EXPRESSION AND SIGNIFICANCE OF PLASMA APOLIPOPROTEIN M AND RETINOL BINDING PROTEIN 4 IN PATIENTS WITH CAROTID ARTERY DISEASE OF TYPE 2 DIABETES

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ABSTRACT

Objective: *To analyze the serum expression and clinical significance of plasma apolipoprotein M (ApoM) and retinol binding protein 4 (RBP4) in patients with type 2 diabetic carotid artery disease.*

Methods: *110 patients treated with type 2 diabetes mellitus (T2DM) in our hospital endocrinology between September 2016 and September 2018 be chosen as the observation group, according to the results of carotid artery color doppler ultrasound, they would be divided into observation group of patients with carotid artery plaque group of 58 cases, normal group of 52 cases of their carotid arteries, while, 50 physical health cases would be chosen as control group, three groups of subjects in clinical general information collection, three groups of subjects was detected in serum ApoM, RBP4 levels, Meanwhile, subjects' fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and other biochemical levels were detected.*

Results: *Waist circumference and waist-hip in the carotid plaque group were significantly higher than those in the control group (P<0.05), and waist circumference in the normal carotid artery group was significantly higher than that in the control group (P<0.05). Serum ApoM in carotid plaque group was significantly lower than that in normal carotid group and control group (P<0.01), and serum ApoM in normal carotid group was significantly lower than that in control group (P<0.01). The serum RBP4 level of carotid plaque group was significantly higher than that of normal carotid artery group and control group (P<0.01). The levels of FBG, TC, TG and LDL-C in the carotid plaque group were significantly higher than those in the normal carotid artery group and the control group (P<0.01), and HDL-C were significantly lower than those in the normal carotid artery group and the control group (P<0.01). Serum ApoM was positively correlated with BMI (r=0.651, P<0.05), and negatively correlated with RBP4, FBG, TC, LDL-C levels (r=-0.257, -0.587, -0.532, -0.428, P<0.05). Serum RBP4 was negatively correlated with LDL-C (r=-0.334, P<0.05), and positively correlated with waist circumference, waist-hip ratio, TC, LDL-C, and BMI (r=0.286, 0.170, 0.210, 0.135, 0.339, P<0.05). Logistic regression analysis results showed that age, course of disease, LDL-C and serum ApoM and RBP4 levels were all risk factors affecting carotid artery lesions in type 2 diabetes mellitus (P<0.05).*

Conclusion: *Serum ApoM and RBP4 levels were decreased in T2DM patients with carotid artery vascular disease, and the mechanism of causing or aggravating vascular disease may be related to insulin resistance and dyslipidemia.*

Keywords: *Plasma Apolipoprotein M, Retinol binding protein 4, Type 2 Diabetes, Carotid Angiopathy, Clinical Significance.*

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Introduction

Type 2 diabetes mellitus (Type 2 diabetes mellitus, T2MD) is a common endocrine metabolic disease with whole body metabolic disease characterized by sustained high blood sugar, mainly due to the defects islet beta cell function and Insulin resistance (Insulin to hold, IR) lead to increased blood sugar of lead, metabolic disorders for a long time can cause eye, kidney, cardiovascular disease and neural system and other systemic organ function obstacle and failure, seriously affecting the quality of life of tens of thousands of people around the world[1,2]. Arteriosclerosis is the main cause of disability and death in T2DM patients, and carotid intima-media thickness is usually used as an indicator to reflect the degree of atherosclerosis in clinical practice[3]. Plasma Apolipoprotein M (ApoM) is a kind of iso-lipid-binding protein in plasma high-density lipoproteins, which belongs to the Lipocalin superfamily. ApoM has high tissue specificity in different tissues of human body, especially in renal tubular tissues

and liver tissues[4]. Relevant data show that it can alleviate the development of aortic atherosclerotic plaques, but whether it is involved in the development of diabetes atherosclerosis is still unclear[5]. Retinol binding protein 4 (RBP4) is an important transporter in the body, which is mainly secreted by liver cells and fat cells. In recent years, many studies have shown that RBP4 can promote obesity and insulin resistance, and jointly promote the formation of atherosclerosis with insulin[6]. In this study, the expression levels of ApoM and RBP4 in serum of type 2 diabetic patients with carotid artery disease were detected to analyze the expression and clinical significance of the two indicators in this disease.

Materials and methods

Research subjects

110 patients with type 2 diabetes mellitus (2TDM) admitted to the endocrinology department of our hospital from September 2016 to September 2018 were selected as the observation group, including 56 males and 54 females, aged from 32 to 70 years, with an average age of (45.27±11.38) years.

- All patients met the WHO diagnostic criteria for T2MD[7];
- Age > 30 years old;
- HbAlc > 7.5%; BMI 19 ~ 35 kg/m2;
- fasting blood glucose > 8.0mmol/L, after meal blood glucose > 11.1mmol/L;
- did not use insulin rosiglitazone and other hypoglycemic drugs;
- patients and their families who give informed consent and cooperate with treatment, this study is approved and approved by the ethics committee of the hospital.

Exclusion criteria:

- patients with other types of diabetes;
- T2DM patients with acute complications;
- severe liver and kidney dysfunction and systemic immune system diseases;
- mental disorders can not normal communication;
- acute infection during hospitalization;
- Pregnant and lactating women.

Observation group patients were performed carotid artery color doppler ultrasound, according to the results divided the patients into carotid plaques group of 58 cases, normal group of 52 cases of their carotid arteries, the other choose to our physical health groups 50 cases as control group, the male 27 cases, 23 cases of female, age 33~75, the average (44.68 ± 10.38) years of age, screening in diabetes, glucose tolerance test eduction groups study generally compare difference has no statistical significance ($P > 0.05$).

Sample Collection

5ml of fasting venous blood of all patients was collected and placed in a heparin anticoagulant tube on the morning of the second day after admission. It was allowed to stand at room temperature for 20min and centrifuged by centrifugal force of 3000r/min for 10min. The supernatant was then taken and stored in a refrigerator at -80°C for later use. The control group drew blood during physical examination. ApoM and RBP4 kits were purchased from hunan yuantai technology co., LTD. The automatic biochemical analyzer is Hitachi 7170A type. Doppler ultrasound instrument (mindray Resona 7) was purchased from hefei sanfeng medical equipment co., LTD.

Methods

- General data examination: subjects' height (cm), weight (kg), waist-hip circumference were measured, body mass index (BMI) and waist-hip ratio were calculated.
- Enzyme linked immunosorbent assay (ELISA) was used to detect the changes of serum ApoM and RBP4 levels in all subjects.
- Detection of biochemical indicators: Using automatic biochemical analyzer test subjects Fasting plasma glucose (Fasting blood glucose, FBG), Total cholesterol, Total cholesterol, TC), three acyl glycerin (Triglycerin, TG), Low density lipoprotein cholesterol, Low density lipoprotein cholesterol, LDL-C), High density lipoprotein cholesterol (High density lipoprotein cholesterol, HDL-C)

Statistical method

SPSS20.0 software package was used to analyze the data of this study. Measurement data were represented by mean standard deviation ($\bar{x}\pm s$), one-way anova was used for comparison between groups, and chi-square test and counting data were used. Pearson correlation analysis method was used to analyze the correlation between various indicators and blood lipid levels, and Logistic regression analysis was used to analyze the risk factors of carotid artery vascular disease in type 2 diabetes mellitus. $P<0.05$ was considered as statistically significant difference.

Results

Comparison of General Conditions

Waist circumference and waist-hip of the carotid plaque group were significantly higher than those of the control group (P<0.05), waist circumference of the normal carotid artery group was significantly higher than that of the control group (P<0.05), and there was no statistically significant difference in BMI of the three groups (P>0.05), as shown in table 1

Group	n	BMI (kg/m²)	Waistline (cm)	Waist hip ratio
Carotid plaque group	58	24.18±5.24	87.14±8.18	0.94±0.05
Normal carotid artery group	52	23.67±2.98	83.14±9.25	0.90±0.47
Control group	50	22.97±3.28	77.12±8.04	0.81±0.03
F		1.22	18.75	3.21
P		0.299	<0.001	0.043

Table 1: Comparison of three groups of general conditions ($\bar{x}$±s).

Comparison of Serum Apom and RBP4 Levels in Each Group

Serum ApoM in carotid plaque group was significantly lower than that in normal carotid group and control group (P<0.01), and serum ApoM in normal carotid group was significantly lower than that in control group (P<0.01). The serum RBP4 level of the carotid plaque group was significantly higher than that of the normal carotid group and the control group (P<0.01), and there was no statistical difference between the normal carotid group and the control group (P>0.05), as shown in table 2

Group	n	ApoM (μg/ml)	RBP4 (ml/L)
Carotid plaque group	58	9.01±1.32	58.54±18.69
Normal carotid artery group	52	11.78±2.05	43.87±14.26
Control group	50	17.16±1.87	41.06±11.47
F		294.40	20.87
P		<0.001	<0.001

Table 2: Comparison of serum ApoM and RBP4 levels in each group ($\bar{x}$±s).

Comparison of biochemical indexes in each group

The levels of FBG, TC, TG and LDL-C in the carotid plaque group were significantly higher than those in the normal carotid group and the control group (P<0.01), and HDL-C were significantly lower than those in the normal carotid group and the control group (P<0.01).

The levels of FBG, TC and TG in the normal carotid group were significantly higher than those in the control group (P<0.01), and HDL-C were significantly lower than those in the control group (P<0.01), as shown in table 3

Group	n	FBG (mmol/L)	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Carotid plaque	58	11.15±5.81	5.92±0.76	3.45±1.23	3.21±0.63	1.02±0.23
Normal carotid artery	52	9.62±5.76	4.49±0.53	2.68±0.77	2.41±0.57	1.22±0.32
Control	50	4.69±0.34	3.17±0.51	2.32±0.24	2.30±0.23	1.47±0.35
F		42.78	266.84	23.97	51.23	29.99
P		<0.001	<0.001	<0.001	<0.001	<0.001

Table 3: Comparison of biochemical indicators in each group ($\bar{x}$±s).

Correlation of Serum Apom and RBP4 with Various Indexes

Serum ApoM was positively correlated with BMI (P<0.05) and negatively correlated with RBP4, FBG, TC, LDL-C levels (P<0.05), while serum ApoM was not correlated with other indicators. Serum RBP4 was negatively correlated with LDD-C (P<0.05), and positively correlated with waist circumference, waist-hip ratio, TC, LDL-C and BMI (P<0.05), while serum RBP4 was not correlated with other indicators. Shown in table 4.

Correlativity test	ApoM		RBP4	
	r	*P*	*r*	*P*
BMI	0.651	0.024	0.339	0.011
FBG	-0.587	0.041	1.274	0.578
TC	-0.532	0.003	0.210	0.026
LDL-C	-0.428	0.034	0.135	0.009
HLD-C	1.278	0.137	-0.334	0.020
Waistline	2.784	1.157	0.286	0.041
Waist Hip ratio	0.747	0.069	0.170	0.039

Table 4: Correlation between serum ApoM and RBP4 and various indexes.

Logistic regression analysis of risk factors affecting carotid artery lesions in type 2 diabetes mellitus

Logistic regression analysis results showed that age, course of disease, LDL-C and serum ApoM and RBP4 levels were all risk factors affecting carotid artery lesions in type 2 diabetes mellitus (P<0.05). Shown in table 5

Psychological risk factors	OR	Wald	*P*	95%CI
Age	1.245	10.337	0.022	0.560~2.078
T2DM pathogenesis	1.081	1.078	0.001	1.044~1.276
LDL-C	5.058	8.362	0.001	3.341~11.304
ApoM	1.754	0.634	0.003	1.124~2.804
RBP4	3.487	1.141	0.024	0.535~21.417

Table 5: Correlation between serum ApoM and RBP4 and various indexes.

Discussion

According to relevant studies, diabetes has become the most common chronic disease in China after cardiovascular and cerebrovascular diseases. With the improvement of people's living standards, the incidence of T2DM has been increasing year by year, accounting for about 1/3 of the world's diabetes patients. Cerebrovascular diseases and renal failure and other complications are the main causes of death of patients, bringing heavy burden to patients' families and society[8]. The main pathological basis of T2DM carotid artery lesions is macro atherosclerosis, which may involve many important arteries such as femoral artery, carotid artery and coronary artery. Studies have shown that the risk of atherosclerosis in T2DM patients is significantly higher than that in non-T2DM patients by more than 3 times[9]. Accordingly, early discovery treats arteriosclerosis early, can reduce or prevent the happening of cardiovascular and cerebrovascular event.

Lipid metabolism disorder is the basis of the pathogenesis of atherosclerosis and serum ApoM mainly exist in the human plasma HDL-C, its molecular weight is 26kDa, located in chromosome 6 short arm, main compatible compound III area, belongs to the Lipocalin protein superfamily[10]. ApoM expression and secretion by the regulation of many biological factors, mainly in the human body or mice in the liver of high expression of its gene promoter region only liver cell nucleus factor 1 (Hepatocyte nuclear factor 1, HNF-1α) binding sites, and liver and kidney are the main transcriptional regulation factor is HNF-1α, its physiological function may have close relationship with the liver and kidney.

Some scholars found through animal experiments[11] that the expression and secretion of ApoM in alloxan diabetic mice were significantly lower than that in normal control mice, the blood glucose of diabetic mice was significantly decreased after insulin injection, and the level of ApoM gene in liver and kidney tissues was significantly increased. Foreign scholars[12] found that feeding high-cholesterol rats with deficient HDL receptors confirmed that overexpression of ApoM can lead to atherosclerosis. In human experiments[13], some scholars have confirmed that the plasma ApoM level of T2DM patients is lower than that of normal people and diabetes patients with good blood glucose control, which indicates that serum ApoM may increase the risk of atherosclerosis in the body.

Serum RBP4 is one of the fat-soluble vitamin carrier proteins and belongs to the retinol binding protein family, which mainly transports retinol from the liver to surrounding tissues. In recent years, studies have confirmed that its secretion imbalance plays an important role in the pathological process of insulin resistance and cardiovascular diseases[14]. It can sensitively reflect the degree of proximal convoluted tubule injury in clinic and can also be used as an indicator of early liver function damage and monitoring treatment. Some scholars have pointed out that serum RBP4 can accelerate endothelial inflammation by increasing oxidative stress, leading to endothelial cell dysfunction, up-regulation of adhesion molecules, and aggravation of atherosclerosis, which can be used as a biomarker for cardiovascular disease of T2DM[15].

This study, according to the results of serum ApoM carotid plaques group was obviously lower carotid artery normal group and the control group ($P < 0.01$), serum RBP4 level carotid plaques group was obviously higher than that of normal carotid artery group and the control group ($P < 0.01$), suggesting that serum ApoM, RBP4 may participate in the development of T2DM patients with carotid artery pathological changes, and associated with the degree of pathological changes. Pearson correlation analysis results showed that serum ApoM was positively correlated with BMI ($r=0.651$, $P<0.05$), and negatively correlated with RBP4, FBG, TC, LDL-C levels ($r=-0.257$, -0.587, -0.532, -0.428, $P<0.05$).Serum RBP4 was negatively correlated with LDC-C ($r=-0.334$, $P<0.05$), and positively correlated with waist circumference, waist-hip ratio, TC, LDL-C, and BMI ($r=0.286$, 0.170, 0.210, 0.135, 0.339, $P<0.05$), suggesting that serum ApoM and RBP4 may aggravate the occurrence of carotid artery lesions in T2DM by regulating blood lipid metabolism. Logistic regression analysis results showed that age, course of disease, LDL-C and serum ApoM and RBP4 levels were all risk factors affecting carotid vascular lesions in T2DM ($P<0.05$), suggesting that the synergistic effect of serum ApoM, RBP4 and other factors could increase the risk of carotid vascular lesions.

In conclusion, the decreased serum ApoM and increased RBP4 levels in T2DM patients with carotid artery disease may be related to insulin resistance and dyslipidemia, providing a new therapeutic target for clinical treatment of T2DM patients with carotid artery disease.

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