CHANGES AND SIGNIFICANCE OF SERUM CMKLR1, MCP-1 AND IL-8 LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS COMPLICATED WITH MICROANGIOPATHY

Dandan Jiang, Liu Xu*, Dawei Jiang, Lan Shen, Guoliang Wang
Department of Hepatobiliary Surgery, First Affiliated Hospital of Jiaxing University, Jiaxing, PR China

ABSTRACT

Objective: *To analyze the changes and significance of serum chemotactic factor receptor-1 (CMKLR1), monocyte chemokines-1 (MCP-1) and interleukin-8 (IL-8) in patients with type 2 diabetes mellitus complicated with microangiopathy.*

Methods: *96 patients with type 2 diabetes mellitusn treated in our hospital endocrinology department from August 2016 to April 2017 were randomly selected. According to whether the patients were complicated with microangiopathy, they were divided into microangiopathy group and non-microangiopathy group. There were 56 patients in microangiopathy group and 40 patients in non-microangiopathy group. At the same time, 50 healthy subjects examined in our hospital were selected as the normal control group. The levels of serum MCP-1, IL-8, tumor necrosis factor-α (TNF-α), homocysteine (Hcy), CD146 and serum CMKLR1, Chemerin were observed in each group. The correlation was analyzed.*

Results: *Compared with the normal control group, the levels of serum MCP-1 IL-8, TNF-α, Hcy, CD146, Chemerin and CMKLR1 in the microangiopathy group and the non-microangiopathy group were significantly higher than those in the non-microangiopathy group, and the above indexes in the microvascular lesion group were significantly higher than those in the non-microangiopathy group (P<0.05). The correlation test of pearson showed that there was a certain correlation between serum MCP-1 and the levels of IL-8, TNF-α, Hcy, CD146, Chemerin and CMKLR1 in patients with type 2 diabetic microangiopathy, and there was a significant positive correlation between them.*

Conclusion: *Serum CMKLR1, MCP-1 and IL-8 are involved in the development of microangiopathy in patients with type 2 diabetes mellitus.*

Keywords: *Type 2 Diabetic Microangiopathy, CMKLR1, MCP-1, IL-8.*

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Introduction

Diabetes is a metabolic disease caused by insufficient insulin secretion or dysfunction of insulin, mainly characterized by long-term hyperglycemia. Type 2 diabetes is also called adult-onset diabetes. The ability of insulin production in patients with type 2 diabetes is not completely lost, and some patients even produce too much insulin, but the effect of insulin is poor, so the insulin in patients is in a relatively deficient state[1]. Long-term hyperglycemia in diabetes causes chronic damage and dysfunction of various tissues, especially eyes, kidneys, heart, blood vessels and nerves. With the application of insulin and various oral hypoglycemic drugs, it is considered that the main factors that seriously affect the quality of life of diabetic patients are various complications of diabetes, whose chronic complications almost involve all organs and tissues of the body[2]. Microangiopathy is the pathophysiological basis of multiple organ injury in diabetes mellitus, and also a typical complication of diabetes mellitus.

Long-term hyperglycemia can damage blood vessels and lead to microangiopathy, which can cause diabetic retinopathy, diabetic nephropathy, diabetic peripheral neuropathy, etc.[3].

Studies have found that the occurrence of diabetic microvessel is closely related to a variety of inflammatory factors, and the inflammatory state can induce the proliferation of vascular endothelial cells, thereby aggravating the occurrence and development of diabetic microvascular disease[4]. Mononuclear chemokine protein-1 (MCP-1), a

representative of the beta subfamily of chemokine cytokines, can chemotaxis monocytes and contribute to cell proliferation and extracellular matrix buildup. Chemerin is a adipofactor formed by the precursor protein pro-Chemerin after the action of protease. It can act on its main functional receptor Chemokine receptor-1 (CMKLR1) through autocrine or paracrine, and plays a role in diabetes and related complications. As shown in the present study, the changes of CMKLR1, MCP-1 and interleukin-8 (IL-8) in serum of patients with type 2 diabetic microangiopathy were mainly studied, and their significance was also discussed.

Basic data and methods

Basic Information

96 patients with type 2 diabetes who were treated in the department of endocrinology of our hospital from August 2016 to April 2017 were randomly selected.

Entry standard:

• All patients met the diagnostic criteria of type 2 diabetes specified by the World Health Organization (WHO)[5];

• In line with the diagnostic criteria for diabetic microangiopathy[6];

• Over 20 years old;

• This study was approved by the hospital ethics committee, and patients and their families all gave informed consent and signed informed consent.

• Abnormal results of nerve electromyography examination.

Exclusion criteria:

• Exclusion of type 1 diabetes or other diabetes patients;

• Serious liver and kidney function, cardiac insufficiency;

• Pregnancy or lactation patients.

Patients were divided into microvascular group and non-microvascular group according to whether patients had microangiopathy or not.

There were 56 patients in the microvascular disease group, including 32 males and 24 females, aged from 35 to 62 years old, with an average age of 52.67±4.22 years old and a body mass index (BMI) of 25.46±2.37 kg/m^2. There were 40 patients in the non-microvascular disease group, including 22 males and 18 females, aged 31-63 years, with an average age of 51.71±4.79 years and a BMI of 24.37±2.05 kg/m^2. At the same time, 50 healthy subjects who were examined in our hospital during the same period were selected as the normal control group, including 24 males and 26 females, with an average age of 53.59±3.98 years old and a BMI of 24.99±2.16 kg/m^2. There was no significant difference in the age, gender, BMI and other basic data of the three groups (P>0.05). See table 1.

Groups	n	Age (year)	Gender (n)		BMI (kg/m^2)
			Man	Woman	
Normal Control	50	53.59±3.98	24	26	24.99±2.16
Microangiopathy Group	56	52.67±4.22	32	24	25.46±2.37
Non-Microvascular Group	40	51.71±4.79	22	18	24.37±2.05
F/t		2.131	0.942		2.833
P		0.123	0.624		0.063

Table. 1: Comparison of three groups of basic data.

Methods

Microvascular disease group, non-microvascular disease group and normal control group personnel are forbidden to drink and fast for 8h, extract fasting elbow median venous blood 5mL, after centrifuge centrifuge, put the supernatant into environmental protection-80°C refrigerator. Centrifuge purchased from Shanghai precision instrument co., LTD., model GL-22MS.

Serum levels of MCP-1, IL-8 and tumor necrosis factor-α (TNF-α) were determined by elisa. All the test kits were purchased from Shanghai tongwei biotechnology co., LTD. The levels of Homocysteine and CD146 were determined by elisa. The test kit was purchased from wuhan mossack biotechnology co., LTD. Serum CMKLR1 level was detected by CMKLR1 detection kit (wuhan bokang biological engineering co., LTD.) and Chemerin detection kit (Shanghai yubo biotechnology co., LTD.). The detection method is carried out in accordance with the operation steps of the kit.

Observation Indexes

Statistical data were analyzed by SPSS24.0 software package, and the counting data were compared by chi-squared test, one-way anova and LSD-t test. Ridit test was used to compare hierarchical data. P<0.05 was considered statistically significant.

Results

Comparison of Serum Levels of MCP-1, IL-8 and TNF-Alpha in Each Group

Compared with the normal control group,

MCP-1, IL-8 and TNF-α levels in the microangiopathy group and the non-microangiopathy group were significantly increased, and the levels of MCP-1, IL-8 and TNF-α in the microangiopathy group were significantly higher than those in the non-microangiopathy group (P<0.05). Shown in table 2.

Groups	n	MCP-1(μg/L)	IL-8(μg/L)	TNF-α(ng/mL)
Normal Control	50	3.55±0.35	8.16±1.54	1.11±0.47
Microangiopathy Group	56	24.05±7.17[ab]	36.29±9.45[ab]	2.49±0.69[ab]
Non-Microvascular Group	40	6.28±1.69[a]	15.56±3.18[a]	1.55±0.49[a]
F		315.79	296.94	72.78
P		<0.001	<0.001	<0.001

Table. 2: Comparison of serum levels of MCP-1, IL-8 and TNF- alpha in each group ($\bar{x}\pm s$).
Note: a means compared with the normal control group, [a]P<0.05, and b means compared with the non-microvascular disease group, [b]P<0.05.

Comparison of Hcy and CD146 Levels in Each Group

Compared with the normal control group, Hcy and CD146 levels in the microvascular disease group and the non-microvascular disease group were significantly increased, and Hcy and CD146 levels in the microvascular disease group were significantly higher than those in the non-microvascular disease group (P<0.05). See table 3.

Groups	n	Hcy(μmol/L)	CD146(μg/L)
Normal Control	50	10.87±4.66	157.33±61.94
Microangiopathy Group	56	18.59±6.51[ab]	297.78±95.35[ab]
Non-Microvascular Group	40	15.51±5.31[a]	194.06±83.12[a]
F		25.19	35.71
P		<0.001	<0.001

Table. 3: Comparison of Hcy and CD146 levels in each group ($\bar{x}\pm s$).
Note: a means compared with the normal control group, [a]P<0.05, and b means compared with the non-microvascular disease group, [b]P<0.05.

Comparison of Serum Chemerin and CMKLR1 Levels in Each Group

Chemerin and CMKLR1 levels in the microvascular disease group and the non-microvascular disease group were significantly higher than those in the normal control group, and Chemerin and CMKLR1 levels in the microvascular disease group were significantly higher than those in the non-microvascular disease group (P<0.05). See table 4.

Groups	n	Chemerin (pg/mL)	CMKLR1 (μg/L)
Normal Control	50	78.36±31.29	32.15±5.38
Microangiopathy Group	56	125.27±34.69[ab]	65.13±8.41[ab]
Non-Microvascular Group	40	109.75±24.25[a]	41.61±5.97[a]
F		30.98	326.34
P		<0.001	<0.001

Table. 4: Comparison of serum Chemerin and CMKLR1 levels in each group ($\bar{x}\pm s$).
Note: a means compared with the normal control group, [a]P<0.05, and b means compared with the non-microvascular disease group, [b]P<0.05.

Correlation Analysis

According to Pearson correlation analysis, serum MCP-1 was significantly positively correlated with IL-8, TNF-α, Hcy, CD146, Chemerin and CMKLR1 levels in patients with type 2 diabetic microangiopathy (P<0.05). As shown in table 5.

Indicator	MCP-1	IL-8	TNF-α	Hcy	CD146	Chemerin	CMKLR1
MCP-1	—	0.667	0.104	0.771	0.081	0.192	0.369
IL-8	0.667	—	0.069	0.517	0.121	0.288	0.554
TNF-α	0.104	0.069	—	0.111	0.007	0.016	0.031
Hcy	0.771	0.517	0.111	—	0.067	0.144	0.277
CD146	0.081	0.121	0.007	0.067	—	0.421	0.219
Chemerin	0.192	0.288	0.016	0.144	0.421	—	0.524
CMKLR1	0.369	0.554	0.031	0.277	0.219	0.219	—

Table. 5: Correlation analysis.

Discussion

Diabetes is a chronic, progressive and lifelong disease caused by a variety of factors. The main clinical manifestation is the continuous increase of blood glucose. The stimulation of continuous high blood glucose in serum will damage the vascular endothelial cells and cause vascular endothelial hyperplasia. Microangiopathy is one of the most common complications in the development of diabetes, and the common damage to organs and tissues includes retina, kidney, nerve and myocardium, etc.

Among them, diabetic retinopathy is the main representative disease of diabetic microangiopathy and one of the main diseases causing blindness[7-8].

It is believed that the occurrence and development of diabetic microangiopathy is related to endothelial cell damage, abnormal polyol metabolism, hyperglycation and other factors, but its specific pathogenesis is not yet clear. In this study, 96 patients with type 2 diabetes were studied to analyze the changes and significance of serum CM-

KLR1, MCP-1 and IL-8 in patients with type 2 diabetes with microangiopathy.

MCP-1, also known as monocyte chemokines and activators, is a member of the beta family of chemokines.MCP-1 is an important inflammatory chemokine regulating monocyte/macrophage migration and infiltration, which can cause vascular endothelial injury and induce vascular smooth muscle cell proliferation and migration[9]. Vasdev et al. found that when the advanced glycation end products formed by long-term hyperglycemia and in the state of diabetes[10], the decrease of nitric oxide synthesis or activity would increase the level of MCP-1.Multiple studies have found that MCP-1 levels increase significantly in patients with diabetic retinopathy. When combined with its receptor CCR2 (CC chemkoine receptor 2), MCP-1 can induce various inflammatory cells, especially monocytes, to accumulate in the lesion site. IL-8 is mainly a cytokine secreted by monocytes/macrophages, etc. IL-8 can have a series of biological reactions after contacting with its specific receptors. TNF-α polypeptide cytokine with various biological activities is an important inflammatory factor closely related to immune response and inflammatory response.

Zorena et al. found in the study of diabetic rats that long-term hyperglycemia can cause glycosylation of various proteins in the body, promote the secretion of TNF-α by cells, and significantly increase the level of TNF-α[11]. Li et al. reported that the occurrence and development of vascular complications in diabetes mellitus is closely related to TNF-alpha level. TNF-α level can induce the release of tissue like precoagulation factor and promote thrombosis. Meanwhile, it can stimulate vascular endothelial cells and significantly increase vascular permeability[12]. Groups, the results of patients with type 2 diabetes, IL - 8, MCP - 1, TNF-α levels were significantly increased, this may be linked to high blood sugar can cause poor cell glucose utilization, cause cells to anaerobic metabolism between intermediate lactic acid accumulation and intracellular acidosis, promote the lipid peroxidation raised concentration of MCP - 1, induction of an increased level of inflammatory factors and so on.

Hcy is a kind of amino acid, reactive vascular injury is a risk factor for cardiovascular diseases and its metabolic enzymes and cofactors required (Folic Acid, VitB12, VitB6) all can affect the concentration of Hcy, the lack of the research thinks, high Hcy can directly through the way such as oxidative stress damage vascular endothelial cells, when in patients with diabetic microvascular lesions, Hcy levels, platelet adhesion and accumulation increased significantly, strengthen blood clotting mechanism, thus increase the microvascular lesion progression[13]. CD146 is a newly discovered cell membrane molecule, which is highly expressed in human vascular endothelial cells and plays an important role in maintaining tissue integrity and regulating cell permeability. Hanefeld et al.[14] found that in diabetic patients complicated with diabetic nephropathy, vascular endothelial cells are damaged and large amounts of CD146 are secreted into peripheral blood.CD146 is an important indicator for evaluating renal injury. Compared with the normal control group, the Hcy and CD146 levels in the microvascular disease group and the non-microvascular disease group were significantly increased, and the Hcy and CD146 levels in the microvascular disease group were significantly higher than those in the non-microvascular disease group ($P<0.05$). It is suggested that the detection of changes in Hcy and CD146 levels can prevent and diagnose the onset of microangiopathy in type 2 diabetes early, so that effective treatment measures can be timely adopted.

Some studies suggest that the occurrence and development of diabetic microangiopathy is closely related to Chemerin level. Chemerin is an adipogen, mainly located in chromosome 7q36,1. The human Chemerin gene encodes a protein containing 163 amino acid residues, which is an inactive protein precursor secreted protein. Teng and Huang[15] studies suggest that Chemerin/CMKLR1 signal pathway is the regulation of adipocyte differentiation, Chemerin also can promote, including macrophages and immature dendritic cells, the high expression of immune cells to raise and migration, in fat, liver, skeletal muscle, pancreas and infiltration of inflammatory cells in various tissues such as blood vessels, in turn, play a role of proinflammatory, and chronic low-grade inflammation is an important characteristic of diabetes and related metabolic diseases. It is similar to the experimental results of Perumalsamy et al.[16] in mice.

In this study, Chemerin and CMKLR1 levels in the microangiopathy group and non-microangiopathy group were significantly higher than those in the normal control group, and Chemerin and CMKLR1 levels in the microangiopathy group were significantly higher than those in the non-microangiopathy group ($P<0.05$). These results suggest that Chemerin and CMKLR1 play an important role in

the occurrence and development of microangiopathy in type 2 diabetes mellitus.

In summary, serum levels of CMKLR1, MCP-1 and IL-8 are closely related to microangiopathy in patients with type 2 diabetes mellitus. MCP-1 and IL-8 levels play an important role in reflecting the degree of vascular endothelial injury. CMKLR1 may affect the development of microangiopathy in diabetic patients by affecting endothelial cell transformation, body glucose homeostasis and other mechanisms.

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Corresponding Author:
Liu Xu
Email: wgl9007@163.com
(China)