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Effect of MATE 1, MATE 2 and OCT1 Single Nucleotide Polymorphisms on Metformin Action in Recently Diagnosed Egyptian Type-2 Diabetic Patients

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ABSTRACT

To study the effect of MATE 1, MATE 2 and OCT1 genetic variants on metformin action in recently diagnosed Egyptian Type-2 diabetic patients. Patients & Methods: One hundred type-2 DM patients and forty healthy control were included in the study. All patients were recently diagnosed receiving no treatment before participation in the study. Three single nucleotide polymorphisms (SNPs) were Genotyped using real time PCR, Sequence Detection System: MATE1 (rs2252281), OCT1 coding variants (rs12208357) (SLC22A1) and MATE2 (rs12943590). There is a significant differences between control and patients regarding MATE2 ($p<0.05$), OCT1 ($P<0.005$) distribution; in which GG (54%), CC (62%) is the most prevalent among studied patients respectively. MATE1 SNP; Patients with CC alleles and TT allele had better HBA1C (8.577±.2924), (8.7±.25) compared to CT allele patients (9.584±.3023) ($P= .04$) ($P=.019$) respectively. OCT1 SNP; CG allele patients showed better RBS (251±9.565) compared to CC allele (294.42±8.476) ($p=0.004$). Logistic regression test showed that RBS ($p=.00001$), ALT ($p=.0001$) and TLC ($p=.025$) are independent factors affecting blood glucose. Conclusion: MATE1 and OCT1 SNPs may have a potential role in metformin efficacy.

Keywords: MATE 1, MATE 2 and OCT1, SNPs, Egyptian, Diabetes**.**

INTRODUCTION

Metformin (1,1-dimethylbiguanide), a biguanide derivative, is the first choice drug prescribed in type II DM patients (T2D), in conjunction with lifestyle modification [1]. It improve insulin sensitivity through up regulation of insulin receptor expression and stimulation of tyrosine kinase activity [2].

About fifty percent of the oral metformin dose is absorbed [3 4] passing to blood followed

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by distribution to different tissues. It is present in unbound form [5], excreted through renal clearance in unchanged form. The efficacy of metformin varies substantially, with more than thirty percent of metformin treated patient are classified as non-responders [6]. The genetic contribution to this response variability has been studied with a focus has been directed twards metformin pharmacokinetics and pharmacodynamics.

Metformin is transferred into the intestinal cells by plasma monoamine transporters or PMAT (*SLC29A4*), then into the blood stream and its active hepatic uptake by the organic cation transporter, OCT1 (*SLC22A1*) [7] Metformin is a good substrate for OCT1, encoded by the solute carrier family 22 member 1 (SLC22A1) gene, which is primarily expressed in the liver [8].

Metformin secretion takes place through two consequent step: First from blood to renal tubule cell which is mediated by organic cation transporter, OCT2 (*SLC22A2*), Second step and final excretion into the urine is mediated by 2 transporters in the multidrug and toxin extrusion family, MATE1 and MATE2-K (*SLC47A1* and *SLC47A2*) [7]. Biliary excretion of the metformin, although insignificant in human, is mediated through MATE1that is located in the canalicular membrane of hepatocytes.

Based on that, polymorphisms on OCTs and MATEs may change the metformin level and metformin efficacy. The present study was carried out to explore the distribution and the effect of MATE1, MATE2 and OCT1 gene polymorphisms on Metformin efficacy among type 2 diabetic patients

Patients and Method

Subjects

One hundred subjects clinically diagnosed as type II diabetes mellitus by clinical and laboratory investigations at internal medicine Department, Faculty of Medicine, Cairo University were enrolled present study. Clinical data of all cases was collected including age, sex and family history. Laboratory data were assessed including: glycosylated Hb (HbA1C), blood glucose level, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALKP), bilirubin, albumin, creatinin, prothrombin concentration (PC), complete blood picture, antinuclear acid (ANA) titer, thyroid stimulating hormone (TSH) and alpha fetoprotein (AFP). None of the patients received treatment before starting this study. In addition, forty age-matched healthy volunteers were recruited after and considered as normal control group. Written informed consent was obtained from each patient and healthy volunteer, this study was approved by the Ethics Committee Board of Faculty of Medicine, Cairo University.

Blood samples collection

Five ml blood samples were withdrawn from all study participants .every blood sample was collected into 2 different tubes, the first was collected into vacationer EDTA tubes to measure the biochemical parameters; AST, ALT, total bilirubin (T. Bil), direct bilirubin (D. Bil), albumin, alkaline phosphatise (ALKP), creatinine, and random blood sugar (RBS) measurement were done using Roche Hitachi 911 Chemistry Analyzer (Bunker Lake Blvd. Greater Minneapolis / St. Paul Area. USA). Hemoglobin A1C (HbA1c), PC, international normalized ratio (INR) were detected using (Stanbio Labrotary, Boerne, TX USA) kits. Hemoglobin (Hb), total leucocytes count (TLC), absolute neutrophil counts (ANC), platelets were detected by cell counter (Sysmex XT-4000*i* Automated Hematology Analyzer Lincolnshire, IL, USA). ELISA kits supplied by (DRG International Inc., Springfield, New Jersey, USA) were used in detection of plasma levels of Thyroid-stimulating hormone (TSH) and alpha-fetoprotein (AFP).

SNP genotyping

DNA was extracted from peripheral blood using Zymo research Quick-gDNA™ MiniPrep kit (Catalog No.D3024) according to the instructions guides of the manufacturer's. All DNA samples extracted were quantitated using the Nano Drop®-1000 spectrophotometer (Nanodrop Technologies, Inc., Wilmington, USA).

Three single nucleotide polymorphisms (SNPs) were screened. Genotyping was identified using real time PCR (StepOne, Applied biosystem) Sequence Detection System (ABI Inc. CA, USA) according to the Applied Biosystem protocol. MATE1 (g."66T>C, rs2252281), OCT1 coding variants (R61C, rs12208357) and MATE2 (g."130G>A, rs12943590) SNPs (Cat No. 4351379) were

subjected to analysis in the extracted DNA using specific primers , FAM and VIC probes (Taqman SNP genotyping assays, Applied Biosystems, Foster City, CA). PCR mixture incorporated 20 ng of whole blood genomic extracted DNA and the following reagents: 1.25 µl FAM and VIC probes and primers (Taqman SNP genotyping assays), 12.5 µl Taqman universal master mix II No UNG (Cat No. 4440040) and complete volume to 25 µl using DNase free water. Negative control was included in each reaction to exclude DNA contamination. The thermal cycling profile was 10 minutes at 95 ºC for enzyme activation then by 40 cycles of 15 seconds DNA denaturation at 95ºC, 20 seconds primers and probes annealing at 55 ºC and 30 second at 72 ºC for the amplification step. The genotyping data were analyzed using SDS 2.1 software (ABI Inc. CA, USA).

RESULTS

Distribution of gene alleles showed significant differences between control and patients as regard MATE2 (rs12943590) ($p<0.05$) in which GG is the most prevalent allele among patients (54%) while the homozygous allele (AA) is the least prevalent one (12%) and OCT1 (rs12208357) ($P<0.005$) in which CC allele is the most prevalent among studied patients (62%), whereas the GG allele is the least prevalent one (12%) Table (1)

Studied patients group showed high significant differences compared to control group as regard AST (44.6±13.6, 32.5±5.2), ALT (52.6±10.1, 30.9±5.4), HBA1C (8.988±1.6907, 5±.6381), RBS (279.66±62.528, 100.7±16.302), TLC (6.634±1.9917, 5.685±.9694), Platelet (272.98±94.1970, 239.050±67.6942), AFP (2.9776±2.04391, 1.7365±.92572) ($P< 0.0001$) Table (2).

Among MATE1 SNP, patients with CC allele showed better HBA1C (8.577±.2924) compared to patients with CT allele (9.584±.3023) ($P= .04$), also patients with TT allele showed better HBA1C (8.7±.25) compared to CT (9.6±.3) ($P=.019$).

While in OCT1 SNP, patients with CG allele showed better RBS (251±9.565) compared to patients with CC allele (294.42±8.476) ($p= 0.004$), Table (3).

Regarding to MATE1 SNP, patients with CC alleles had better HBA1C (8.577±.2924) compared to patients with CT alleles (9.584±.3023) ($P= .04$), also patients with TT allele showed better HBA1C (8.7±.25) compared to CT (9.6±.3) ($P=.019$).

While in OCT-1 SNP, patients with CT allele showed better RBS (251±9.565) compared to patients with CC allele (294.42±8.476) ($p= 0.004$), Table (3).

Logistic regression test was done showed the independent factors that may affect blood glucose level as indicated by the HBA1C are RBS (.00001), ALT (.0001) and TLC (.025) Table (4).

Comparison between MATE1 variance and references who are MATE2 references showed insignificant differences except TSH ($p<0.0002$). Comparison between MATE2 variance and references who are MATE1 references showed significant differences regarding total bilirubin ($p<0.033$), Direct Bilirubin ($p<0.029$) and Albumin ($p<0.011$), Table (5).

Table 1: Subject genetic distribution of the studied population

Group		MATE1rs2252281			MATE2rs12943590**			OCT1rs12208357**		
		CC	CT	TT	AA	AG	GG	CC	CT	TT
Control	Count (40)	6	16	18	20	12	8	6	10	24
	%	15%	40%	45%	50%	30%	20%	15%	25%	60%
Patients	Count (100)	26	38	36	12	34	54	62	26	12
	%	26%	38%	36%	12%	34%	54.0%	62.0%	26%	12%

DISCUSSION

Individualized glycemic control is essential in patients' with DM2 to make an equilibrium between age, comorbidities, and the risk of development of hypoglycemia. Proper control of blood glucose by keeping the HbA1c below 6.5 % significantly decrease nephropathy and cardiovascular complications development [9].

Table 2: Comparison between Patients and control group

	Group	Mean
Age	Control	43.25±9.71
	Patients	40.12± 11.459
AST **	Control	32.45±5.208
	Patients	44.58±13.559
ALT **	Control	30.85±5.447
	Patients	52.58±10.071
Bilirubin Total *	Control	.849±.22942
	Patients	.742±.16834
Direct Bilirubin	Control	.226±.11082
	Patients	.2208±.10017
Albumin *	Control	4.295±.4082
	Patients	4.058±.4795
Alk Phosphatase	Control	71.4±22.74
	Patients	67.18±26.44
Creatinine	Control	.949±.1838
	Patients	.912±.291
HBA1C ***	Control	5±.6381
	Patients	8.988±1.6907
RBS ***	Control	100.7±16.302

Although the metformin neither bound to plasma protein nor metabolized [7], about sixty third percent of metformin treated patients experience gastrointestinal symptoms leading to discontinuation of the metformin by about from 5-10 % of these patients [10]. However, it is actively transported and distributed [11], with respected inter-individual variability in metformin's action[12].

These change in metformin efficacy is primarily due to the variation in the activity of the transporters either at the action site (hepatocyte) or at the excretory site (renal tubules).

In the present study; the distribution of gene alleles showed significant differences between control and patients as regard MATE2 (rs12943590) ($p<0.05$) and OCT1 (rs12208357) ($P<0.005$).

The studied patients group in the present work showed the MATE1 allele distribution heterozygous CT allele is the most prevalent one (38%), whereas homozygous variant CC is the least prevalent one (26%), while MATE2 alleles distribution showed that GG is the most prevalent alleles among patients (54%) while the homozygous variant (AA) is the least prevalent variant (12%); whereas OCT1 allele distribution showed that CC allele is the most prevalent among studied patients (62%), while the GG is the least prevalent one (12%).

OCT1, located on the enterocytes basolateral membrane, may be responsible for transport of metformin into the intersitial fluid [13] and

Table 3: Effect of MATE 1, AMTE 2 and OCT-1 gene polymorphisms on metformin actions

	MATE 1			MATE 2			OCT-1		
	CC	CT	TT	AA	AG	GG	CC	CT	TT
	26	38	36	12	34	54	62	26	12
RBS	281.8	281.68	276	264.17	268.12	290.37	294.42	251	265.5
	±10.3	±11.594	±10.094	±18.360	±8.696	±9.2	±8.476£	±9.565	±11.89
HBA1C	8.577	9.58±	8.656	9.033	8.741	9.133	9.235	8.438	8.9
	±.2924	0.3 * ¥	±.2473	±.5322	±.22	±.26	±.8	±.2469	±.4777

* Significant compared to CC (P= .040)

¥ Significant compared to TT (P=.019)

£ Significant compared to CT (P=0.004).

Table 4: Logistic Stepwise Regression Test

	R	R^2	Adjusted R^2	Coefficient	SE	Standardized coefficient	p
RBS	.734[a]	.539	.536	11.142581	1.5858	0.734	.00001
ALT	.763[b]	.583	.577	9.096	1.5146	0.248	.0001
TLC	.773[c]	.598	.589	-2.259263	1.4924	-0.1307	.025

Table 5: Comparison between MATE 1, MATE 2 references and variants effect on metformin efficacy

	MATE1		MATE2	
	Variant (TC & CC) (n=64)	Reference (TT) (n=36)	Variant (AA) (n=12)	Reference (GG&GA) (n=87)
HBA1C	9.175 ±.2225	8.656±.2473	9.03±.5322	8.979±.1811
RBS	281.72±7.998	276±10.094	264.17±18.36	280.92±6.679

on the hepatocytes sinusoidal membrane, mediates the first step of metformin entry into hepatocytes [14].

The action of metformin may depend on the expression of OCTs which act as influx transporters to transport metformin intracellularly [15]. T allele of SLC22A1 rs12208357 (Arg61Cys) polymorphism is strongly correlated with decreased OCT1 protein expression in Caucasian liver tissue samples [16] .

OCT transporters are Na + -independent carriers that mediate the facilitated uniport transport of different organic cations through the plasma membrane [17].

OCT1 showed high expressions variability among different individuals' human liver samples [16].

Wang et al., approve the role of OCT1 in active uptake of the metformin into hepatocyte as the lactate production is completely abolished in a transgenic mouse model, knockout of liver SLC22A1 [18]. The polymorphisms of SLC22A1 gene that results in change in the OCT1 function are associated with changes in metformin pharmacokinetics and dynamic [19].

A non-synonymous coding variants of OCT1 (rs12208357) is associated with reduction in OCT1 expression [16].

In the present study, OCT-1 SNP, patients with CT allele showed better RBS compared to patients with CC allele (p= 0.004), where there is insignificant differences regarding the effect of different alleles on HbA1c, RBS couldn't be considered as a respected parameter for determine the effect, hence OCT1 SNPs in our study didn't result in any differences in metformin action.

In agreement with our results was the GoDARTS study which didn't showed an associations between SLC22A1 rs12208357 variants and glycemic response [14].

On the other hand, our result is against shu et al., who showed the total abolishment of metformin action in the knockout mice [15], or those which showed association with rs12208357 variants and impaired or reduced glucose response [14 20 21-23] in healthy volunteer?? [15, 24] and diabetic patients [25 21].

On the same way, Nies et al., showed decreasing OCT1 protein expression in Caucasian liver sample in T allele of SLC22A1 subject. [16]

Many factor can affect OCT1 action; it showed substrate specificity, where 41C > T variant was associated with increased uptake of MPP + [26] but diminished metformin uptake [15]. Races also affect OCT1 genetic variants distribution and function; where the genetic variants R61C, G401S, 420del, and G465R, which are related to decreased metformin uptake, have not been identified in Korean, Asian American or Japanese populations and four variants of SLC22A1 (480C > G, 848C > T 1022C > T and 1222A > G) which are found in the Korean population were not associated with functional changes of metformin uptake, suggesting that OCT1 polymorphisms may not be the main contributors to the inter-individual pharmacokinetic variation of metformin in Korean and other East Asian populations [27], although this can't be considered as a rule all over the world.

The SLC47 family has two members named and toxin extrusion 1 (MATE1) and 2 (MATE2) [28]. MATE2 has two variants, MATE+2-K and MATE2-B, which have a different splicing pattern between exons 6 and 7. This codes for a 566 amino acid protein (MATE2-K) and a truncated protein with 220 amino acids (MATE2-B).

While MATE1 is expressed in many tissues specially the liver and kidney [29], MATE2 and MATE2-K are predominantly expressed in the human kidney [29]. MATE2-B has been detected in many organs but not in the kidney [29]; however, the physiological roles of MATE2 and MATE2-B remain unclear [28].

Metformin is excreted by kidney through action of OCT2 [30], and MATE1 and MATE2 K [31 32] [29]. MATE1 acting as an efflux pump in many tissue tissues such as the liver which play an important role in metformin pharmacodynamics [31 32].

The reduction of promoter expression of MATE1 result in reduction of transport level leading to decrease metformin efflux and increase tissue level which are predicted to result in greater metformin pharmacological action. The MATE1 variant appear to affect mainly metformin pharmacodynamics rather than kinetic as it may be the single metformin transporter on the bile. [33]

In the present work, Among MATE1 SNP, patients with CC or TT allele responds better to metformin compared to those with CT allele patients as evident by better HBA1C level (P= .04) and (P=.019) respectively.

Our result is against Stocker et al., who showed the T/C variant in the promoter of SLC47A1 gene is associated with a greater metformin action in T2DM patients using HbA1c as an indicator of efficacy [33].

Comparison between MATE1 variance and references who are MATE2 references nullify such effect.

Our present results are against Stocker et al., 2013 which showed that both MATE genotypes were associated with altered post-metformin glucose tolerance, with variant carriers of MATE2 reduced response in healthy volunteer [33].

MATEs are inhibited by many drugs e.g., pyrimethamine, baclofen, ketoconazole, propranolol, naloxone [34] and cimetidine [35] that may result in significant drug-drug interaction.

All patients' included in the current study didn't receive the above mentioned drugs prior or during this study.

In the current study, MATE2 SNPs or reference to variant alleles has insignificant differences in the effects on metformin action. Comparison between MATE2 variance and references who are MATE1 references showed insignificant effect differences.

Our results are against results of Stocker et al., 2013 which showed alteration of glucose level in both MATE genotypes of metformin treated healthy volunteer [33].

The expression of MATE in the apical part and the expression of OCT in the basolateral may result in the transcellular movement of

cations substrates that play an important role in transportation of these cations from the blood to the urine or bile[36].

In the present study, comparing MATE1 and MATE2 reference to variant alleles showed insignificant differences in their effects on metformin action

Our result is against Stocker et al., who showed that GG and GA carriers experience more improvement in HbA1c level following metformin administration compared to AA carriers [33], and Choi et al., in metformin treated patients, demonstrated that GG carriers better HbA1c level compared AA carriers after metformin treatment [37].

The beneficial effect of MATE2 could be explained by increase in renal gluconeogenesis and glucose uptake increases in type II diabetes, [38]. Thus, enhanced-expression of MATE2 associated with homozygous variant would have lower renal metformin level resulting in reduction of its pharmacologic effect.

The conflicting results between the present study and other studies may be related to small number included in the present study, different parameters used to evaluate the metformin effect, and racial factor; however multicenter study is needed to clarify such conflict.

In the present study, some limitation should be acknowledged such evaluation of metformin level which didn't assessed and highly encouraged to be explored in further studies.

From the current work we can conclude that MATE1 and OCT1 SNPs may have a potential role in metformin efficacy; however multi-centre study is needed for more clarification.

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