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Correlation of MMP-2, TIMP-1, β2-MG and hs-CRP with the progression of retinopathy in patients with type 2 diabetes

Xiaobo Shi, Min Yang, Xuguang Jiang, Yanlong Li, Li Meng*

Department of Fundus Disease, Xi'an Aier Ancient City Eye Hospital, Xi'an 710014, Shaanxi Province, China

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ABSTRACT

This study aimed to investigate the relationship between MMP-2, TIMP-1, β2-MG, hs-CRP and the progression of type 2 diabetic retinopathy (T2DM). For this purpose, 68 patients with T2DM retinopathy treated in our hospital were selected as the retinopathy group (REG), and 68 T2DM patients without retinopathy were selected as the control group (CDG). The serum levels of MMP-2, TIMP-1, β2-MG and hs-CRP were compared between the two groups. According to the international clinical classification of T2DM non-retinopathy (NDR), the patients were divided into non-proliferative T2DM retinopathy group (NPDR) (n=28) and proliferative T2DM retinopathy group (PDR) (n=40). The levels of MMP-2, TIMP-1, β2-MG and hs-CRP in patients with different conditions were compared. In addition, the Spearman method was used to analyze the correlation between the levels of MMP-2, TIMP-1, β2-MG, hs-CRP and glucose and lipid metabolism and the course of disease in patients with T2DM retinopathy (DR). Logistic multiple regression was used to analyze the risk factors of DR. Results showed that the levels of serum MMP-2, β2-MG and hs-CRP in PDR groups were raised than those in NPDR and NDR, while the serum TIMP-1 level was reduced. The levels of MMP-2, β2-MG and hs-CRP were positively correlated with the levels of HbA1c, TG and the course of disease in DR patients, while the levels of TIMP-1 in DR patients were negatively correlated with the levels of HbA1c, TG and the course of disease. The results of multivariate Logistic regression model showed that MMP-2, β2-MG and hs-CRP were independent risk factors for DR, and TIMP-1 was the protective factor for DR. In conclusion, the changes of peripheral blood MMP-2, TIMP-1, hs-CRP and β2-MG levels are closely related to the progression of T2DM retinopathy.

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Introduction

Type 2 diabetes mellitus (T2DM) can cause damage to many systems and tissues, such as autonomic nerves, peripheral nerves, microvessels and microvessels, thus causing a variety of diabetic complications and seriously endangering the life and health of patients. T2DM retinopathy (DR) is one of the most common microvascular complications in patients with T2DM and the first cause of blindness in people aged 30 to 70 years old. Therefore, how to prevent and treat DR has become the focus of clinical diabetic complications. At present, the pathogenesis of DR has not been fully explained, but as a chronic inflammatory immune disease, retinal microvascular inflammatory disease, infection and immune abnormality are important pathogenic factors affecting DR. Matrix metalloproteinase-2 (MMP-2) is a proteolytic enzyme, which can participate in the destruction of the microvascular structure by regulating the production and degradation of extracellular matrix, and then participate in the progression of T2DM vascular disease (1). Tissue inhibitor 1 (TIMP-1) is a specific inhibitor of MMP-2, which can regulate the biological activity of MMP-2 (2). In addition, β2-microglobulin (β2-MG) is a tiny protein formed by platelets, polymorphonuclear leukocytes and lymphocytes. Recent studies have shown that serum β2-MG is closely related to microangiopathy in patients with diabetic nephropathy. Hypersensitive C-reactive protein (hs-CRP) is a kind of C-reactive protein synthesized by the liver. Its level can reflect the severity of inflammation and has been proven to be closely related to the occurrence and development of microangiopathy in patients with diabetes (3). The purpose of this study was to explore the correlation between MMP-2, TIMP-1, β2-MG, hs-CRP and the occurrence and development of T2DM, in order to provide a reference for clinical diagnosis and treatment of patients with DR.

Materials and Methods

General information

68 patients with DR diagnosed and treated in our hospital from January 2021 to December 2022 were selected as the retinopathy group, and 68 T2DM patients without retinopathy were selected as a control group. The general data between the two groups were no difference (P>0.05), and it was comparable, as shown in Table 1. This study was approved by the Ethics Committee of our hospital, and all patients voluntarily participated and signed the informed consent form. Diagnostic criteria: the diagnosis conforms to the diagnostic criteria of DR in the international guidelines for Diabetes Mellitus. Inclusion criteria: in accordance with the diagnostic criteria of DR; age 20-65 years old; course of disease more than 2 years; complete clinical data and informed consent of patients. Exclusion

* Corresponding author. Email: kanpianzhang183@163.com

criteria: patients with severe dysfunction of organs such as heart, brain, liver and kidney; patients with severe infectious diseases; those with a history of mental illness and cognitive impairment, who are difficult to complete the test; retinopathy caused by non-diabetes; women during lactation or pregnancy.

Methods

Specimen collection

The fasting peripheral venous blood 5ml was taken from all subjects in the morning, and the serum was collected by centrifugation. The levels of MMP-2, TIMP-1, β2-MG and hs-CRP in peripheral blood were determined by enzyme-linked immunosorbent assay (Elisa). The detection instrument was Varioskan LUX automatic enzyme labeling instrument (Thermo Fisher Scientific, USA). The levels of serum triglyceride (TG) and glycosylated hemoglobin (HbAlc) were measured by an automatic biochemical analyzer and immunoturbidimetry respectively.

Diagnostic criteria

According to the T2DM international clinical classification criteria for non-retinopathy (4): fundus photography or fundus fluorescein angiography was performed: stage 5: retinal hemorrhage or neovascularization and/or vitreous hemorrhage; stage 4: no signs of proliferative diabetic retinopathy, but with intraretinal microvascular abnormalities, venous bead-like abnormalities in 2 quadrants, and intraretinal hemorrhage in any quadrant > 20. Stage 3: a mild manifestation of severe non-proliferative diabetic retinopathy and formation of microaneurysms; stage 2: mild formation of fundus microaneurysms; stage 1: no abnormal changes in fundus of patients. Stage 5 was proliferative diabetic retinopathy and stage 2-4 was non-proliferative diabetic retinopathy.

Statistical method

SPSS20.0 was used for statistical analysis, chi-square test was used for counting data, mean ± standard deviation was used for measurement data, t-test was used for comparison between two groups, ANOVA was used for comparison among groups, LSD test or Tamhane test was used for pairwise comparison between groups, Spearman method was used for correlation analysis, and multivariate analysis was used for Logistic regression analysis.

Results

Comparison of clinical data between the two groups

The sex, age, course of the disease, HbAlc, TG and other clinical data between the T2DM retinopathy group (DR) and T2DM non-retinopathy group (NDR) were no different (P>0.05). Table 1.

Comparison of serum indexes between the two groups

The levels of serum MMP-2, β2-MG and hs-CRP in DR were raised than those in NDR, while the serum TIMP-1 level in DR was reduced than that in NDR (P<0.05). Table 2.

Comparison of serum indexes in patients with DR with different severity

The serum levels of MMP-2, β2-MG and hs-CRP in the non-proliferative T2DM retinopathy group (NPDR) were raised than those in the NDR, while the serum TIMP-1 level was reduced than that in the NDR. The serum levels of MMP-2, β2-MG and hs-CRP in the proliferative T2DM retinopathy group (PDR) were raised than those in NPDR and NDR. The serum TIMP-1 level in PDR was reduced

Table 1. Comparison of clinical data between the two groups.

Project	DR (n=68)	NDR (n=68)	$\chi^2/t/Z$	*P*
Gender (case)			0.119	0.731
Male	38	36		
Female	30	32		
Age (year)	54.26±9.27	55.35±8.68	0.708	0.480
Duration of diabetes (year)	6.57±2.12	6.84±2.20	0.729	0.467
HbAlc (%)	9.22±1.51	9.42±1.21	0.852	0.396
TG (mmol/L)	1.75±0.33	1.78±0.40	0.477	0.643

Table 2. Comparison of serum indexes between the two groups.

Group	Number	MMP-2 (ng/ml)	TIMP-1 (ng/ml)	β2-MG (mg/L)	hs-CRP (mg/L)
DR	68	89.87±22.82	12.42±4.76	2.72±0.84	11.10±1.73
NDR	68	36.92±8.12	31.25±10.14	2.37±0.72	9.46±1.42
t		18.027	-13.862	2.609	6.042
P		0.000	0.000	0.010	0.000

Table 3. Comparison of serum indexes in patients with DR with different severity.

Group	Number	MMP-2 (ng/ml)	TIMP-1 (ng/ml)	β_2-MG (mg/L)	hs-CRP (mg/L)
NDR	68	36.92±8.12	31.25±10.14	2.37±0.72	9.46±1.42
NPDR	28	83.98±19.00[a]	15.03±4.38[a]	2.69±0.62[a]	10.83±1.61[a]
PDR	40	98.19±27.25[ab]	10.50±3.02[ab]	3.04±0.61[ab]	12.65±1.84[ab]
F		166.153	106.771	12.738	50.710
P		0.000	0.000	0.000	0.000

Note: Compared to the NDR group: [a]*P*<0.05; Compared to the NPDR group: [b]*P*<0.05.

than that in NPDR and NDR(P<0.05). Table 3.

Correlation of MMP-2, TIMP-1, β2-MG and hs-CRP levels with glycolipid metabolism and disease course in patients with DR

The levels of MMP-2, β2-MG and hs-CRP were positively correlated with the levels of HbA1c, TG and the course of disease in patients with DR, while the levels of TIMP-1 in patients with DR were negatively correlated with the levels of HbA1c, TG and the course of disease(P<0.05). Table 4.

Logistic regression analysis of related factors of DR

Taking DR as dependent variable, multivariate Logistic regression model showed that MMP-2, β2-MG and hs-CRP were independent risk factors of DR, and TIMP-1 was protective factor of DR (P<0.05). Table 5.

Discussion

As people's living standards improve and their habits change, the incidence of T2DM in China is increasing, and cases account for more than 90% of diabetes patients (5). Microangiopathy and microcirculatory disturbance are not only the main pathological manifestations of diabetic patients but also the pathological basis of late DR and other complications. Clinical studies have shown that (6) angiopathy in patients with diabetes can lead to functional and organic damage of related blood vessels of nutritive nerves, neuro dystrophy and ischemic neuritis, and neuronal micro-damage one after another. as a result, the defense mechanism of the body is disturbed, and the formation of retinal angiopathy is aggravated. With the disease progression of patients with DR, retinal angiopathy will deteriorate rapidly, and patients will have retinal detachment, resulting in rigid exudation, glass sphere proliferation and so on, resulting in further aggravation of the disease, and even complete loss of vision in patients. Therefore, an in-depth study of microangiopathy to avoid the deterioration of patients with DR is the focus of clinical research. The pathogenesis of DR has not been fully explained, but as a chronic inflammatory immune disease, retinal vasculitis, infection, dyslipidemia and immune abnormality are important pathogenic factors in patients with DR (7). MMP-2, TIMP-1, β2-MG and hs-CRP are widely studied in recent years to evaluate chronic inflammation and autoimmune status. The detection method is simple, only a blood routine is needed, and can better reflect the inflammatory and immune status of the body.

MMPs is a kind of endogenous proteolytic enzyme containing zinc ions. Because it needs zinc, calcium and other metal ions as cofactors to exert its biological ability, it is named matrix metalloproteinases (8). MMP-2 is an important member of the MMPs family, also known as gelatinase A, which belongs to the gelatinase family, is mainly located in the protruding part of the cell-penetrating the matrix (9). MMP-2 has a wide variety of degradation substrates, which can degrade not only type IV, V, VII, and X collagen, but also denatured collagen, basement membrane, elastin and so on. It has been found that the serum expression of MMP-2 is significantly increased in patients with macrovascular diseases, and can participate in the formation of oxidized low-density lipoprotein in the arterial basement membrane and atherosclerotic plaque together with inflammatory factors such as tumor necrosis factor so that the increase of its level is easy to increase the incidence of vascular occlusion in patients with DR (10). TIMPs are an endogenous specific inhibitor of MMPs, which is also synthesized and secreted by inflammatory cells such as macrophages, neutrophils, lymphocytes, fibroblasts and so on. The main biological function is to regulate the normal metabolism of the extracellular matrix by inhibiting MMPs.

In the physiological state, TIMPs and MMPs work together to maintain the dynamic balance in the body, which is beneficial to coordinate the degradation and reconstruction of the extracellular matrix, prevent excessive destruction and excessive accumulation of local tissue, maintain the integrity of tissue structure and the stability of the internal environment, thus provide nutrients and growth space for neovascularization, and reduce the incidence of microvascular thrombosis (11). When the body is in pathological conditions such as inflammation and abnormal glucose and lipid metabolism, the dynamic balance between TIMPs and MMPs is broken, resulting in an imbalance in the synthesis and degradation of extracellular matrix, resulting in endothelial cell increase, basement membrane thickening, microvascular thrombosis, retinal tissue ischemia and hypoxia, thus promoting the progression of DR (12). The results showed that the serum MMP-2 level in the PDR group was raised then that in NPDR and NDR, while the serum TIMP-1 level in PDR was reduced than that in NPDR and NDR. The level of MMP-2 in patients with DR was positively correlated with the level of

Table 4. Correlation of MMP-2, TIMP-1, β2-MG and hs-CRP levels with glycolipid metabolism and disease course in DR.

Indicator	MMP-2		TIMP-1		β2-MG		hs-CRP	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
HbA1c	0.315	0.000	-0.350	0.000	0.233	0.000	0.294	0.000
TG	0.327	0.000	-0.356	0.000	0.315	0.026	0.356	0.000
Disease course	0.246	0.000	-2.121	0.000	0.291	0.000	0.315	0.000

Table 5. Logistic regression analysis of related factors of DR.

Factor	*P*	*Exp (B)*	*b*	Waldχ^2	*SE*	95%CI
MMP-2	0.007	12.849	2.988	7.338	1.103	2.285-37.452
TIMP-1	0.005	0.050	-2.998	7.786	1.074	0.006-0.410
β2-MG	0.028	4.406	1.483	4.806	0.676	1.170-16.589
Hs-CRP	0.020	0.376	2.789	6.473	0.879	0.699~1.471

HbA1c, TG and the course of the disease, while the TIMP-1 level in DR patients was negatively correlated with the HbA1c level, TG and the course of the disease. MMP-2 is an independent risk factor for DR and TIMP-1 is a protective factor for DR. It is suggested that the MMP-2 level in the peripheral blood of DR patients is raised, the TIMP-1 level is decreased, and the proportion of MMP-2/TIMP-1 is out of balance, and the changes of MMP-2 and TIMP-1 in peripheral blood are related to the progression of DR.

β2-MG is a single-chain polypeptide low molecular protein synthesized mainly in the liver. As the light chain of the major histocompatibility antigen, it exists in the cell membrane. The function of β2-MG is related to the major histocompatibility antigens expression and plays a very important role in iron metabolism, immunoglobulin transport and antigen expression. It is a protein related to the immune mechanism. Recent clinical studies have found that β2-MG may be involved in the pathophysiological process of diabetic foot disease and has a certain impact on the disease prognosis. β2-MG is mainly produced by lymphocytes, and the activation of CD4+ helper T lymphocytes plays a critical role in the formation of atherosclerosis (13). At present, studies have confirmed that DR is related to abnormal blood lipid metabolism, endothelial cell injury and the imbalance of inflammatory factors. Hs-CRP is an acute-phase reactive protein synthesized by the liver. It is a non-specific marker of acute inflammation and participates in the progression of inflammatory response. Studies have found that there is a significant increase in serum hs-CRP levels in patients with diabetic foot (14). It has been found that hs-CRP can reach an abnormally high level in a short time after the occurrence of acute inflammation, vascular endothelial injury or tissue necrosis, and then activate the complement system, promote the production of inflammatory mediators, release oxygen into the blood freely, damage vascular intimal cells, lead to vasospasm and plaque shedding, thus induce vascular thrombosis, cause plaque rupture, and lead to vascular endothelial injury. Retinal ischemia and hypoxia participate in the progression of DR (15-21). The results showed that the hs-CRP and β2-MG levels in PDR were raised than those in NPDR and NDR. The levels of hs-CRP and β2-MG in patients with DR were positively correlated with the levels of HbA1c, TG and the disease course. Hs-CRP and β2-MG are independent risk factors for retinopathy in T2DM. It is suggested that the levels of hs-CRP and β2-MG in the peripheral blood of patients with DR are significantly increased, and the changes in hs-CRP and β2-MG levels in peripheral blood are closely related to the progression of DR.

To sum up, the levels of MMP-2, hs-CRP and β2-MG in the peripheral blood of patients with DR were raised, TIMP-1 levels were reduced, and the proportion of MMP-2/TIMP-1 was out of balance, and the changes of peripheral blood MMP-2, TIMP-1, hs-CRP and β2-MG levels were closely related to the progression of DR.

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