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Effects of the drugs gliclazide and metformin on solute carrier family 2 member 2 (SLC2A2) gene expression in type 2 diabetic patients in Vietnam

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ABSTRACT. In recent years, the number of people with type 2 diabetes mellitus has increased rapidly and it has become an important public health problem in Vietnam. To provide valuable information on the efficacy and safety of diabetes medications (metformin and gliclazide), we evaluated how they affected the expression level and mutations of the SLC2A2 gene in the liver of T2DM patients. The SLC2A2 gene was analyzed in 165 patients with T2DM and 54 control subjects. Anthropometry, clinical parameters, molecular analysis, and gene expression were examined. We discovered high levels of mRNA SLC2A2 expression in the livers of people with T2DM who did not receive drug treatment. These levels were 1.5 times higher when compared to other groups that received drug treatment. Mutations of the SLC2A2 gene sequence were recorded in two T2DM patients belonging to the therapy group (c.1127T>G (p.Met376Arg) in exon 9 and c.609T>C (p.Ser203Ser) in exon 5). Finally, we found a strong and

significant relationship between the glycemic control index and two enzymes, alanine aminotransferase and aspartate aminotransferase, with mRNA SLC2A2 expression under therapy. Our findings identified mutations of the SLC2A2 gene and a positive correlation between SLC2A2 gene expression and the effects of gliclazide and metformin in the liver of T2DM patients. This might contribute to a better understanding of the SLC2A2 gene, and the safety and tolerability of metformin and gliclazide therapy.

Key words: Gliclazide; Metformin; Type 2 diabetes mellitus; SLC2A2 gene; Vietnam

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the non-communicable diseases that is increasing in Vietnam. According to a published 2020 report, Vietnam has 5.76 million people with diabetes (Ngoc et al., 2020). Most of them have T2DM (Nguyen et al., 2015; Huynh et al., 2021). T2DM is caused by a disorder in the use of insulin in the body's tissues, also known as insulin resistance and/or impaired insulin secretion by the pancreas (Amin, 2018). Insulin resistance, which is the inability of cells to respond adequately to normal levels of insulin, occurs primarily within the muscles, liver, and fat tissue (Dadich, 2007). In the liver, insulin normally suppresses glucose release. However, in the setting of insulin resistance, the liver inappropriately releases glucose into the blood (Larsen et al., 2011).

T2DM typically accounts for 80-90% of all diabetes cases, with common complications including heart disease, kidney failure, peripheral neuropathy, diabetic retinopathy of the eye, or poor blood flow in the limbs, which may lead to amputations (WHO, 2011). The general goals of treatment for T2DM are to stabilize fasting plasma glucose (FPG) levels and weight, raise HbA1c to the ideal level to minimize complications, lose weight in obese people, and maintain a healthy weight (Simó and Hernández, 2002). Treatments should be based on certain principles, such as a combination of drugs to treat T2DM, diet and exercise, control FPG and blood lipids, using insulin as needed, especially during exacerbations of chronic conditions, infections, or severe cardiovascular disease (Maruthur et al., 2016).

Nowadays, metformin (MET) and gliclazide (GLIC) are most commonly used in the treatment of T2DM in Vietnam. Although some evidence suggests that MET reduces mortality (Ripsin et al., 2009; Palmer et al., 2016), it should not be used in people with severe kidney or liver disease (Vijan, 2010). Pharmacological studies have shown that metformin acts by (a) improving peripheral sensitivity to insulin, (b) inhibiting gastrointestinal absorption of glucose (Jackson et al., 1987; Klip and Leiter, 1990), and (c) decreasing hepatic glucose production. Excessive hepatic glucose production (HGP) is an important factor in the pathogenesis of T2DM (De Frenzo, 1988). Treatment with MET at dosages of 1000-2550 mg/day for up to three months significantly reduced basal HGP (typically by 10-30%) (Stumvoll et al., 1995). MET- induced suppression of hepatic gluconeogenesis, mediated in part by reductions in free fatty acids and lipid oxidation, has been suggested as the primary cause of the reduction in fasting hyperglycemia in T2DM. MET is recommended for the treatment of T2DM patients who are overweight or obese

because it helps maintain or reduce weight. However, the side effects of MET are gastrointestinal, so it should be started at a low dose (500 mg per day).

Meanwhile, GLIC is a sulfonylurea (SU) type of anti-diabetic medication, used to treat T2DM. The drug is taken orally and is used when dietary changes, exercise, and weight loss are not enough. The side effects include low blood sugar, vomiting, abdominal pain, rash, and liver problems (Kalra et al., 2018). GLIC is recommended to be used with caution in elderly patients, patients with significant kidney and liver problems (blood creatinine > 200 μmol/L, , while it is not recommended to be used in pregnant patients. The mechanism of GLIC is to stimulate the pancreas to produce insulin, which can reduce glucose in the blood by 50 to 60 mg/dl, reducing HbA1c levels by about 2%. GLIC selectively binds to sulfonylurea receptors (SUR-1) on the surface of the pancreatic beta-cells but does not link with sulfonylurea receptors (SUR-2A) in the heart, thus, it can protect cardiovascular (Lawrence et al., 2001). This binding effectively closes these K+ ion channels. This decreases the efflux of potassium from the cell, leading to the depolarization of the cell. This causes voltage-dependent Ca^{2+} ion channels to open, increasing the Ca2+ influx. Calcium can then bind to and activate calmodulin, which in turn leads to exocytosis of insulin vesicles, leading to insulin release (Urbanova et al., 2015). However, its classification is ambiguous, as the literature uses it as both a first-generation and a second-generation (Shimoyama et al., 2006) sulfonylurea.

Glucose transporter 2 (GLUT2) was also known as solute carrier family 2 (facilitates glucose transporter), which is encoded by the solute carrier family 2 member 2 (SLC2A2) gene (Joost et al., 2002). The SLC2A2 gene was expressed in tissues involved in glucose homeostasis, i.e., the liver, pancreatic β cells, kidney, and intestine (Thorens et al., 1990). Previous experiment models revealed increased SLC2A2 gene expression in diabetic kidneys (Vestri et al., 2001; Freitas et al., 2005; Freitas et al., 2009), as well as in T2DM tubular cells (Rahmoune et al., 2005). Furthermore, insertion of the GLUT2 protein into the proximal tubule brush border membrane in the kidney of diabetic rats has been described, providing an additional glucose route of entry into proximal tubule cells (Marks et al., 2003; Freitas et al., 2005). In addition, this gene is also highly expressed in hepatocytes and in pancreatic β cells (Wright, 2001). GLUT2 plays an important role in the glucose uptake by the hepatocyte after meals and in insulin secretion by β cells. Experimental diabetes identified an increased expression of the SLC2A2 gene in hepatocytes (Brichard et al., 1993), whereas it is decreased in β cells (Thorens, 1996).

Recognizing the important role of the SLC2A2 gene in encoding the GLUT2 protein, our study was conducted with the aim of investigating a possible correlation between the gene expression level and lipid profiles and glycemic control in the non-treatment and MET or GLIC therapy groups. Mutations of the SLC2A2 gene in the liver of T2DM patients were also analyzed.

MATERIAL AND METHODS

Patients

Our study focused on a total of 165 patients diagnosed with T2DM and 54 control subjects. In the group of diabetic patients, we divided into 3 groups, including 52 patients without treatment, 59 patients treated with MET drugs, and 54 patients treated with GLIC

drugs. All patients were randomly selected from those presenting to the Endocrinology Department of Hospital 19/8 at the Ministry of Public Security in 2020 with clinical exclusion criteria including type 1 diabetes mellitus, a history of endocrine disorders, pregnancy, heart failure, and cancer. The study protocol was approved by the Research Ethics Committee of Vietnam Military Medical University (Decision Number 2883/QĐ-HVQY), and all patients gave their informed consent before study commencement.

Anthropometry and Clinical parameters

Body mass index (BMI) was a person's weight in kilograms divided by the square of their height in meters. The waist-to-hip (W⁄H) ratio was calculated as the waist measurement divided by the hip measurement. Venous blood was collected after a minimum of 10 hours of fasting prior to the lipid profiles and glycemic control analysis. Fasting plasma glucose (FPG) and total cholesterol (TC) were measured in EDTA-plasma samples by using the hexokinase and cholesterol oxidase methods, respectively. Serum triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using the enzymatic peroxidase method by using an auto biochemistry instrument (Hitachi 7170, Tokyo, Japan). Glycosylated hemoglobin (HbA1c) was measured by high-performance liquid chromatography (HPLC) (Ultra Primus 2, Trinity Biotech Affinity Baronate Colons, U.S.A.). All patients' liver functions were determined by biomarkers, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), with normal ranges (ALT: 10-40 international units per liter (IU/L) and AST: 10-35 IU/L).

Molecular analysis and gene expressions

The patient's liver tissue was provided by the Genome Laboratory of Hospital 19/8. Then, total RNA was extracted using the RNAiso Plus reagent (Takara, Japan) according to the manufacturer's protocol. RNase-Free DNase (Promega, USA) was used to remove contaminating DNA. Total RNA (5μg) was reverse-transcribed into cDNA using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, USA) and stored at −20°C until analyzed.

The primers designed for qRT-PCR were used to clone part of the SLC2A2 coding sequence. To eliminate the possibility of interference from genomic DNA, these sequence and sense primers were designed for different exons. The following polymerase chain reaction (PCR) protocol was used. PCR products were then separated on agarose gels and purified using the TaKaRa MiniBEST Agarose Gel DNA Extraction Kit (Takara, Japan). Quantitative real-time reverse transcriptase PCR (qRT-PCR) was conducted on a 7300 Real-time PCR system (Applied Biosystems, USA) with two pairs of primers, each for the amplification of SLC2A2 and GAPDH. All amplifications were repeated twice. Amplifications were carried out with the SYBR$^®$ Premix Ex TaqTM Kit (Takara, Japan) at a final volume of 20 μL, containing 1 μL of cDNA sample, 10μL of SYBR Premix ExTaq (Takara, Japan), 0.4 μL of ROX Reference Dye, 1 μL of each primer, and 6.6 μL ddH$_2$O. Reactions without cDNA templates were used as controls. A dissociation curve analysis was performed after each assay to determine target specificity.

In addition, we sequenced the SLC2A2 gene for all patients who had T2DM. We amplified the 11 coding exons of the SLC2A2 gene by PCR (primers available on request). PCR products were sequenced using standard methods on an ABI 3700 (Applied Biosystems, Warrington, UK) and were compared with the published sequence NM_000340.1 using Mutation Surveyor v3.2 (SoftGenetics, State College, PA, USA).

Statistical analysis

Statistical analysis was performed using R and R-studio software (Team RC, 2018). Data were expressed as mean ± SD or frequency (%). One-way ANOVA with post hoc (least significant difference) analysis to assess for differences in body composition, anthropometric, metabolic, and hormonal parameters among the T2DM and control groups. The correlation of mRNA SLC2a2 gene expression with other clinical characteristics was determined using Pearson correlation analysis, with a significant value of $P < 0.05$. The sequenced PCR products were analyzed by Mutation Surveyor v3.2 (SoftGenetics, State College, PA, USA). Multiple sequence alignments were performed using Clustal-W tools, and this result was used to determine the nucleotide and amino acid mutations. The BioEdit bioinformatics tool was used for the nucleic acid sequences of two groups (patient and control) to translate and get protein sequences. The Ensemble database software supported all the exon and protein sequences. These sequences were used to determine the location of 11 exons in our study.

RESULTS

The clinical and blood characteristics analyses

The data on some clinical characteristics and biochemical parameters of each group are presented in Table 1. The ratio between male/female of control, non-treatment, and MET or GLIC drugs were 26/28, 20/23, 29/30 and 27/27, respectively. The lowest average age was in the control group (58.04 ± 10.7 years), and the highest was 61.96 ± 9.16 years for GLIC drug patients. The non-treatment, MET-treated, and GLIC-treated groups had disease durations of 4.5 ± 2.9, 5.9 ± 4.0, and 6.3 ± 4.2 years, respectively. BMI and WHR were recorded at 21.79 ~ 22.10 kg/m^2 and 0.82 ~ 0.85 in the control and treatment groups, respectively. The BMI of non-treatment patients was highest, at 23.5 ± 2.6 kg/m^2, and signaled the phenomenon of overweight for patients in this group.

Meanwhile, HbA1c, FPG, TC, and TG are the most commonly ordered tests for the diagnosis of diabetes and are also used for the prevention of microvascular complications associated with diabetes. The highest concentration of HbA1c in the non-treated group was recorded at 6.64 ± 0.9%, the lowest in the control group was 5.44 ± 0.59%. This was also observed for the FBG value in 3 groups of non-treated and using MET or GLIC patients: 7.52 ± 2.76, 7.15 ± 1.27, and 7.18 ± 1.99 mmol/L, respectively. They were much higher than those of the control group (5.33 ± 0.42 mmol/L). The value of TC showed that the mean value of the non-treated group was one and a half times higher than that of the control group and the group of patients assigned to MET or GLIC drugs 6.13 ± 0.77 mmol/L vs. 4.99 ± 0.74 mmol/L, 4.43 ± 1.1 mmol/L, or 4.7 ± 1.01 mmol/L. The concentration of TG used to screen lipid status in order to detect atherosclerotic risks and monitor lipid-lowering

measures in order to determine blood fat level was also tested in all four groups. The lowest value was 1.7 ± 0.51 mmol/L for the control. Under the effect of MET or GLIC, the TG value decreased lightly to 3.18 ± 2.50 mmol/L, 2.21 ± 1.43 mmol/L from 3.45 ± 2.38 mmol/L with non-treatment.

Table 1. Demographic, anthropometric, and metabolic characteristics of the study groups.

Group	Control	Non treatment	Metformin	Gliclazide
Numbers	54	52	59	54
Male/ Female	26/28	20/23	29/30	27/27
Age (years)	58.04 ± 10.70	61.58 ± 9.32	61.61 ± 8.23	61.96 ± 9.16
Duration time (years)	-	4.5 ± 2.9	5.9 ± 4.0	6.3 ± 4.2
BMI (kg/m^2)	21.79 ± 1.92	23.5 ± 2.6	21.79 ± 1.74	22.1 ± 1.81
WHR	0.82 ± 0.08	0.82 ± 0.09	0.85 ± 0.1	0.85 ± 0.11
HbA1c (%)	5.44 ± 0.59	6.64 ± 0.9	6.42 ± 1.33	5.61 ± 1.33
FPG (mmol/L)	5.33 ± 0.42	7.52 ± 2.76	7.15 ± 1.27	7.18 ± 1.99
ALT (Ul/L)	28.41 ± 6.8	39.54 ± 5.19	29.79 ± 8.34	31.93 ± 12.35
AST (Ul/L)	27.43 ± 4.33	31.87 ± 6.96	26.76 ± 6.36	28.48 ± 5.54
HDL Cholesterol (mmol/L)	1.58 ± 0.36	1.32 ± 0.44	1.36 ± 0.52	1.35 ± 0.12
LDL Cholesterol (mmol/L)	2.49 ± 0.59	2.72 ± 0.39	2.58 ± 0.67	2.69 ± 0.34
Total cholesterol (mmol/L)	4.99 ± 0.74	6.13 ± 0.77	4.43 ± 1.1	4.7 ± 1.01
Triglycerides (mmol/L)	1.7 ± 0.51	3.45 ± 2.38	3.18 ± 2.5	2.21 ± 1.43

Values are mean $\pm$ SD or frequency (%); *P < 0.01 vs Control group.

The findings also revealed a comparison of drug effects on plasma lipid levels. MET reduced LDL-C levels by about 0.14 mmol/L, whereas GLIC slightly decreased LDL-C levels. MET and GLIC had similarly minimal or no effect on HDL-C levels by a mean of 0.03 to 0.04 mmol/L compared with the non-treatment group. Two biochemical markers (AST and ALT), which are reasonably sensitive for liver damage, were also recorded in all 4 study groups. The quantification of these indicators can be used to diagnose the disease and distinguish the degree of cell damage in the liver. The highest AST and ALT values expressed in the non-treated group were 31.87 ± 6.96 Ul/L and 39.54 ± 5.19 Ul/L. Despite being treated with MET or GLIC, however, these values were still higher than those of the control group, AST: 27.43 ± 4.33 and ALT: 28.41 ± 6.8 Ul/L.

Molecular genetics and qRT-PCR

In our study, mutations were recorded in two T2DM patients in the MET therapy group. One of them had a homozygous SLC2A2 mutation c.1127T > G (p.Met376Arg) in exon 9 (Table 2) and another had a homozygous SLC2A2 mutation c.609T > C (p.Ser203Ser) in exon 5; however, this mutation resulted in no change in the amino acid sequence of the protein of the SLC2A2 gene (Table 2). Re-checking the blood parameters of the two patients mentioned above, there were no abnormalities in both cases of mutations in the SLC2A2 gene.

Meanwhile, the expression levels of the $_m$RNA SLC2A2 gene were also determined in all groups. There was no significant difference in the groups of patients treated with MET or GLIC drugs compared with the control group. However, we observed that the expression of mRNA SLC2A2 was markedly increased in the liver of patients with long-standing type 2 diabetes who were not indicated for treatment with either drug. The mRNA expression

level of the SLC2A2 gene in this group of subjects was 1.5 times higher than that of other groups of patients (Figure 1).

Table 2. The sequence mutations on the exon of the SLC2A2 gene.

| No	Ref Seq	Allele 1	Allele 2	Amino acid 1	Amino acid 2	Position in cDNA	Amino acid position	Exon	Change
Patient 1	T	T	C	Ser	Ser	609	203	5	c.609T>C p.Ser203Ser
Patient 2	T	T	G	Met	Arg	1127	376	9	C1127T>G p.Met376Arg
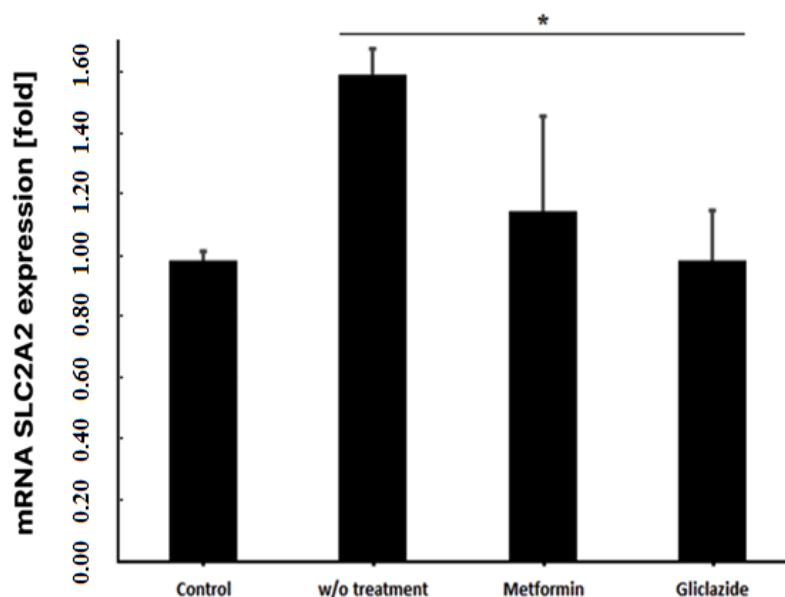
Figure 1. Real-time RT-PCR analysis of mRNA SLC2A2 gene expression. Data are means ± SE; *P < 0.05 vs Control.

The correlation of mRNA SLC2A2 expression with glycemic control, lipid profiles and clinical characteristics in all group

To evaluate the correlation of mRNA SLC2A2 gene expression with glycemic control, linear regression analysis was performed. Our results showed that in both treatment groups, mRNA SLC2A2 expression was positively correlated with glycemic control markers such as FPG and HbA1c, including MET drugs (FPG: $r = 0.268$, P = 0.041 and HbA1c: $r = 0.444$, P < 0.001) and GLIC drugs (FPG: $r = 0.567$, P < 0.001 and HbA1c: $r = 0.439$, P < 0.001), respectively (Figure 2).
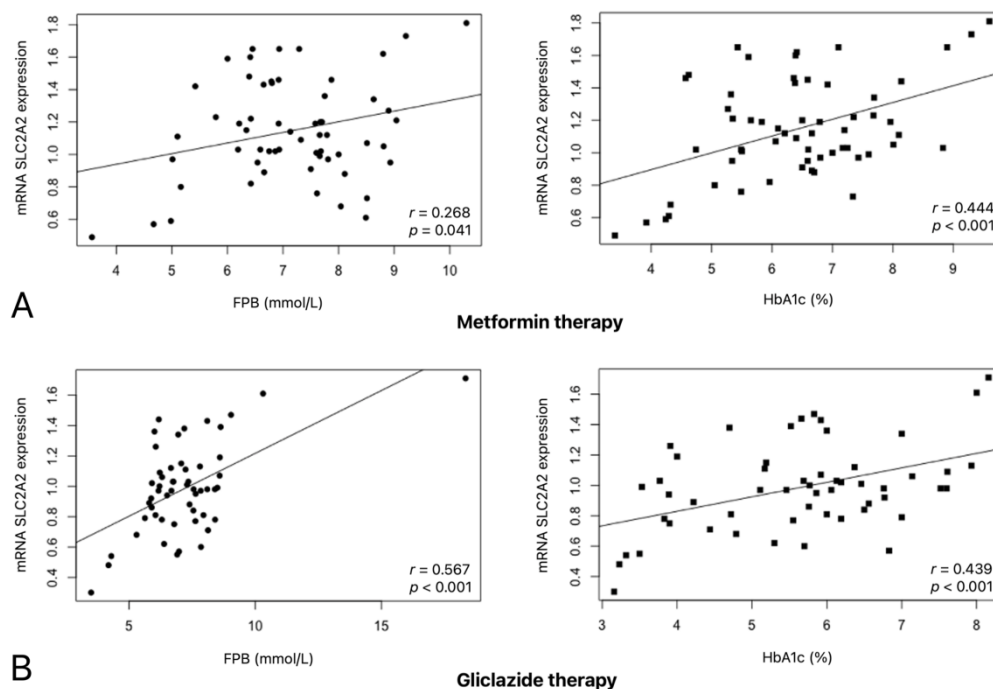
Figure 2. Correlations between mRNA SLC2A2 gene expression with FPG and HbA1b in the treatment group: Metformin group (A), and Gliclazide group (B).

In addition, a linear regression analysis was also used to evaluate the correlation between SLC2A2 gene expression with other metabolic parameters, and lipid profiles related to T2DM. Our results showed that in both MET and GLIC therapy groups, mRNA SLC2A2 gene expression was positively correlated with BMI, TC and LDL-C. In GLIC treatment, our results reported that SLC2A2 is positively correlated with BMI, WHR, TC and LDL-C, however, these results were not significantly different (P > 0.05). The *p*-value was greater than 0.05 for other parameters such as age, duration, and the triglyceride index, all of which were confirmed to be negatively correlated with the mRNA expression of SLC2A2 (Table 3).

Table 3. The correlation analysis results between clinical variables and mRNA SLC2A2 expression gene level in all groups.

Parameters	Control		Non-treatment		Metformin		Gliclazide	
	r	P-value	r	P-value	r	P-value	r	P-value
Age	-0.165	0.232	0.055	0.098	-0.046	0.079	-0.170	0.218
BMI	0.220	0.109	-0.051	0.074	0.015	0.109	0.117	0.401
WHR	0.016	0.108	-0.026	0.056	-0.154	0.243	0.087	0.058
Duration time	-	-	0.133	0.348	-0.036	0.085	-0.044	0.051
TC	-0.236	0.086	-0.037	0.093	0.183	0.165	0.082	0.553
TG	-0.079	0.565	-0.193	0.069	-0.066	0.119	-0.038	0.283
HDL-C	0.095	0.062	0.106	0.149	0.125	0.075	-0.082	0.063
LDL-C	0.032	0.168	-0.025	0.054	0.071	0.085	0.039	0.203

In addition, two hepatic enzyme markers (AST and ALT), usually measured in the blood to assess liver health, were found to be strongly and significantly correlated with mRNA SLC2A2 expression under therapy, $P < 0.001$ (Figure 3).
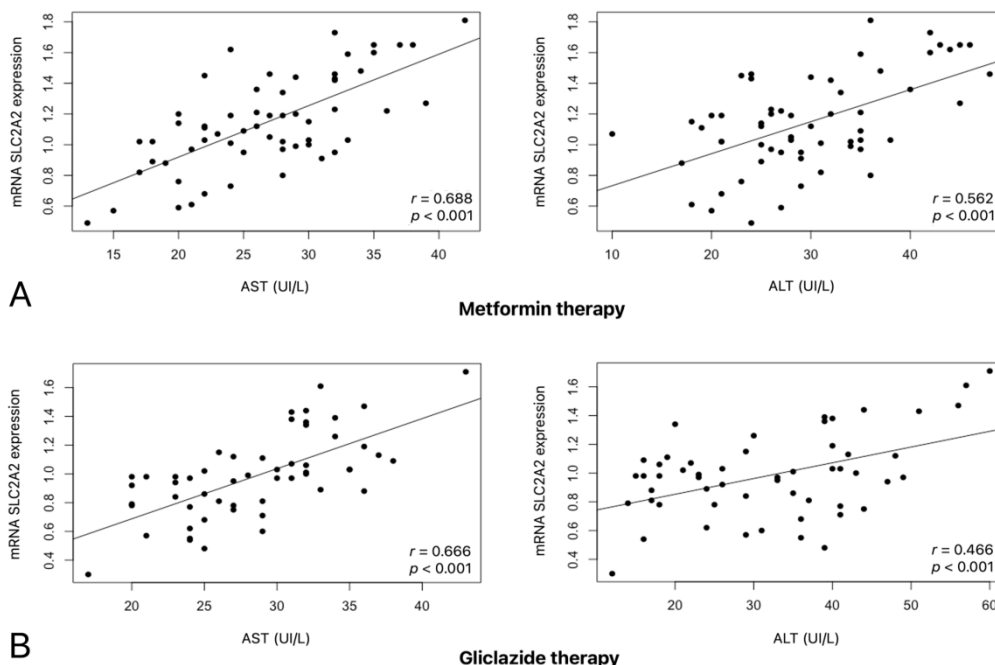
Figure 3. Correlations between SLC2A2 mRNA gene expression with Liver index (AST and ALT) in the treatment group: Metformin group (A), and Gliclazide group (B).

DISCUSSION

Metformin or gliclazide monotherapy is considered first-line treatment in patients who are prone to weight gain and/or are dyslipidemic and who have failed to achieve adequate glycemic control on dietary management alone (Sulfate, 2016). Our results suggested an effect on reducing fasting plasma glucose (FPG) concentration, glycated hemoglobin (HbA1c) levels, and maintaining stable body weight, although BMI index in this study not clear yet. For the group of patients assigned to be treated with MET or GLIC, HbA1c decreased by 0.22% in the MET group and 1.03% in the GLIC group from the original 6.64% in the non-treatment group. Although the FBG reduction in both groups was identified, FBG still maintained a higher rate than that of the control group.

Patients evaluated the antihyperglycemic and antihyperlipidemic efficacy, as well as the effect on body weight and blood pressure, in MET therapeutic trials. In controlled clinical studies, MET monotherapy (0.5–3 g/day) reduced FPG concentrations and HbA1c levels to a significantly greater extent than placebo (De Fronzo et al., 1995; Hermann et al., 1994). Over a one-year period, Harrower (1985) compared the glycemic efficacy of five

SUs (n = 112): chlorpropamide, glibenclamide, glipizide, glycineone, and gliclazide. The results showed that GLIC was significantly superior in HbA1c reduction compared to the other four SUs (Harrower, 1985). In a randomized double-blind multicentric study by Perreillo et al. (2006), they found similar reductions in HbA1c and FPG with gliclazide and pioglitazone (Perriello et al., 2006). The study by Foley et al. (2009) compared gliclazide with vildagliptin in treatment-naive T2DM subjects. The mean reduction in HbA1c from baseline was similar in both groups (-0.5% in vildagliptin vs. -0.6% in gliclazide). Moreover, FPG was significantly lower in the GLIC arm (Δ -0.5 mmol/l, $P < 0.025$) compared with vildagliptin. However, weight increase was significantly less with vildagliptin compared to GLIC (0.8 vs. 1.6 kg, $P < 0.01$) and fewer minor hypoglycemic episodes were observed in vildagliptin arm compared to GLIC (0.7 vs. 1.7%). Notably, no severe hypoglycemic episode was observed in either group (Foley and Sreenan, 2009). In another study, Filozoff and Gautier (2010) compared vildagliptin to GLIC in a background MET therapy. This study showed a similar reduction in HbA1c (Δ -0.03, 95% CI -0.11 to 0.20) in both arms. A similar reduction in FPG was also observed in both arms (1.31 vs. 1.52 mmoИ, $P = 0.257$). Although the hypoglycemic events (6 vs. 11 events in GLIC) and body weight gain (+0.08 vs. +1.36 in gliclazide, $P < 0.001$) were lower in the vildagliptin group; higher number of patients discontinuing therapy in the vildagliptin group (22 vs. 13 in GLIC) was due to an unsatisfactory glycemic effect (Filozoff and Gautier, 2010).

However, Jerums et al. (1987) discovered no significant difference in glycemic control in the GLIC arm over others in a prospective double-blind controlled study. In a retrospective chart review, they evaluated the prevalence of hypoglycemia in older (age 40–65) patients taking oral hypoglycemic agents. This study showed a significantly higher prevalence of hypoglycemic symptoms in patients treated with glibenclamide compared to GLIC ($P < 0.01$) or chlorpropamide ($P < 0.05$), despite similar HbA1c level (Jennings et al., 1989). Van Staa evaluated hypoglycemia in patients treated with SUs and found a higher rate of hypoglycemia for glibenclamide compared to other SUs, including GLIC (Van Staa et al., 1997). Inukai et al. (2005) showed no difference in glycemic control (HbA1c and fasting plasma glucose) when patients were switched to glimepiride from GLIC and glibenclamide. However, the HbA1c reduction was significantly better in the group switching to glimepiride.

Besides, the total cholesterol (TC) and triglyceride (TG) lowering effects of the treatment with MET and GLIC were also determined. TC levels were maintained at 4.43 mmol/L, compared with 4.99 mmol/L in the control group and 6.13 mmol/L in the non-treated group. The TG concentration was 3.18 mmol/L, a decrease of 0.27mmol/L compared with the non-treated group, which was still 1.7 mmol/L higher than the control group. In the Landin et al (1991) study, nondiabetic and nonobese untreated hypertensive, MET therapy reduced TC and TG, while improving insulin sensitivity, decreasing plasma insulin, and markedly blood pressure. In addition, the relationships between FPG, HbA1c control, TC, TG and lipid profiles were evaluated for T2DM patients in some studies reported. Mullugeta et al. (2012) reported that HbA1c was positively associated with TC but not with LDL-C. However, some studies showed a positive correlation between HbA1c, LDL-C, and TC (Ozder, 2014). Other studies reported that LDL-C was not associated with HbA1c (Begum et al., 2019) or T2DM (Gupta et al., 2008; Qi et al., 2012), and even that lower LDL-C could increase the incidence of T2DM (Sattar et al., 2010; Andersson et al., 2015).

In general, no primary effects on the lipid profile induced by MET or GLIC were seen in our study. However, in some studies, improvement in lipid profile abnormalities associated with T2DM has been reported with GLIC therapy (Cathelineau et al., 1997). Previous studies have demonstrated improved lipid profiles in patients with type 2 diabetic dyslipidemia receiving MET (Scarpello and Howlett, 2008). In the present study, we demonstrated significant improvements in lipid profile with gliclazide-MR monotherapies, whereas lipid profile was not significantly different from baseline in the MET group. TC and TG (borderline) in the gliclazide-MR group decreased (Erem et al., 2014). In either group, no patient had an AST or ALT level that was more than twice the normal.

In the current study, liver SLC2A2 mRNA expression was significantly increased in non-treatment T2DM patients. Previous studies showed that changes in SLC2A2 expression partially contribute to the glucose regulation by SLC2A2, which is a membrane-bound, insulin-independent glucose transporter that is mainly expressed in the liver. The protein coded by SLC2A2 (GLUT-2) was evaluated as a major contributor to glucose and fructose homeostasis in the liver (Wood and Trayhurn, 2003; Manolescu et al., 2007). GLUT-2 is the main fructose transporter in hepatocytes, where most of the ingested fructose is metabolized. It plays an important role in the metabolic disorders associated with fructose consumption (Douard and Ferraris, 2013). In studies on the renal proximal tubule of diabetic rats, Freitas et al. (2005; 2009) showed that HNF-1α and HNF-3β play an important role in the overexpression of the SLC2A2 gene when treated with insulin. Besides, GLUT-2 may be involved in insulin resistance where its expression increases in the liver (Liu et al., 2015; Mathur et al., 2015; Narasimhan et al., 2015). In this study, SLC2A2 expression was significantly reduced by MET and GLIC. Interestingly, SLC2A2 expression was nearly normal in the GLIC treated group. These findings are consistent with those obtained by Wang et al. (2017), who discovered that T2DM patients have a significant increase in SLC2A2 mRNA levels. In addition, the results of our study may indicate that an association between the two hepatocellular nuclear factors (HNF-1α and HNF-3β) and the treatment of T2DM patients with MET and GLIC. However, further studies are needed to determine this potential relationship.

Mutations in the SLC2A2 gene of patients with type 2 diabetes in Vietnam were also determined in our study by the Sanger sequencing method. In which the mutation of the SLC2A2 gene leading to the replacement of an amino acid in the protein translation process, c.1127T > G (p.Met376Arg) in exon 9, was also demonstrated in a study on neonatal diabetes patients by Sansbury et al. (2012). Other studies showed that homozygous recessive inactivating mutations in humans resulted in less frequent and less severe neonatal diabetes than biallelic inactivation of SLC2A2 in mice (Guillam et al., 1997). This is in keeping with GLUT2 being produced in lower amounts and having a less important role in glucose-stimulated insulin secretion in humans than in rodents (De Vos et al., 1995). In addition, the results also found a mistaken position in the SLC2A2 gene; however, there was no error in the amino acid translation process. It has been proposed that GLUT2 may have a role as a signaling molecule as well as a simple transporter (Leturque et al., 2009). GLUT2 supports insulin secretion when expression levels are adjusted to ensure a similar glucose flux (Hughes et al., 1993). A common SNP near SLC2A2 was found to influence fasting glucose (Dupuis et al., 2010), but further investigation revealed no evidence of a liver function defect. The two patients carrying the above mutation were in the long-term

treatment group with MET, so it was hard to determine the effect of this mutation on the overexpression of the SLC2A2 gene in the liver and other biochemical indices.

Our study found that mRNA SLC2A2 expression was high in the T2DM lever without drug treatment. Mutations of the SLC2A2 gene were identified in the therapy group. A strong correlation was found between gene expression and the effects of gliclazide and metformin in the liver of T2DM. Regarding safety and tolerability, GLIC and MET were well tolerated. There were no adverse events or side effects (hypoglycemia, gastrointestinal side effects, skin rash, ankle edema, pulmonary edema, congestive heart failure, myocardial infarction or cardiovascular death) in all treatment groups. Although there are still disagreements in previous studies, our results also contribute significantly to the safety of GLIC and MET being used in the treatment of T2DM in Vietnam.

ACKNOWLEDGMENTS

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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