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Effect of Metformin Treatment On Multiple Cytokines And Oxidative Stress In Type 2 Diabetes Mellitus Patients

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Abstract

Objective: To investigate the effect of metformin treatment on multiple cytokines and oxidative stress in type 2 diabetes mellitus (T2DM) patients.

Methods: The 130 newly diagnosed T2DM patients were randomly divided into an observation group and a control group, with 40 cases in each group. The patients in the control group were treated conventionally, and the patients in the observation group were given 500 mg of metformin twice a day after meals for 3 months. The ankle-brachial index, oxidative stress index level, inflammatory cytokine level, pain degree (VAS score), clinical symptom scores and treatment effect were compared between the two groups before and after treatment.

Results: The results showed that the clinical symptom scores were significantly lower, and the ABI index was significantly higher in the observation group after treatment than those in the control group and before treatment ($P < 0.05$). The levels of isoprostaglandin F2α and oxidized low-density lipoprotein were lower and the superoxide dismutase level was higher in the observation group after treatment than those in the control group and before treatment ($P < 0.05$). After treatment, the pain degree score in the observation group was lower than that in the control group ($P < 0.05$). The total effective rate in the observation group was 97.50%, which was higher than the 72.50% in the control group.

Conclusion: Metformin treatment can significantly improve the clinical symptoms of T2DM patients and sufficiently reduce the level of oxidative stress, while has no significant effect on the inflammatory cytokines in patients.

Keywords: type 2 diabetes mellitus; metformin; inflammatory cytokines; oxidative stress

Introduction

In recent years, with the rapid development of society and economy, the substantial improvement of living standards and the continuous changes in lifestyles, the incidence of diabetes mellitus has shown a rapid increase worldwide and has become the third chronic non-communicable disease that seriously threatens human health after cardiovascular and cerebrovascular diseases and tumors [1, 2]. The expansion and rapid development of the population of diabetic patients has brought serious economic burdens and social pressures to countries [3].

Insulin resistance and insufficient secretion of pancreatic β-cells are the main pathogenesis of type 2 diabetes mellitus (T2DM) [4]. Adipose tissue is closely related to the formation of insulin resistance. It can not only actively participate in the balance of energy metabolism, but also secrete many adipocytokines to regulate the functions of itself and other tissues. However, with the development of urbanization and changes in people's lifestyles, the intake of high-calorie foods and lack of exercise lead to excessive accumulation of adipose tissues [5], resulting in overweight or obesity. The adipose tissues of these overweight or obese people have a certain degree of endocrine regulation dysfunction, which leads to the imbalance in the secretion of adipocytokines. Studies have shown that the oxidative stress

process triggered by the mutual activation of adipocytokines through the regulation of immune and inflammatory responses, is one of the causes of a series of changes in the body environment, leading to B cell apoptosis and impaired insulin secretion stimulated by glucose [6, 7]. Metformin can reduce weight and improve insulin resistance. It is jointly recommended by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) as the first-line medication for early T2DM patients [8]. It can increase the sensitivity of the body to insulin through regulation on nuclear receptor gene transcription. A study has shown that it can affect the serum levels of some adipocytokines [9]. Therefore, studying the physiological effects of adipocytokines and the mechanism of the drugs on adipocytokines can further clarify the pathogenesis of diabetes and insulin resistance, which may provide new ideas for the development of new drugs for the treatment of insulin resistance and the treatment of diabetes.

Herein, our study aimed to explore the the effect of metformin treatment on multiple cytokines and oxidative stress in T2DM patients.

1. Materials and methods

1.1 Clinical data

The 130 newly diagnosed T2DM patients from May 2019 to May 2021 in outpatient department of our hospital were randomly divided into an observation group and a control group. The method of randomization was that an independent researcher who had no direct contact with the participants used random computer-generated numbers to divide the participants into the two groups. A total of 80 patients in the two groups who had enough blood samples for subsequent experiments were included in this study. There were 40 cases in the observation group, including 18 males and 22 females, aged 53.48 ± 13.64 years old. There were 40 cases in the control group, including 26 males and 14 females, aged 49.90±9.46 years old. No significant difference was discovered in the general data of patients between both groups ($P > 0.05$). Inclusion criteria: 1) Newly diagnosed T2DM, which met the diagnosis criteria of T2DM published by the WHO in 1999 [10] (diagnosed as T2DM within 3 months before enrollment); 2) Those who were voluntary and able to provide the signed informed consent, and willing to cooperate to complete the trial; 3) 18-70 years old; 7% ≤ HbAlc ≤ 11%; 4) Those who did not take oral hypoglycemic drugs (including Chinese patent medicines) or insulin therapy on the basis of diet and exercise control. Exclusion criteria: 1) Patients with type 1 diabetes, gestational diabetes and other special types of diabetes; 2) Those who were not able to cooperate to complete the trial due to inability to communicate normally or for other reasons; 3) Those who had incomplete data with a missing rate of >5%; 4) Those who combined with severe chronic complications such as insufficiency of heart, liver and kidney; 5) With other endocrine system diseases, such as hyperthyroidism and hypercortisolism; 6) With stress conditions such as surgery, severe trauma, etc.; 7) Combined use of drugs affecting glucose metabolism; those with tumors; 8) Those who were not able to cooperate to complete the follow-up.

1.2 Treatment methods

The patients in the control group were treated conventionally, which mainly focused on the reduction of blood lipid level, blood sugar level, and blood pressure level, etc. [8-9]. The patients in the observation group were given 500 mg of metformin (Merck Pharmaceutical (Jiangsu) Co., LTD) twice a day after meals for 3 months of continuous treatment. During the treatment, angiotensin-converting-enzyme (ACE) inhibitors, aspirin, adrenergic receptor agonists, glucocorticoids, insulin, female, and male hormones, etc., were not used. If there was dizziness, diarrhea, vomiting, edema, and other discomforts during the course of treatment, patients could decide whether to withdraw from the study according to the severity.

1.3 Sample collection

All subjects were fasted overnight for more than 10 h. In the morning, 6 ml of cubital venous blood was taken, and 2 ml of blood sample was collected and injected into the anticoagulation test tube for the following detection of hemoglobin A1c (HbA1c). The remaining blood sample was injected into the non-anticoagulation test tube and centrifuged at 300 rpm/min for 10 min within 2 h. The serum was separated, and 2 ml of serum was collected and stored in the refrigerator at -70ºC for the following concentrated detection of serum adiponectin (ADP), visceral adipose-specific SERPIN (Vaspin), and interleukin-6 (IL-6). The remaining serum was used to determine fasting plasma glucose (FPG) and blood lipid profile: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The cubital venous blood was drawn 2 h after the meals, and the serum was collected by centrifugation in the same way, which was used for the determination of 2-hour postprandial blood glucose (2hPG) and 2-hour postprandial insulin (2hFINS). All indicators were reviewed in the same way after three months of treatment.

1.4 Detection of ankle brachial index (ABI)

The automated arteriosclerosis monitor measures the patient's ABI, which is the ratio of the systolic pressure of the ankle artery of the lower limb to that of the brachial artery of the upper limb. The measurement method was as follows: Before the measurement, the patient was instructed to stay in a quiet state for at least 5 minutes, lie supine on the diagnosis and treatment bed, and bind the blood pressure cuff to the brachial artery of both upper arms and ankle arteries of both lower limbs respectively. The balloon mark of the cuff cuff in the upper arms corresponded to the brachial artery, and the balloon mark of the cuff cuff in the lower limbs was located inside the ankle of the lower limbs. The normal ABI value ranged from 0.91 to 1.40, and an ABI lower than 0.91 indicated the possibility of lower limb artery stenosis or obstruction.

1.5 Observation indicators

The clinical symptom scores, ABI, levels of oxidative stress indicators (8-isoprostaglandin F2α, oxidized low-density lipoprotein, and superoxide dismutase), levels of inflammatory cytokines (IL-10, IL-6 and TNF-α) and pain degree were compared between the two groups before and after treatment, and the treatment effect of the two groups were observed.

1.6 Evaluation criteria

(1) The total clinical symptom scores were 1-16, and the score was directly proportional to the severity of the patients' condition.

(2) The visual analogue scale (VAS) score was selected to assess the pain degree of the two groups of patients [11]. It had 10 scales, ranging from 0 to 10. According to the patients' conscious pain level, the corresponding number was chosen to reflect the patients' own degree: 0 meant no pain, and 10 meant the most painful.

(3) Efficacy judgment criteria: the patients' clinical symptoms

were improved at level 2, and the patients' cold sensation, resting pain symptoms, and numbness were significantly relieved or disappeared, which was regarded as "significantly effective"; the patients' clinical symptoms were improved at level 1, and the patients' cold sensation, resting pain symptoms, and numbness were alleviated, which was regarded as "effective"; the patients' clinical symptoms were not relieved, which was regarded as "ineffective"; the severity of the patients' clinical symptoms after treatment exceeded level 1, and the patients' cold sensation, resting pain symptoms, and numbness aggravated, which was regarded as "severely worsened". Total effective = significantly effective + effective.

1.7 Statistical analysis

SPSS 20.0 software was used to perform statistical analysis on the obtained data. Comparison of measurement data was represented by (x±s) using t test; comparison of count data was represented by rate (%) using X^2 test. The difference was statistically significant when $P < 0.05$.

2. Results

2.1 Comparison of clinical symptom scores and ABI between the two groups before and after treatment

Before treatment, there was no significant difference in clinical symptom scores and ABI between the two groups ($P > 0.05$). After treatment, the ABI was higher, and the clinical symptom score was lower in the observation group ($P < 0.05$). Compared to the control group, the ABI in the observation group was higher, and the clinical symptom score were lower after treatment ($P < 0.05$). The ABI and clinical symptom scores before and after treatment in the control group were compared, and the differences were not statistically significant ($P > 0.05$), as shown in Table 1.

Table 1. Clinical symptom scores and ABI of the two groups before and after treatment

Groups	*ABI*		*Clinical symptom score*	
	Before treatment	*After treatment*	*Before treatment*	*After treatment*
Observation group (n=40)	*0.75 ± 0.12*	*0.95 ± 0.16**	*8.99 ± 3.43*	*3.43 ± 1.90**
Control group (n=40)	*0.77 ± 0.15*	*0.83 ± 0.13*	*8.45 ± 3.52*	*7.90 ± 1.65*
t	*0.658*	*3.681*	*0.694*	*11.234*
P	*0.5122*	*0.0004*	*0.4892*	*0.0000*

*Note: *Versus before treatment, P < 0.05.*

Comparison of the levels of inflammatory cytokines between the two groups before and after treatment

There were no statistical differences in IL-10, IL-6, and TNF-α between the two groups before and after treatment ($P > 0.05$), as shown in Table 2.

Table 2. The levels of inflammatory cytokines of the two groups before and after treatment

Groups	IL-10 (pg/ml)		IL-6 (pg/ml)		TNF-α (pg/ml)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group (n=40)	60.13 ± 7.25	61.59 ± 7.76	18.25 ± 6.15	17.09 ± 6.27	54.53 ± 21.99	53.25 ± 22.15
Control group (n=40)	59.45 ± 8.15	59.35 ± 8.15	17.73 ± 5.82	15.73 ± 5.76	53.75 ± 20.49	51.35 ± 20.16
t值	0.3942	1.2589	0.3884	1.0102	0.1641	0.4012
P值	0.6945	0.2118	0.6988	0.3155	0.8701	0.6894

Comparison of oxidative stress indicators between the two groups before and after treatment

Before treatment, there was no statistically significant difference

in the levels of oxidative stress indicators between the two groups (P > 0.05). After treatment, the levels of 8-isoprostaglandin F2α and oxidized low-density lipoprotein were lower, and the superoxide dismutase was higher in the observation group after treatment (P < 0.05). Compared to the control group, the levels of 8-isoprostaglandin F2α and oxidized low-density lipoprotein were declined, and the superoxide dismutase was elevated in the observation group after treatment (P < 0.05). The levels of oxidative stress indicators before and after treatment in the control group were not statistically significant (P > 0.05), as shown in Table 3.

Table 3. Oxidative stress indicators of the two groups before and after treatment

Groups	8-isoprostaglandin F2α (pg/ml)		Oxidized low-density lipoprotein (U/L)		Superoxide dismutase (μ/ml)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group (n=40)	3.85 ± 0.49	3.13 ± 0.39	1.79 ± 0.56	0.81 ± 029	75.13 ± 8.25	94.25 ± 9.79
Control group (n=40)	3.75 ± 052	3.85 ± 0.43	1.87 ± 0.59	1.72 ± 0.42	76.25 ± 8.13	81.33 ± 9.46
t	0.8851	7.8441	0.6219	11.2763	0.6115	6.0022
P	0.3788	0.000	0.5358	0.000	0.5426	0.000

Comparison of pain degree scores between the two groups before and after treatment

Before treatment, there was no statistically significant difference in the pain degree score between the two groups (P > 0.05). After treatment, the pain degree score in the observation group was lower than that in the control group (P < 0.05), as shown in Table 4.

Table 4. Pain degree scores of the two groups before and after treatment

Groups	VAS score	
	Before treatment	After treatment
Observation group (n=40)	9.29 ± 0.35	2.52 ± 0.25
Control group (n=40)	9.31 ± 0.25	6.39 ± 0.39
t	0.2940	52.8355
P	0.7695	0.000

Comparison of clinical efficacy between the two groups

The total effective rate in the observation group was 97.50%, which was higher than that of 72.50% in the control group (X^2 = 9.8039, P = 0.0017), as shown in Table 5.

Table 5. Clinical efficacy of the two groups

Groups	Significantly effective (%)	Effective (%)	Ineffective (%)	Total effective rate (%)
Observation group (n=40)	30 (75.00)	9 (22.50)	1 (2.50)	39 (97.50)
Control group (n=40)	18 (45.00)	11 (27.50)	11 (27.50)	29 (72.50)

Discussion

T2DM, as one of the low-level inflammatory diseases, is closely related to a variety of inflammatory cytokines. For such patients,

in the early stage of atherosclerosis, inflammation has been shown [12-14]. As an anti-inflammatory cytokine, IL-10 plays a significant role in inhibiting the production of proinflammatory cytokines and the proliferation and activation of T lymphocytes [15]. In addition, IL-6 can play a central role in the inflammatory response of patients, and also plays a major role in the development of atherosclerosis in patients, causing damage to the blood vessel wall. As a pro-inflammatory cytokine, TNF-α shows the characteristics of diversified functions. It can correspondingly stimulate the release of platelet growth factor, so that patients show smooth muscle cell proliferation phenomenon, and eventually lead to vascular disease in patients [16, 17]. After the occurrence of high blood sugar, the antioxidant level and oxidation level of patients will be out of balance, so that the blood vessel membrane of patients will show lipid peroxidation and cell damage, so that the level of free radicals in patients will increase to a certain extent. Under this situation, the patients' vascular permeability and cell membrane permeability show a corresponding increase, which promotes cell proliferation and atherosclerosis, making the patient appear the phenomenon of diabetic lower extremity vascular disease [18, 19]. As an end product of unsaturated fatty acid lipid peroxidation, the appearance of 8-isoprostaglandin F2α is correlated with the peroxide damage shown by patients, which can reflect the level of oxidative stress in the patients' body. Oxidized low-density lipoprotein may play a direct role in the process of atherosclerosis. In particular, oxidized low-density lipoprotein can promote the aggregation of smooth muscle cells in patients and inhibit endothelial relaxation function, thereby increasing the permeability of endothelial cells to a certain extent, and finally binding with 8-isoprostaglandin F2α, significantly promoting atherosclerosis and corresponding vascular disease.

For diabetes, drug therapy is the main method in the study of treatment options. As one of the analogues of insulin resistance, metformin is selected to treat patients with diabetic lower extremity vascular disease, which can directly act on patients with plaque arteries and stenotic arteries, and significantly improve the arterial resistance of patients [20].

The results of this study showed that the ABI was higher, and the clinical symptom score was lower in the observation group after treatment than those in the control group and before treatment, which was in line with previous studies [21]. The levels of 8-isoprostaglandin F2α and oxidized low-density lipoprotein were lower, and superoxide dismutase was higher in the observation group after treatment than those before treatment and in the control group, which was consistent with the relevant literatures [22]. Besides, after treatment, the pain degree score in the observation group was lower than that in the control group, which was similar to former studies [23]. In addition, the total effective rate in the observation group was 97.50%, which was higher than the 72.50% in the control group, which further validated the therapeutic effect of metformin in T2DM [24]. Therefore, it could be confirmed that the application of metformin could significantly reduce the level of oxidized low-density lipoprotein and 8-isoprostaglandin F2α, and increase the level of superoxide dismutase, thus significantly improving the oxidative stress of patients and hinding the progression of the disease.

The limitations of the present study included the small number of patients, as well as lack of follow-up, which might be insufficient to assess the benefits of metformin in T2DM.

In conclusion, a reasonable choice of metformin combined with conventional therapies to treat diabetes can effectively improve the blood flow of the patients' lower limbs and clinical symptoms, and fully reduce their oxidative stress level, while there is no significant influence on the inflammatory cytokines in the patients.

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Data availability statement

Data sets for the duration of this study and/or the analysis period are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no competing interests.

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