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The effect of the Organic Cation Transporters1 (OCT1) gene polymorphism on the therapeutic efficacy of metformin in type 2 diabetic patients in Basrah/ Southern Iraq

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Abstract

Metformin does not undergo metabolism inside the body and is excreted in the urine unchanged. The organic cation transporter1 (OCT1) is one of the most numerous hepatic transporters and has an essential function in the hepatic transport of metformin. The study objectives are to research the prevalence of two (single nucleotide polymorphism) SNP [rs12208357] and [rs72552763] of (OCT1) of SLC22A1 gene in type 2 diabetes mellitus (T2DM) patients in (Basrah city)/South of Iraq and to investigate the association between these SNPs and metformin efficacy. It is a prospective cohort study. The study involved one hundred and two adult patients recruited from two large tertiary care centers in Basrah city. All the patients received immediate-release metformin tablets 1g twice daily for three months. Laboratory data included (HbA1C) and fasting blood glucose (FBG) initially and later after three months of the study. Metformin responders are patients whose HbA1c values are reduced by ≥1% after 90 days of metformin monotherapy. All enrolled patients were genotyped for two of the most prevalent SNPs in the OCT1 gene by using ARMS- PCR genotyping assays. Inclusion criteria include: newly diagnosed (drug naïve) T2DM patients with HbA1C range (6.5-9.9), ages ranging from 25 and 75 years old, and signed consent from all the participants. This study had 33 non-responders, and the reduction in FBG and HbA1C levels in the responder's group was significant (p-value < 0.05) after 90 days of treatment. The patients with homozygous genotype (CC) of rs12208357 and (AA) of rs72552763 gene polymorphism were characterized by good therapeutic efficacy of metformin.

Keywords

Type 2 diabetes mellitus, Metformin, SNP, rs12208357/R61C, rs72552763/Met420del.

List of abbreviations

Δ%: first measure minus second measure
ADEMC: Almawani Specialized Diabetes, Endocrine, and Metabolism Center
AMPK: Activated protein kinase, complex 1, respiratory-chain complex 1
ARMS-PCR: The Amplification Refractory Mutation System PCR
Bp: base pair
Del: deletion

DNA: deoxyribonucleic acid
dsDNA: Double-strand DNA
EDTA: Ethylene diamine tetraacetic acid
FBG: fasting blood glucose
FDEMC: Faiha Specialized Diabetes, Endocrine, and Metabolism Center
F: forward
GC: columns Gas chromatography columns
G.I.: Gastrointestinal
GoDARTs: The Genetics of Diabetes Audit and Research Tayside
GSB: Gel Sample Buffer
G465R: Glycin 465 arginine
G401S: Glycin 401 serine
HbA1c: Glycated Hemoglobin (Hb) A1C
Hb: Hemoglobin
IRB: Institutional review board
M420del: The methionine420 deletion
NCBI: The National Center for Biotechnology Information
OCT1: Organic cation transporters
R61C: Arginin 61 cysteine
R: reverse
Rs: number Reference SNP
SD: Standard deviations
SLC22A1: Solute Carrier Family 22 Member 1
SNP: Single Nucleotide Polymorphism
SPSS: Statistical Package for the Social Sciences
TAE: tetra ethyl ammonium
TBE: Tris-borate-EDTA
T2DM: type 2 diabetes mellitus
USA: the United States of America

Introduction

Metformin is the first oral glucose-lowering treatment for T2DM [1]. It incorporates safety, cheapness, and effectiveness [2]. The main mechanism of action is to decrease hepatic glucose production, although the precise pharmacological action has not yet been completely understood [3]. It decreases hepatic gluconeogenesis and promotes insulin sensitivity by increasing peripheral glucose uptake and use, reducing basal and postprandial plasma glucose [4].

Metformin is not metabolized in vivo and is excreted unchanged in urine [5]. The passive diffusion of metformin through the cell membranes is limited [6]. The distribution, transmission to the target tissues, and later excretion of metformin from the body are carried out by organic cation transporters (OCTs) [4]. OCT1 is one of the numerous transporters in the liver and has an important role in the hepatic uptake of metformin [7]. In humans, OCT1 is denoted by the SLC22A1 gene which is present at chromosome 6q25.3 [8]. SLC22A1gene is highly polymorphic, and many single nucleotide polymorphisms (SNPs) have been reported to be associated with metformin activity. This might explain some of the inter-individual variability in metformin efficacy. [9].

WANG and his colleagues found in a study that the existence of one of these four SNPs; (R61C/rs12208357, G401S/rs34130495, M420del/rs72552763, and /or G465R/rs34059508) reduces the therapeutic effects of metformin [10]. A study found that the mice without the organic cation transporter 1 (OCT1) have few or no metformin in the liver [11]. In a study of 138 types 2 diabetic patients randomized to metformin 1000 mg/day for four weeks duration and, after that, increased dose to 2000 mg/day, reduced-function haplotypes consist of the alleles (R61C, G401S, G465R, and M420del) in OCT1 were accompanied with a significant decrease in metformin steady-state concentration and a reduced absolute decrease in HbA1c [12].

On the other hand, Zhou et al., in one of the largest studies to research OCT1 variants and response to metformin (GoDARTs). The researchers studied the two most popular OCT1 SNPs; R61C/rs12208357, and Met420del/ rs72552763], in more than one thousand and half T2DM patients who received metformin. They observed that there was no influence on metformin response [13]. So in our study, we want to investigate the reason for this variation in the effect of OCT1 polymorphism on the therapeutic efficacy of metformin among studies and to show if this genetic polymorphism present in Iraqi patients and does it affect the therapeutic efficacy of metformin in T2DM.

Objectives of the study

The objectives of this prospective population-based cohort study are

1 To study the prevalence of SNP ŧR61C/rs12208357] and [Met420del/rs72552763] of OCT1 (SLC22A1 gene) in T2DM patients in Basra/Southern Iraq.
2 To investigate the association between these SNPs and metformin efficacy in T2DM patients.

Patients And Methods

The present study is a prospective study. The study was done on one hundred and two adult patients (men and women) with T2DM recruited from two large tertiary care centers; Al-Mawani Specialized Diabetes, Endocrine, and Metabolism Center, and Faiha Center (FDEMC) in Basrah City, South of Iraq from January 2022 to December 2022. All recruited patients received immediate-

release metformin tablets available in government primary, secondary, and tertiary care clinics, including our institutions where the study was conducted. (In slow titration) to ensure patient compliance as follows: 500mg tablet once a day for (7) days, (500mg) tablet two times a day for (7) days, and (1g) two times a day for three months. The flow diagram of the patients is explained in Figure (1).
Figure (1) flow diagram of the patients

Ethical approval

The institutional review board (IRB) of the Ethical Committee at (Al Nahrain College of Medicine) approved the study design at Al Nahrain University/ Baghdad/ Iraq, number:20211030, dated 16/12/2021. And each patient gave written informed consent.

Inclusion criteria

Newly diagnosed (drug naпve) T2DM patients with HbA1C range (6.5-9.9), 2. Ages ranged from 25 and 75 years old, male and female, 3. They signed informed consent.

Exclusion criteria

Patients who are currently on any glucose-lowering agents, 2. Patients with chronic kidney disease, liver cirrhosis, pregnancy, endocrine disorders, malignancies, or systemic inflammatory diseases.

Data collection

Data was immediately taken from the patients during interviews with them. A questionnaire was designed to register patient information (Appendix 1), which included name, age (years), sex, family history of DM, drugs used and doses, any microvascular complication, blood pressure, ethnicity, Smoking history, make sure that the patients were drug naive and did not take any medication to treat diabetes before and while using metformin.

Laboratory data were assessed, including glycosylated Hb (HbA1C) and fasting blood glucose (FBG). All the above parameters were assessed initially, before any intervention, and after three months of the study.

Blood Sampling

After approval by the Institutional Review Board of Al-Nahrain Medical College, blood samples were collected from recruited patients at zero time and after three months of treatment with metformin monotherapy. 1 ml of venous blood was withdrawn from all patients in this study. 2 ml was put in EDTA- tube for genetic testing. Three ml of whole blood is used with an anticoagulant tube for HbA1c analysis at zero time and after three months of the study.

Genetic analysis and primer design

Polymerase Chain Reaction (PCR) was accomplished using pairs of primers specifically prepared for the (SLC22A1) gene. Using the database of the national center for biotechnology information, and Primer Three software. The sequences of these primers (forward 'and reverse) with their product size are mentioned below:

The primers sequences [Forward (F) and Reveres (R)] that were used for the amplification analysis of the OCT1 (SLC22A1) gene for two SNPs identifications and allele detection were designed at AL Bayan private lab in Basrah. SNP1 (rs72552763) (M420del) Axon 7 ----- A/-, M420del-F (A): GGGCA GCCTG CCT CGCCA,
M420del-F (del): GGGCAGCCTGCCTCGCCT,
M420del-R: GCCTGAGGGAGGCTTTGGAG.
Product size: 645 BP (base pair).

SNP2 (rs12208357) (R61C) Axon 1 ----- C/T, R61C-F(C): GGTGGC TGAGCTGAGCCGGC, R61C-F (T): GGTGGCTGAGCTGAGCCGGT, R61C-R:
ACACAGCCAGACACCCACGA. Product size: 385 BP (base pair).

Main genetic polymorphisms of the SLC22A1 gene

Gene	Axon	Rs number	Nucleotide position and variation	Amino acid position	Amino acid variation
SLC22A1	Axon 1	rs12208357	181C > T	61	Arg → Cys
	Axon 7	rs72552763	1258A > del	420	Met → del

Extraction of the DNA

DNA Genome was extracted from the blood sample using the Geneaid DNA extraction protocol. The principles of DNA Extraction are carried out in 4 stages, including lyses, binding, washing, and elution.

Quantitation of DNA

The concentration of extracted DNA was detected and recognized using Quantus Fluorometer and measured by QuantiFluor®ONE dsDNA System kit.

Polymerase Chain Reaction (PCR) program

1. (PCR) Thermocycler program for (SLC22A1) gene (Met420del) SNP:

Stages	Temperature(°C)	minute: second	Cycles
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	52	00:30	
Extension	72	00:30	
Final extension	72	05:00	1
Hold	4	∞	∞

2. (PCR) Thermocycler Program for (SLC22A1) gene for (R61C) SNP:

Stages	Temperature(°C)	minute: second	Cycles
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	62	00:30	
Extension	72	00:30	
Final extension	72	05:00	1
Hold	4	∞	∞

Agarose Gel Electrophoresis

We used agarose gel electrophoresis to assure the existence of PCR amplification. Solutions used include TBE Buffer (Tris-borate-EDTA) (10X), DNA ladder marker, and Ethidium bromide (10 mg/ml).

The casting of the agarose gel:

The solution of the agarose flowed into the plate and let the gel solidify at room temperature for 30 minutes. The tank was topped up with 1X TBE buffer until the buffer extended 5 mm above the gel surface.

PCR products:

10µl of PCR products and a (100 BP) ladder were immediately loaded into the well. Electrical power was 100 volts for 40 min. DNA shifts from Cathode to the Anode poles. The Gel imaging system was used to visualize the bands.

Statistical analysis:

The data were analyzed by using a statistical package for the social sciences software, Version 26. Quantitative data were measured as (Mean± S.D), while qualitative data were given as frequencies. We used (Independent Student t., Mann Whitney U, Wilcoxon Signed Ranks, and ANOVA) tests to investigate the association between quantitative data. In contrast, (Chi-square or Fisher's Exact) test was employed for qualitative data. A p-value of <0.05 was considered significant.

Results

A total of 1650 patients were interviewed from two large tertiary care centers in Basrah/Iraq, 750 patients from Almawani Specialized Diabetes, Endocrine, and Metabolism Center from (December 2021 to March 2022) and 900 patients from Faiha Specialized Diabetes, Endocrine, and Metabolism Center (FDEMC) from (April 2022 to December 2022).

Only one hundred and two patients completed the study; 54 men and 48 women. The age of the patients was between (33 and 70) years, and a mean age of (52.87±10.91) for women and (51.7±10.63) for men. About 18% of the participants had hypertension. More than 50% of the recruited patients had a family history of DM, and two-thirds were of low social level. The demographic and baseline characteristics of the enrolled patients were illustrated in TABLE (1).

TABLE (1) Baseline characteristics of the enrolled patient

The clinical parameters before and after metformin treatment:

The mean HbA1c level reduced from 8.18% (S.D. 1.06%) before treatment to 7.8% (S.D. 1.40%) after three months of metformin treatment. (p-value 0.004), the mean FBG level decreased from 191 mg/dl (S.D. 32.40) before treatment to 177 mg/dl (S.D. 43.19) after three months. (P-value=0.020). As presented in Table (2).

Table (2) Clinical parameters before and after metformin treatment* Wilcoxon Signed Ranks test.

Metformin Response in Treatment of T2DM Patients:

Based on the response to metformin monotherapy, we divided the patients recruited in this study (drug naïve patients) into two groups, the responders and the non-responders group, and the

response to metformin therapy depends on HbA1c value. The non-responders included patients with HbA1c levels that decreased by <1% after three months of treatment. Furthermore, the responders included patients whose HbA1C levels decreased by 1% or more after three months of treatment [14]. After three months of metformin treatment, we had 69 responders and 33 non-responders. No significant differences between groups regarding (age, sex, social status, marital status, family history of DM, or hypertension of the patients) (p-value >0.05). Also, the present study showed no differences between the two groups regarding the patients' baseline HbA1C and FBG (p-value> 0.05). In contrast, the reduction in (FBG and HbA1C) in the responder's group was significant (p-value < 0.05) after three months of metformin treatment. As well as the Δ% HbA1c and Δ % FBG (Δ%: first measure minus second measure) were also significantly different between groups. As summarized in Table (3).

Table (3) Characteristics of the patients before and after metformin treatment (n=102)

*Independent sample T-test, ** Chi-Square Test, *** Mann-Whitney U-test tests

Molecular Assays

Two SNPs were investigated in the present study for their association with metformin efficacy. The genotyping was performed by the ARMS-PCR method. The genotypes and allelic frequencies of detected polymorphisms were done by finger counting, as shown in Table (4), and the characteristics of SNPs were summarized in Table (5)

Table (4) Characteristics of single-nucleotide polymorphisms used in the study

SLC22A1 Gene polymorphism

Gel electrophoresis of the PCR products is shown in Figures (2, 3). Specific pairs of primers were used by conventional PCR for gene amplification.

The rs12208357 had three genotypes showed by PCR: CC, CT, and TT, and the rs72552763 had three genotypes showed, including AA, A/del, and del/del, and the fragment lengths of (rs12208357 and rs72552763), were 888bp and 621bp respectively.

Figure (2) gel electrophoresis of (rs12208357) gene polymorphism.

Figure (3) the gel electrophoresis of (rs72552763) gene polymorphism.

Allele frequency for rs12208357 (R61C) reference allele frequency of "C" was 0.94, and the alternative allele frequency of "T" was 0.06, and for rs72552763 (met420del), the reference allele frequency of "A" was 0.74, and the alternative allele frequency "del" was 0.26. as shown in Table (4).

Regarding genotypes frequency rs12208357 (R61C), the frequency of CC genotypes was 0.91, CT genotypes were 0.06, and TT genotypes were 0.03. While for rs72552763 (Met420del) "AA" genotype was 0.73, for the del/del genotype was 0.24, and for the A/del genotype was 0.03. as shown in figure (4).

Figure (4) Genotypes frequencies of rs12208357 and (rs72552763) genes among studied T2DM patients.

The prevalence of alleles of rs12208357 (R61C) within the enrolled patients:

In the present study for rs12208357 (R61C) polymorphism, the reference allele frequency (reference allele) "C" was 0.94, and the alternative allele frequency (alternative allele) "T" was 0.06, and these frequencies are consistent with other populations in a study of 1000 genome like; European "T" = 0.06, African "T" =0.003, East Asian" T" =0.000. (NCBI database).

The prevalence of alleles of rs72552763 (Met420del) within the enrolled patients

In the present study for rs72552763 (Met420del) polymorphism, the reference allele frequency (reference allele) "A" was 0.74, and the alternative allele frequency (alternative allele) "del" was 0.26 and the frequency different among different populations; in a study of sample size 1000 genome the alternative allele was del GAT= 0.18 for European, del GAT= 0.045 for African, East Asian del GAT=0.0005. (NCBI database).

Demographic and baseline clinical variables based on OCT1 genetic variants

No significant differences in sex, age, and presence of hypertension were observed at baseline based on rs12208357 and rs72552763 polymorphism of OCT1. As described in Table (5).

Table (5) Comparison of demographic and baseline clinical variables based on OCT1 genetic variant* Fisher Exact Test, ** independent sample T-test.

Effect of genetic polymorphism on the therapeutic activity of Metformin in Type 2 Diabetic Patients

Interestingly, carriers of two copies of the "C" allele homozygous (CC) of rs12208357 polymorphism were more common among the responders than the non-responders (73.1% vs. 26.9) (P-value 0.001). Carriers of variant (CT) are more common among the non-responders than the responders (83.4% vs. 16.6%). All patients with two

copies of the alternative allele "T" homozygous (TT) genotypes were present among non-responders. (100.0% vs. 0.0%). Also, allele frequency showed differences between the responders and the non-responders groups, in which "C" reference alleles were more common among the responders, and all "T" alternative alleles were present in the non-responders groups. (P-value=0.032).

These data suggested that T2DM patients who carried homozygous genotype (CC) wild type of SLC22A1 rs12208357 gene polymorphism or the allele C were associated with good therapeutic efficacy (p-value <0.05) of metformin in Basrah Southern Iraq, As shown in Figure (5).

Figure (5) The influence of (rs12208357) genetic polymorphism on the therapeutic effectiveness of metformin* (Fisher Exact Test).

A similar thing was observed with the (rs72552763) gene polymorphism, In which the responder's groups were associated with wild-type (AA) genotypes (p-value= 0.0001) and reference allele A (p-value= 0.000), As presented in figure (6).

Figure (6) Effect of the SLC22A1 rs72552763 genetic polymorphism on the therapeutic efficacy of metformin* Fisher's Exact Test, ** ANOVA test.

Clinical effects of the genetic variants:

We compared changes in the levels of FBG and HbA_{1c} of the patients from baseline levels vs. after three months of treatment among different genotypes of the rs12208357 (R61C) and rs72552763 (M420del) genetic variants of OCT1. The T2DM patients with the C/C genotypes of rs12208357 exhibited more significant reductions Δ% in their FBG (p-value=0.019) and HbA1c (p-value=0.010) levels in comparison to those carrying (C/T) and (T/T) rs12208357 genotypes following metformin treatment, As illustrated in Table (6).

The T2DM patients with the A/A genotype of rs72552763 exhibited more significant reductions in their FBG level (p-value 0.002) and HbA1c (p-value 0.000) compared to patients carrying (A/del) and (del/del) of rs72552763 genotypes, As shown in Table (6).

Table (6) Comparison of study variables based on rs12208357 and rs72552763 genotypes. Δ= 1st measure minus 2nd measure of both parameters (HbA1c, FBG),*ANOVA test.

In addition, we compared changes in the levels of FBG and HbA1c of the patients between baseline and later after three months of treatment based on different alleles of the (R61C): C, T, and (M420del): A and del of OCT1. Statistically, no significant differences between C and T alleles depending on HbA1C, Δ%HbA1c, FBG, and ΔFBG%,

Patients with reference alleles A in our study showed a significant decrease in HbA1c (p-value=0.001) and FBG (p-value =0.001) after 90 days of treatment. As prescribed in Table (7).

Table (7) Comparison of study variables based on alleles of rs12208357 and rs72552763.

Δ= 1st measure minus 2nd measure of both parameters (HbA1c, FBG), independent sample T test.

Discussion

T2DM is a common chronic illness distinguished by hyperglycemia resistance to insulin [15]. It constitutes more than 90% of all types of diabetes worldwide. [16]. Metformin is considered the first-line treatment of T2DM unless there are contraindications, according to the current guidelines from the Iraqi expert consensus on the management of type 2 diabetes. [17].

The glucose-lowering effect of metformin has a broad interindividual variability due to several causes, one of them being genetic factors [18]. More than 30% of diabetic patients can not attain good glycemic control on metformin treatment. [19, 20].

The (OCT1) is a protein that takes metformin to the liver, the primary target of metformin activity, which is an essential step in decreasing hepatic glucose production [7]. The OCT1 (SLC22A1) gene is highly polymorphic [9]. The genetic differences in the OCT1 coding gene can alter the protein function and reduction in the amount of metformin at the receptors hence causing a decline in the therapeutic response [21, 12].

Several studies [10, 21]. have investigated the association with response to metformin, while other studies have found no association with response to metformin. [10, 21, 13]. This variability in the results can be manifested by the variation in the designing of the research, which includes the type of the study, size of the study population, characteristics of the cohort, methods of the analysis, and manner of assessment of treatment efficiency [22].

Several studies have declared that there is no agreeable standard for dividing type 2 diabetic patients into responders and non-responders., Shikata et al. chose a decrease of HbA1c values by greater than 0.5 % as a cutoff point for classifying patients into responders and non-responders. The researcher's choice was dependent on their clinical experience [23]. However, it was noticed in a methodical review that after three months of metformin monotherapy (doses of 1,500 mg/day), the HbA1c levels were reduced by about 1 % in comparison with placebo [24]. Previous studies

demonstrated that oral glucose-lowering drugs reduce HbA1c levels effectively by 0.5−1.5% [24]. Thus, in the current study, a decrease of ≥1 % in HbA1c was considered a response to metformin therapy. The current study was designed to assess the glycemic control of metformin monotherapy by differences in HbA1c from baseline to three months of metformin treatment in a dose of (2g/day metformin) was associated with about three-quarters of the responders to metformin monotherapy. In the current study, we used metformin in doses of 2g/day to estimate a better glycemic response, so we used to increase the dose level to the best possible level.

The same findings were obtained by Rashid et al., who showed that 59.5% of the patients were classified as responders and 40.5% as non-responders to metformin therapy [25]. While in a study done in South India. 76% of the patients were classified as responders and 23% as non-responders [26]. However, this inconstantly non-responsiveness may be explained by other factors like genetic factors, diabetes duration, or patient compliance.

Our results reported that after three months of metformin monotherapy, HbA1c and FBG levels were significantly decreased. Furthermore, the responders had significantly lesser HbA1c and FBG levels than the non-responders. The same results were observed in Mahrooz et al. study, which investigated that HbA1c and FBG levels were significantly reduced in responders than non-responders [27].

OCT1 polymorphisms in humans modified the therapeutic efficacy of metformin. Several studies indicate that OCT1 intercedes the initial stage in the metformin action, and the genetic variability in OCT1 may modify the metformin efficacy. [10].

Our study showed that the mean decrease in HbA1c after metformin therapy was (1.033± 0.866) among responders; the value was increased to (-1.003± 0.692) in non-responders. The mean reduction in HbA1c levels in our study is higher than in the previous one conducted by Mahrooz. et al., In which the mean decrease in HbA1c was 0.67 % (0.67 ± 0.58) among metformin-responders, and was -0.12 % (-0.12 ± 0.9) in metformin non-responders [27], the use of metformin together with dietary and lifestyle modification may be the probable reason for the most significant reduction of HbA1c levels.

To date, most of the studies about genetic variation in OCT1 have concentrated on European, American, and Japanese populations. The current study determined the genotype and allele frequencies of the two most common SNPs (rs12208357 and rs72552763) of OCT1 in a cohort of Iraqi subjects in Basrah/ Iraq. We investigated the effects of these SNPs of OCT1 on the glycemic response to metformin treatment in T2DM patients. The alternative allele frequencies of our study were 6% and 26% for R61C and Met420del, respectively. Shu et al. study indicates that SNPs in OCT1 may participate in the differences in metformin efficacy. They observed that the two variants (R61C and Met420del) of OCT1 are the most prevalent SNPs of OCT1, with frequencies of alleles (7.2% and 19%), respectively, in European-origin people. [28].

In mouse hepatocytes, the OCT1 deletion caused a decrease in the action of metformin on AMPK phosphorylation and gluconeogenesis. In OCT1-absent mice, the glucose-lowering effects of metformin were abolished entirely [10].

The present study established a significant relationship between SLC22A1 (rs12208357/R61C) and (rs72552763/Met420del) gene polymorphism and metformin response. We observed that single nucleotide polymorphisms in the SLC22A1 gene are associated with differences in HbA1c and FBG values because patients with the homozygous CC-rs12208357 genotype and the homozygous AA-rs72552763 genotype had a significant decrease in HbA1c and FBG levels after follow-up from baseline compared to patients with other genotypes of rs72552763 and rs12208357. Minimal metformin is predicted to be transferred into the hepatocytes in patients with OCT1 reduced-function alleles. The assumption is that the cellular uptake of metformin constitutes the initial step in AMPK activation. The liver is the initial target of metformin action [3]. The current study hypothesizes that the carriers of the reduced function variants need an elevated metformin dose to recover their glycemic control.

Becker et al. revealed a significant relationship between patients with the CC-rs622342 genotype and an average increase of 0.02% in the HbA1c levels [21].

In 2007, Shu et al. supported the association between SLC22A1 polymorphisms and metformin response. Their results proposed decreased acute response to metformin in subjects with defective variants after a glucose load by OGTT in healthy recruited people (N = 12) taken two subsequent metformin doses (1000 mg at night and 850 mg in the morning. [10]. Becker et al. showed that polymorphism of rs622342 of OCT1 has been related to the glucose-lowering effect of metformin [21].

On the other hand, Zhou et al., in one of the largest studies to investigate OCT1 variants and glycemic response to metformin (GoDARTs). The researchers studied the two most common OCT1 variants, R61C, and 420del, in more than a thousand and a half T2DM patients who received metformin. They showed no effect on metformin response. [13]. Also, a prospective study

done by Ningrum and his colleagues for (81) T2DM patients in the Javanese-Indonesian population who received 500 mg metformin twice daily for at least two weeks, found that Met420del has no effects on the pharmacokinetics of steady-state metformin concentrations [29]. Davis et al. also found no relation between OCT1 variants and changes in HbA1c [30]. Furthermore, another study investigating eleven SNPs of the SLC22A1 gene also could not detect any significant relationship with response to metformin [21].

This variability in results among different studies may be attributed to several factors, such as genetic factors, involving the number and type of SNPs investigated in the study, SNPs interactions, number of SNPs present in the patients, ethnicity, and drug interaction with OCT1 as substrate or inhibitors or non-genetic factors include duration and onset of diabetes, a dose of metformin, use of metformin monotherapy or combination with other glucose-lowering drugs and finally compliance of the patients, in addition to design and duration of the study all of these factors lead to variation in the results. The restriction of the current study is due to the small sample size of the individuals involved, and future studies needed to use other SNPs related to the efficacy of metformin.

Conclusion

The current study showed that the reduction in HbA1C values by ≥1% from baseline data could be considered a response to metformin therapy. The current study found there was a significant decrease in the HbA1c and FBG levels in the responder's group compared with the non-responders. The reason for non-responsiveness to metformin therapy may be due to a collection of genetic and non-genetic factors. We found in the current study that the (rs12208357/R61C) and (rs72552763/Met420del) gene polymorphism are significant modulators of metformin response in T2DM patients.

This study may help a physician in clinical decision-making for the treatment of T2DM by determining a recently diagnosed patient to receive metformin or other antihyperglycemic drugs, and this may decrease the cost and complaints of the patients.

Conflict of Interest

No conflict of interest.

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Tables:

Table (1) Baseline characteristics of the enrolled patients

Parameters	Mean ± S.D
Age (year)	52.24 ± 10.746
Sex Men% Women %	(54) 52.9% (48) 47.1%
HbA1c%	8.188± 1.066
FBG mg/dl	191.00± 32.401
Hypertension Yes No	(18) 17.6% (84) 82.4%
Family history of DM Yes No	56.9% 43.1%
Marital status Married Divorced Widowed Single	(80) 78.4% (4) 3.9% (14) 13.7% (4) 3.9%
Social level Low Medium High	(71) 69.6% (31) 30.4% (0) 0.00%

Table (2) Clinical parameters before and after metformin treatment

Parameters	Baseline	After 3 months of treatment	P value*
HbA1c%	8.18± 1.066	7.81± 1.400	0.004
FBG (mg/dl)	191.00± 32.401	177.07± 43.196	0.020

* Wilcoxon Signed Ranks test

Table (3) Characteristics of the patients before and after metformin treatment (n=102)

Parameter		Responders (n=69)	Non-responders (n=33)	P value
Age (Mean ±SD)		53.04±9.88	50.55±12.34	0.274*
Sex	Men %	55.1%	48.5%	0.533**
	Women %	44.9%	51.5%	

Social level	Low	(52) 75.4%	(19) 57.6%	0.068**
	Medium	(17) 24.6%	(14) 42.4%	
Marital status	Married	(53) 76.8%	(27) 81.8%	1.000**
	Divorced	(3) 4.3%	(1) 3.0%	
	Widowed	(10) 14.5%	(4) 12.1%	
	Single	(3) 4.3%	(1) 3.0%	
Family history of DM	Yes	(35) 50.7%	(23) 69.7%	0.070**
	No	(34) 49.3%	(10) 30.3%	
Hypertension	Yes	(13) 18.8%	(5) 15.2%	0.648**
	No	(56) 81.2%	(28) 84.8%	
HbA1C% baseline		8.298±1.045	7.957±1.090	0.910***
HbA1C% after 3 months		7.265±1.027	8.960±1.394	0.025***
Δ change %		1.033± 0.866	-1.003± 0.692	0.000***
FBG (mg/dl) baseline		195.065±31.489	182.967±33.1838	0.860***
FBG (mg/dl) after 3 months		159.645±31.398	211.935±42.825	0.033***
Δ change %		33.500± 29.706	-30.965± 21.499	0.000***

*Independent sample T test, ** Chi-Square Test, *** Mann-Whitney U-test tests

Table (4) Characteristics of single-nucleotide polymorphisms used in the study

Gene	SNP code	Position	AA change	Reference allele	Reference allele frequencies	Alternative allele	Alternative allele frequencies
OCT1	rs12208357	chr6:160122116 (GRCh38.p13) c.181C > T	R61C	C	0.94	T	0.06
OCT1	rs72552763	chr6:160139849 (GRCh38.p13)	Met420del	A	0.74	Del	0.26

Table (5) Comparison of demographic and baseline clinical variables based on OCT1 genetic variant

Variables	Genotypes			p-value
rs12208357	CC	CT	TT	
Sex Men Women	50 (53.8%) 43 (46.2%)	3 (50.0%) 3 (50.0%)	1 (33.3%) 2 (66.7%)	0.872*
Age (y)	52.30± 10.618	57.67± 6.947	39.33±13.650	0.052**
Hypertension Yes No	16 (17.2%) 77 (82.8%)	2 (33.3%) 4 (66.7%)	0 (0.0%) 3 (100.0%)	0.460*
rs72552763	AA	A/ deletion	Deletion	
Sex Men Women	40 (54.1%) 34 (45.9%)	1 (33.3%) 2 (66.7%)	13 (52.0%) 12 (48.0%)	0.859*
Age (y)	52.34± 10.194	46.33±15.177	52.64± 12.086	0.627**
Hypertension Yes No	12 (16.2%) 62 (83.8%)	0 (0.0%) 3 (100.0%)	6 (24.0%) 19 (76.0%)	0.570*

* Fisher Exact Test, ** ANOVA test

Table (6) Comparison of study variables based on rs12208357 and rs72552763 genotypes

Parameters	CC	CT	TT	P-value*
rs12208357				
FBG (mg/dl) baseline	191.81± 32.02	176.33± 33.50	197.33± 46.60	0.501
FBG (mg/dl) after 3 months	173.72± 40.82	202.00± 59.27	221.00± 59.27	0.059
ΔFBG (mg/dl)	15.35± 40.68	-25.66± 27.81	-23.66± 12.66	0.019
HbA1c baseline %	8.20± 1.06	7.73± 1.05	8.43±1.48	0.530
HbA1c% after 3 months	7.71± 1.33	8.58± 1.81	9.20± 1.90	0.074

Δ HbA1c %	0.49± 1.23	-0.85± 0.91	-0.76± 0.41	0.010
rs72552763	A/A	A/del	Del/del	
FBG mg/dl Baseline	190.77± 32.22	182.00± 47.44	192.78± 32.47	0.862
FBG mg/dl After 3 months	167.40± 31.66	172.66± 54.22	205.82± 58.09	0.001
ΔFBG (mg/dl)	20.32± 33.32	9.34 ± 23.15	-15.04± 51.59	0.002
HbA1c% baseline	8.17± 1.08	7.90± 1.49	8.26± 1.01	0.845
HbA1c% after three months	7.49± 1.04	7.60±1.70	8.77±1.84	0.000
Δ HbA1c %	0.67± 1.00	0.30± 0.72	-0.51± 1.55	0.000

Δ= 1st measure minus 2nd measure of both parameters (HbA1c, FBG),*ANOVA test

Table (7) Comparison of study variables based on alleles of rs12208357 and rs72552763

Parameters	C allele	T allele	p- value	A allele	Del allele	p- value*
HbA1C baseline %	8.180±1.061	8.433±1.484	0.584	8.164±1.087	8.260±1.019	0.643
HbA1C after 3 months%	7.771±1.374	9.200±1.374	0.503	7.501±1.062	8.776±1.845	0.001
Δ HbA1C %	0.4091±1.256	-0.766±0.416	0.238	0.6636±.993	-0.5160±1.551	0.214
FBG baseline (mg/dl)	190.775±32.169	197.333±46.608	0.548	190.391±32.592	192.782±32.476	0.956
FBG after 3 months mg/dl)	175.611±42.222	221.000±59.270	0.509	167.628±32.336	205.826±58.098	0.001
ΔFBG (mg/dl)	12.275±41.195	-23.666±12.662	0.200	19.790±32.852	-15.047±51.597	0.299

Δ= 1st measure minus 2nd measure of both parameters (HbA1c, FBG),* independent sample T test

Figures

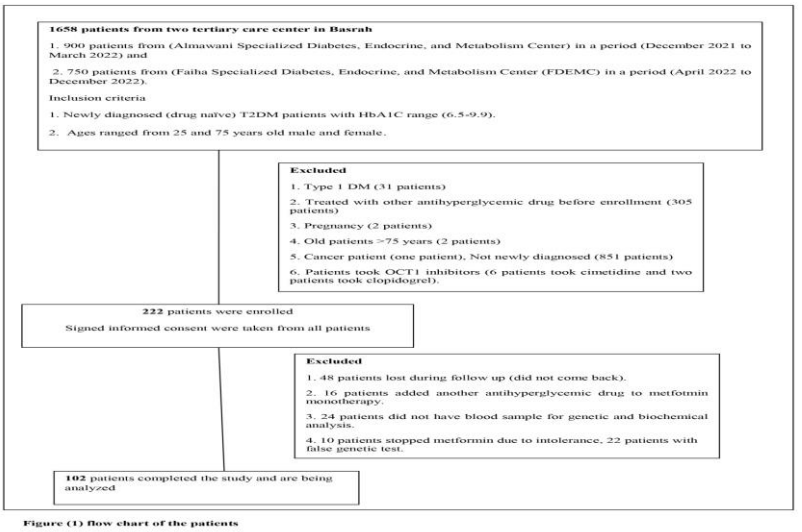

Figure (1) flow chart of the patients

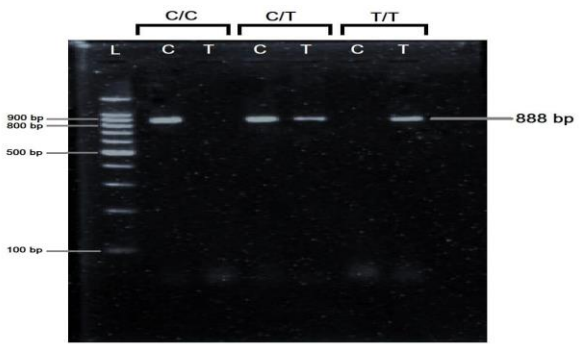

Figure (2) gel electrophoresis of (rs12208357) gene polymorphism. The fragment length was 888bp. Three genotypes CC, CT, TT of (rs12208357).

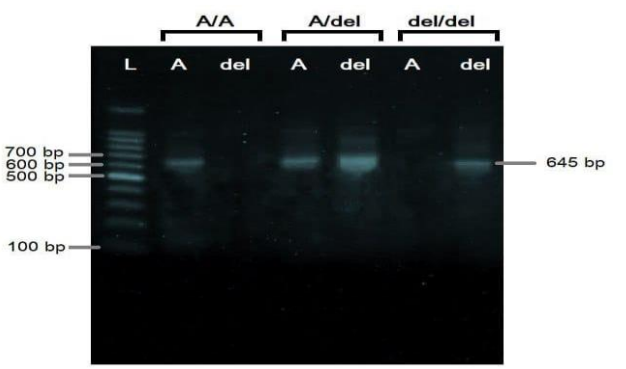

Figure (3) the gel electrophoresis of (rs72552763) gene polymorphism. The fragment length was 621bp.

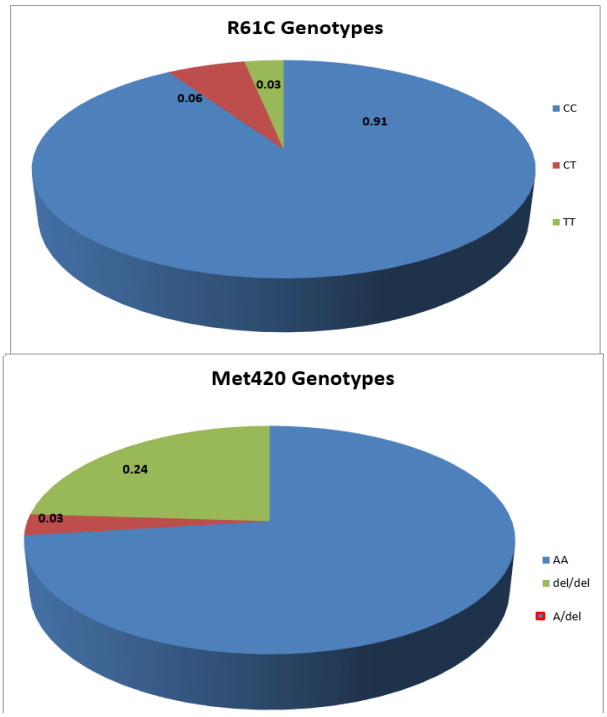

Figure (4) Genotypes frequencies of (R61C) rs12208357 and (Met420del) rs72552763 gene among studied T2DM patients.

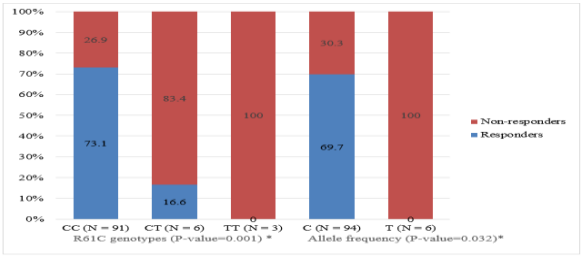

Figure (5) Effect of SLC22A1 rs12208357 genetic polymorphism on the therapeutic efficacy of metformin * Fisher Exact Test

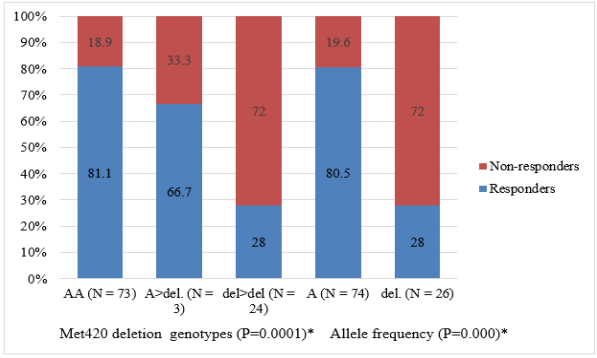

Figure (6) Effect of the SLC22A1 rs72552763 genetic polymorphism on the therapeutic efficacy of metformin* Fisher Exact Test

Appendix 1

Questionnaire

Name
Age
Sex
Smoking history
Medical history
Any microvascular complication
Blood pressure

Biochemical analysis: HbA1C, fasting blood glucose (FBG) at baseline and after three months of metformin monotherapy
Genetic test to detect SNPs

Appendix 2

Informed consent form:

My name is Rawnaq Adil Aladhab, and I am inviting you to participate in a research study. Involvement in the study is voluntary, so you may choose to participate or not. You will be asked (some questions listed above in the questionnaire form). And we will take the remaining blood from your routine investigation in the hospital lab in order to make the genetic test in a private lab at the beginning of the study after three months of treatment for follow-up. If you no longer wish to continue, you can withdraw from the study, without penalty, at any time. All information will be kept confidential. If you have any questions about the research, please contact (name and email address of person doing project). By signing below, indicate that you understand the information printed above and wish to participate in this research study.

Name of participant_____ _____
Signature of participant
Date _____ Name of investigator
Date