1.91) and the adjusted odds ratio

was 1.23 (95% CI 1.09-1.71). Low physical activity the unadjusted odds ratio was 1.20 (95% CI 1.11–1.89) and the adjusted odds ratio was 1.18 (95% CI 1.05-1.81).

Gene sequencing results in T2DM patients receiving metformin (Table 3).

Table 3. Gene sequencing results in T2DM patients receiving metformin

Exon/ Intron	SNPs	Genotyped	f	%
Exon 1	rs200710420 (G>A)	GG	47	94.0
		GA	3	6.0
		AA	0	0
	rs1867351 (T/C)	TT	28	56.0
		TC	17	34.0
		CC	5	10.0
Intron	rs4646272 (T>G)	TT	21	42.0
		TG	21	42.0
		GG	8	16.0
	rs74795793 (T>C)	TT	47	94.0
		TC	2	4.0
		CC	1	2.0
Exon 2	rs683369 (G>C)	GG	2	4.0
		GC	13	26.0
		CC	35	70.0
	rs201942835 (G>T)	GG	49	98.0
		GT	1	2.0
		TT	0	0
Intron	rs4646273 (G>A)	GG	29	58.0
		GA	18	36.0
		AA	3	6.0

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 current study is extremely important since it gives information on the relationship between metformin metabolism and Indonesian genetic profiles.

This study found 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 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's also crucial to understand the influence of these variables on T2DM patients' genetic connections with fasting blood glucose levels. One possible weakness of the current 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.

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Conflict of interest statement

There were no potential conflicts of interest stated by the authors.

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