Effects of Metformin Combined with Dapagliflozin on Homocysteine, Cystatin C and Beta-2 Microglobulin Levels in Patients with Diabetes Mellitus

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We attempt to discuss the effect of metformin combined with dapagliflozin on homocysteine, cystatin-c and beta-2 microglobulin levels in patients with type 2 diabetes mellitus. We selected 150 type 2 diabetes mellitus patients treated in our hospital from July 2018 to October 2021. According to random number table method, we divided them into observation group (metformin tablets combined with dapagliflozin tablets) and control group (metformin hydrochloride tablets), with 75 cases in each group. All were continuously treated for 24 w. After treatment, compared both groups on blood glucose level, islet function indexes levels before and after treatment, homocysteine, cystatin-c, beta-2 microglobulin and lipid metabolism indexes, and then recorded adverse reactions rate of both groups. After treatment, observation group possessed lower fasting blood glucose, 2 h postload blood glucose, hemoglobin A1c and body mass index levels than control group ($p<0.05$), and lower homocysteine, cystatin-c and beta-2 microglobulin levels than control group ($p<0.05$). Observation group possessed higher high-density lipoprotein cholesterol, homeostasis model assessment-beta cell function and low-density lipoprotein cholesterol levels than control group, but lower triglyceride, homeostasis model assessment-insulin resistance and total cholesterol levels than control group ($p<0.05$). The adverse reactions rate of observation group (13.33 %) had no remarkable difference with control group (9.33 %) ($p>0.05$). Metformin combined with dapagliflozin can effectively reduce homocysteine, cystatin-c and beta-2 microglobulin levels in type 2 diabetes mellitus patients with good safety.

Key words: Metformin, dapagliflozin, diabetes mellitus, homocysteine, cystati-C, beta-2 microglobulin

Diabetes Mellitus (DM) is a type of heterogeneous chronic metabolic disease characterized by hyperglycemia caused by defects in insulin secretion and utilization, which is related to genetic, environmental and autoimmune factors, among which Type 2 Diabetes Mellitus (T2DM) is more common[1,2]. Studies have found that T2DM can develop macrovascular, microvascular and neuropathy with the progression of the disease. Among them, hyperglycemia with Homocysteine (HCY) can remarkably increase the risk of cardiovascular complications, while hyperglycemia with increased Cystatin-c (Cys-C) and beta-2 Microglobulin (β2-MG) levels suggests diabetic nephropathy in T2DM patients[3,4]. Metformin is widely adopted in treating T2DM patients clinically by enhancing the glucose uptake ability of peripheral tissues and improving insulin sensitivity, which can achieve the effect of regulating insulin resistance and lowering blood glucose concentration. However, there are still some patients whose clinical application is limited due to the poor efficacy of single drug use and the greater damage to gastrointestinal function caused by long-term drug use[5]. Dapagliflozin is a sodium-glucose co-transporter inhibitor hypoglycemic drug, which can achieve hypoglycemic effect by promoting the excretion of urine glucose[6]. This study mainly attempted to explore and analyze the effect of metformin combined with dapagliflozin on HCY, Cys-C and β2-MG levels in T2DM patients. We selected 150 patients with T2DM treated in our hospital from July 2018 to

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October 2021. Inclusion criteria meets the diagnostic criteria of T2DM[7]; course of disease ≥12 mo; patients and their families know about the situations and give informed consent. Exclusion criteria, combined with acute and chronic infectious diseases of various tissues and organs; combined with malignant tumors; combined with immune system diseases; have allergic reactions to the drugs adopted in our study. We randomly divided the patients in this study into control group (75 cases) and observation group (75 cases). 39 males and 36 females included in observation group, ages were from 32 to 65 y old and average was about (47.69±5.32) y old. 40 males and 35 females included in control group, ages were from 33 y to 65 y old and average was about (47.72±5.26) y old. Both groups possessed no remarkable difference on general data ($p>0.05$). The hospital ethics committee approved this study. After admission, checked blood glucose regularly for both groups and received basic treatments such as anti-infection, fluid replacement, maintenance of acid-base balance, DM exercise and diet. Control group received metformin hydrochloride tablets (Youcare Pharmaceutical Group Co., Ltd., gyzz H20051289, 60 tablets 0.5 g each) orally, 250 mg/time twice daily. Observation group received oral treatment with metformin hydrochloride tablets combined with dapagliflozin tablets (AstraZeneca Pharmaceuticals Co., Ltd., gyzz J20170040, 14 tablets 10 mg each), in which the usage of metformin tablets was the same as that of the control group and dapagliflozin tablets 10 mg/time once daily. Both groups received continuous treatment for 24 w. Before and after treatment, extracted fasting venous blood and 2 h postprandial venous blood from both groups for spare use, adopted blood glucose meter to detect FBG and 2hPG levels and glycation analyzer to detect Hemoglobin A1c (HbA1c) levels; recorded the body mass changes of both groups and calculated Body Mass Index (BMI); adopted chemiluminescence immunoassay to detect Fasting Insulin (FINS) level, and calculated Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) and Homeostasis Model Assessment-beta cell function (HOMA-β); adopted enzyme-linked immunosorbent assay (all kits were produced by Bohui Biotechnology (Guangzhou) Co., Ltd.) to detect serum HCY, Cys-C and β2-MG levels; Used 7600 automatic biochemical analyzer produced by Hitachi to detect Triglyceride (TG), Total Cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C) and LDL-C levels and recorded the occurrence of adverse reactions of both groups. Adopted Statistical Package for the Social Sciences (SPSS) 22.0 to process and analyze data. Expressed the measurement data (blood glucose levels after treatment and pancreatic islet function indexes before and after treatment, HCY, Cys-C, β2-MG and lipid metabolism indexes) by ($\bar{x}\pm s$). Expressed the difference by t-test. Expressed the counting data (the number of adverse reactions during treatment) by percentage (%) and expressed the difference by Chi-square (χ^2) test. $p<0.05$, indicating that the divergence possessed statistical significance. After treatment, observation group possessed remarkably higher Fasting Blood Glucose (FBG), 2 h Postload Blood Glucose (2hBG), HbA1c and BMI levels than control group ($p<0.05$). As shown in Table 1. After treatment, observation group possessed lower HOMA-IR level than control group, but higher HOMA-β level than control group ($p<0.05$). As shown in Table 2. Before treatment, both T2DM patients had no significant difference in HCY, Cys-C and β2-MG lelvels ($p>0.05$); after treatment, observation group possessed remarkably lower HCY, Cys-C and β2-MG levles than control group ($p<0.05$). As shown in Table 3. After treatment, observation group possessed remarkably lower TG and TC levels than control group, but remarkably higher HDL-C and Low-Density Lipoprotein Cholesterol (LDL-C) levels than control group ($p<0.05$). As shown in Table 4. The adverse reactions rate of observation group was 13.33 %, which was not remarkably different from 9.33 % of control group ($p>0.05$). As shown in Table 5. DM is a chronic metabolic disease caused by the body's insulin secretion disorder or relative underutilization, it mainly appears as T2DM in clinic[8,9]. At present, biguanide hypoglycemic drugs represented by metformin are the first choice drugs for treating T2DM patients, but patients with a longer course of disease have poor clinical efficacy due to the progressive decline of islet β-cell function[10]. Dapagliflozin inhibits the renal reabsorption of glucose in a non-insulin-dependent manner, promotes the timely excretion of urine glucose and is beneficial to hypoglycemic therapy[11,12]. We attempt to discuss the influence of metformin combined with dapagliflozin on HCY, Cys-C and β2-MG levels in T2DM patients. Our results showed that observation group possessed remarkably higher FBG, 2hBG, HbA1c and BMI levels than control group after treatment, indicating that metformin combined with dapagliflozin could effectively reduce the blood sugar level and improve the body mass index of T2DM patients. Metformin can directly act on the body's glucose metabolism, promote glycolysis, reduce glycogenolysis and delay the damage of insulin β

cells[13]. Dapagliflozin is an Sodium-Glucose Cotransporter-2 (SGLT2) inhibitor, which can effectively reduce the sugar absorption of renal tubular epithelial cells, so that excess glucose can be quickly excreted through urine. Moreover, because it does not depend on insulin secretion, it has the advantage of not increasing BMI of patients and effectively exerts its hypoglycemic effect[14,15]. Lu *et al.*[16] and other studies found that dapagliflozin combined with metformin could effectively reduce the blood glucose level in T2DM patients, which was basically consistent with the results of this study, confirming the exact efficacy of metformin combined with dapagliflozin in treating T2DM patients. In this study, HOMA-IR level of observation group decreased, but HOMA-β level increased, indicating that metformin combined with dapagliflozin had a definite improvement effect on insulin β cells in T2DM patients. Insulin resistance and insulin β-cell damage are the main factors leading to the accelerated progression of T2DM and laboratory tests can be manifested as increased HOMA-IR and decreased HOMA-β. Analysis of the reasons may be related to that dapagliflozin can effectively control HbA1c level and promote the secretion of intestinal hypoglycin and the synthesis of glucokinase. In this study, observation group possessed remarkably lower HCY, Cys-C and β2-MG levels after treatment than control group, indicating that metformin combined with dapagliflozin could remarkably reduce HCY, Cys-C and β2-MG levels, which was beneficial to improve the risk of cardiovascular and renal complications in T2DM patients. HCY is an intermediate metabolite produced by methionine metabolism, which is closely related to insulin sensitivity and Cys-C has a significant promoting effect on the secretion of HCY. Elevated HCY and Cys-C levels can lead to aggravation of vascular endothelial damage in patients and increase the risk of cardiovascular complications[17,18]. β2-MG is a low molecular weight serum globulin with good stability, which can be absorbed by the proximal convoluted tubule after being filtered by the glomerular filtration membrane. The increase of serum β2-MG in T2DM patients often indicates that the glomerular filtration function of the patient is damaged[19]. In this study, observation group possessed remarkably higher HDL-C and LDL-L levels than control group, indicating that metformin combined with dapagliflozin could effectively improve the lipid metabolism in T2DM patients. Due to the low insulin content in the body, T2DM patients need to consume a large amount of fat for energy, resulting in abnormal lipid metabolism in the body, which further increases the risk of cardiovascular events. The blockade of SGLT2 receptors in dapagliflozin is beneficial to rapid osmotic diuresis, significantly increasing urinary glucose excretion, increasing lipid utilization and improving the body's lipid metabolism. Li *et al.*[20] and other studies found that dapagliflozin combined with metformin could increase HDL-C and LDL-L levels in T2DM patients, which was basically consistent with our results, confirming that dapagliflozin combined with metformin has a high improvement effect on lipid metabolism in T2DM patients. In this study, the adverse reaction rates of both groups had no remarkable difference, indicating that the combined treatment of T2DM patients can avoid serious adverse reactions and the treatment safety is good. In conclusion, metformin combined with dapagliflozin is effective in treating T2DM, can effectively reduce blood glucose level, HCY, Cys-C and β2-MG levels, and improve lipid metabolism and insulin β cells, with good safety. The disadvantage of this study is that the number of samples is limited and the changes of oxidative stress indicators in T2DM patients with combined therapy have not been analyzed. In the follow-up, it is necessary to increase more samples for further research and verification.

TABLE 1: COMPARISON OF BLOOD GLUCOSE LEVELS AND BMI OF BOTH GROUPS FOR T2DM PATIENTS BEFORE AND AFTER TREATMENT (x̄±s)

Group	Cases	FBG (mmol/l)		2hBG (mmol/l)		HbA1c (%)		BMI (kg/m^2)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	75	7.54±0.83	6.17±0.52	11.49±0.98	9.76±0.83	8.33±0.92	5.45±0.56	27.69±1.45	25.42±1.25
Control group	75	7.56±0.89	6.68±0.64	11.47±1.03	8.47±0.78	8.34±0.95	6.67±0.62	27.61±1.49	26.33±0.97
t		0.142	6.356	0.121	9.808	0.065	12.646	0.333	4.980
p		0.887	<0.001	0.903	<0.001	0.947	<0.001	0.739	<0.001

Note: FBG: Fasting Blood Glucose; 2hBG: 2 h Postload Blood Glucose; HbA1c: Hemoglobin A1c and BMI: Body Mass Index

TABLE 2: COMPARISON OF FINS, HOMA-IR AND HOMA-β LEVLES OF BOTH GROUPS FOR T2DM PATIENTS BEFORE AND AFTER TREATMENT (x̄±s)

Group	Cases	FINS (mU/l)		HOMA-IR		HOMA-ß		BMI (kg/m²)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	75	8.93±0.82	7.86±1.45	3.53±0.25	1.85±0.36	39.83±4.15	145.27±16.13	27.69±1.45	25.42±1.25
Control group	75	8.84±1.17	7.59±1.38	3.47±0.34	2.19±0.42	38.91±4.24	106.95±10.07	27.61±1.49	26.33±0.97
t		0.545	1.168	1.231	5.323	1.342	17.452	0.333	4.980
p		0.586	<0.001	0.220	<0.001	0.181	<0.001	0.739	<0.001

Note: FINS: Fasting Insulin; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance and HOMA-ß: Homeostasis Model Assessment-beta cell function

TABLE 3: COMPARISON OF HCY, Cys-C and β2-MG LEVELS OF BOTH GROUPS FOR T2DM PATIENTS BEFORE AND AFTER TREATMENT (x̄±s)

Group	Cases	HCY (mg/l)		Cys-C (μmol/l)		ß2-MG (mg/l)		BMI (kg/m²)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	75	1.36±0.24	0.61±0.13	14.53±0.69	8.12±0.31	4.25±0.99	2.25±0.17	27.69±1.45	25.42±1.25
Control group	75	1.35±0.28	0.96±0.19	14.55±0.72	11.04±0.56	4.24±0.93	3.28±0.33	27.61±1.49	26.33±0.97
t		0.235	13.166	0.173	39.507	0.063	24.029	0.333	4.980
p		0.814	<0.001	0.862	<0.001	0.949	<0.001	0.739	<0.001

Note: HCY: Homocysteine; Cys-C: Cystatin C and ß2-MG: Beta-2 Microglobulin

TABLE 4: COMPARISON OF BLOOD LIPID INDEX LEVELS OF BOTH GROUPS FOR T2DM PATIENTS BEFORE AND AFTER TREATMENT (x̄±s)

Group	Cases	TG (mmol/l)		TC (mmol/l)		HDL-C (mmol/l)		LDL-C (mmol/l)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	75	3.03±0.28	1.45±0.47	6.17±0.33	4.81±0.25	1.15±0.85	1.94±0.73	1.16±0.35	1.82±0.45
Control group	75	3.05±0.21	2.26±0.34	6.15±0.34	5.36±0.29	1.16±0.83	1.57±0.71	1.14±0.36	1.49±0.43
t		0.495	12.092	0.365	12.440	0.073	3.147	0.344	4.591
p		0.621	<0.001	0.715	<0.001	0.942	<0.001	0.730	<0.001

Note: TG: Triglyceride; TC: Total Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol and LDL-C: Low-Density Lipoprotein Cholesterol

TABLE 5: ADVERSE REACTIONS RATE OF BOTH GROUPS FOR T2DM PATIENTS (cases %)

Group	Cases	Bloating	Nausea	Fever	Hypoglycemia	Total adverse reaction rate
Observation group	75	5 (6.67)	2 (2.67)	1 (1.33)	2 (2.67)	10 (13.33)
Control group	75	3 (4.00)	2 (2.67)	1 (1.33)	1 (1.33)	7 (9.33)
χ^2						0.597
p						0.440

Authors' contributions:

Hui Zhou and Jinya Ding have contributed equally to this work.

Conflict of interests:

The authors declared no conflicts of interest.

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