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Original Article

Retinol binding protein 4 correlates with and is an early predictor of carotid atherosclerosis in type 2 diabetes mellitus patients

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Abstract

The association of retinol binding protein 4 (RBP4) with atherosclerosis of the carotid artery in type 2 diabetes mellitus (T2DM) remains undefined. We aimed to investigate the correlation of RBP4 expression with atherosclerosis of the carotid artery in T2DM. A total of 1,076 subjects were investigated for intima-media thickness of the bilateral common carotid arteries, and they were divided into three groups: in group I, patients had normal neck vascular ultrasound, in group II, intimal carotid artery media thickness was equal to or more than 1 mm, and in group III, carotid artery plaque was present. Height, weight, blood pressure (BP), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A-1 (apoA-1), apolipoprotein B (apoB) and lipoprotein (a) [Lp(a)] were determined by routine laboratory methods. RBP4 and high sensitivity C reactive protein (HsCRP) were measured by an enzyme-linked immuno-sorbent assay, and insulin concentration was measured by an electrochemiluminescence sandwich immunoassay. Duration of diabetes, waist and BP, FPG, HbA1c, TG, TC, LDL-C, APOB, Lp(a), HsCRP, RBP4 and homeostasis model assessment insulin resistance index (HOMA-IR) were significantly lower in group I than in the other two groups ($P<0.01$, $P<0.01$). Plasma levels of HbA1c, RBP4, LDL-C, TC, HOMA-IR, HsCRP and Lp(a), waist and BP were significantly increased in group III than in group II ($P<0.01$). Multivariate logistic regression analysis showed that there were seven factors associated with the occurrence of carotid artery atherosclerosis and its risks in descending order were: high LDL-C, high waist, high HsCRP, duration of diabetes, high HOMA-IR, HbA1c and high RBP4. Our finding supported that RBP4 was positively correlated with carotid atherosclerosis in patients with T2DM and could be used as an early predictor of cardiovascular disease.

Keywords: type 2 diabetes mellitus, retinol binding protein 4, subclinical atherosclerosis

Introduction

Diabetes mellitus (DM) is a metabolic disorder that can be affected by genetic factors, environment and life styles. The incidence of DM, especially type 2, is dramatically increasing due to the growing obesity and immobility in China[1-2]. Epidemiologic surveys have recently reported a high incidence of atherosclerosis in patients with DM which is an artery damage attributing to cholesterol deposition, immune substance infiltration, connective tissue hyperplasia and can cause severe ischemic heart disease, cerebrovascular disease and death. Therefore, quantitative assessment of atherosclerosis and its risks is very important. Carotid ultrasound for measurement of intima-media thickness (IMT) and detection of atheroma plaques is a

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non-invasive, well standardized and validated imaging technique that is currently recommended by clinical guidelines for cardiovascular risk assessment[3]. RBP are a family of proteins with diverse functions. They are carrier proteins that bind to retinol. RBP4 may play an important role in the origin of insulin resistance and metabolic syndrome[4-5]. However, there is scanty data on the relationship between RBP4 and IMT and subclinical atherosclerosis in type 2 diabetes mellitus (T2DM).

Patients and methods

Patients

A total of 1,076 inpatient patients (578 men) with a mean age of 62.8±13.6 (ranged from 35 to 82) years were admitted for this study at the authors' affiliated hospital. All patients were diagnosed with T2DM. Type 1 DM and secondary DM were excluded. All patients provided informed consent and the study was approved by the local Institutional Review Board at the authors' affiliated institution.

Carotid intima-media thickness assessment

IMT of the bilateral common carotid arteries was measured using a GE Logiq7 linear array probe (7.5 MHz). Patients were examined in the supine position with their head tilted back. Each common carotid artery was evaluated with the subject's head turned slightly to the contralateral side. The field depth, gain, and near- and far-field gain controls were optimized to enable visualization of the far wall of the common carotid artery. All images were interpreted by a single technician, specifically trained in the assessment of carotid intima-media thickness (CIMT). The patients were stratified into 3 groups: neck vascular ultrasound showed no abnormalities (Group I), intimal thickening was defined as carotid artery media thickness ⩾1 mm (Group II), and carotid artery plaque was defined as a focal area of arterial wall thickness ⩾1.5 mm (Group III).

Laboratory and clinical data

In all patients, fasting plasma glucose (FPG), baseline total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A-1 (apoA-1) and apolipoprotein B (apoB) were measured from fasting blood samples with the enzymatic colorimetric method by using commercial kits on a OLYMPUS AU2700 (Japan). Glycosylated hemoglobin A1c (HbA1c) was determined by high pressure liquid chromatography (HPLC, Bio-Rad, Hercules, CA, USA). RBP4 and high sensitivity C reactive protein (hsCRP) were measured by an enzyme-linked immuno-asorbent assay (ELISA, Phoenix, American). Insulin concentration (mIU/L) was measured from fasting blood samples using an electrochemiluminescence sandwich immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany) on a MODULAR ANALYTICS SWA Modular DDPP+EE. Homeostasis model assessment insulin resistance index (HOMA-IR) was defined as (fasting plasma glucose (mmol/L) × serum insulin (mIU/L))/22.5[6].

Statistical analysis

All statistical analyses were performed using SPSS (version 13.0; SPSS Inc, Chicago, IL, USA). The data were expressed as mean±SD, except for data that did not have a normal distribution, which were expressed as median (interquartile range). Variables were tested for normality using Kolmogorov-Smirnov Z statistic. Between-group differences were analyzed with one-way ANOVA in conjunction with SNK-q test. Correlation relationships between plasma RBP4 concentrations and various metabolic parameters were analyzed with Spearman correlation analysis. Multivariate analysis was used with logistic regression analysis. RBP4, duration of DM, waist, HsCRP, HOMA-IR, LDL-C, Lp(a), DBP and HbA1c were independent variables in the multivariable-adjusted models with carotid artery plaque as the dependent variable, including Group II and Group III, and age and sex as the covariates. Two-tailed significance values were given with $P<0.05$ regarded as significant.

Results

There were 116 men (34.9%) and 216 women (65.1%) in Group I, 192 men (49.7%) and 194 women (50.3%) in Group II, 201 men (56.1%) and 157 women (43.9%) in Group III. The mean age, BMI, HDL-C and apoA-1 were not different among the three groups. Duration of diabetes, waist, BP and the serum levels of FPG, HbA1c, TG, TC, LDL-C, apoB, Lp(a), HsCRP, RBP4 and HOMA-IR were significantly lower in normal controls than in the other two groups ($P<0.01$). Although duration of diabetes, FPG, apoB and TG showed no significant difference between Group II and Group III, waist, BP and plasma levels of HbA1c, Lp(a), RBP4, LDL-C, TC, HOMA-IR and HsCRP were significantly higher in patients with carotid artery plaque than in intimal thickening ($P<0.01$) (***Table 1***).

Spearman correlation showed that RBP4 concentrations were weakly correlated with duration of diabetes, BP, TC, LDL-C, apoB and Lp(a), while no associations were found between RBP4 and age, BMI, FPG, HDL-C and apoA-1; waist circumference, HbA1c,

Table 1 **Baseline characteristics of the study subjects**

	Group I	Group II	Group III
Number	332	386	358
Age (years)	62.70±8.40	63.80±9.20	63.10±7.20
Duration (years)	5.80±3.20	8.60±4.80*	8.90±4.40**
BMI (kg/m^2)	27.50±4.20	27.90±3.40	27.60±3.60
W (cm)	81.60±9.20	86.70±7.50*#	94.80±9.80**
FPG (mmol/L)	8.07±3.02	9.63±4.61*	9.34±4.22**
HbA1c (%)	7.52±0.84	8.07±1.03*#	8.92±0.96**
SBP (mmHg)	126.80±10.30	132.00±8.40*#	138.70±12.40**
DBP (mmHg)	78.40±7.20	86.50±8.60*#	90.60±10.50**
TG (mmol/L)	1.89±0.66	2.32±0.72*	2.16±1.08**
TC (mmol/L)	4.18±0.96	5.07±0.68*#	5.45±0.85**
HDL-C (mmol/L)	1.32±0.78	1.27±0.69	1.21±0.72
LDL-C (mmol/L)	2.53±0.86	3.78±0.59*#	3.95±0.82**
apoA-1 (g/L)	1.38±0.32	1.34±0.41	1.32±0.51
apoB (g/L)	0.68±0.49	0.82±0.58*	0.86±0.35**
Lp(a) (mg/L)	179.30±65.70	382.90±92.70*#	399.60±78.50**
RBP4 (mg/L)	32.10±10.3	38.20±8.30*#	46.90±7.60**
Ln (HOMA-IR)	0.87±0.49	1.10±0.19*#	1.18±0.21**
HsCRP (mg/L)	1.78±0.87	3.41±1.06*#	5.62±0.91**

Duration: duration of diabetes; BMI: body mass index; W: Waist circumference; FPG: fasting plasma glucose; HbA1c: hemoglobin A1c; HOMA-IR: homeostasis model assessment insulin resistance index; SBP: systolic pressure; DBP: diastolic pressure; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; apoA-1: apolipoprotein A-1; apoB: apolipoprotein B; Lp(a): lipoprotein a; RBP4: retinol-binding protein 4; HsCRP: high sensitivity C reactive protein. *$P<0.01$, *vs*. Group I: normal neck vascular ultrasound; #$P<0.01$, *vs*. Group III: carotid artery plaque; **$P<0.01$, *vs*. Group I: normal neck vascular ultrasound.

HOMA-IR, TG and HsCRP demonstrated moderate correlation with RBP4 of all investigated cardiovascular risk factors (***Table 2***).

Table 3 summarizes the risk factors of carotid artery atherosclerosis by using multiple logistic regression analysis, which showed that there were seven factors associated with the occurrence of carotid artery atherosclerosis and its risk in descending order were: high LDL-C, high waist, high HsCRP, duration of diabetes, high HOMA-IR, HbA1c and high RBP4 while Lp(a) and BP showed no significant association with carotid artery atherosclerosis.

Discussion

The prevalence of diabetes is increasing throughout the world, which has a high incidence of macro-vascular complication. Macroangiopathy is a heterogeneous disorder characterized by multifactorial etiology and various processes, including changes in adipocytokines, activation of low-grade inflammation, and others. RBP4 was discovered as an adipocytokine that bound specifically to vitamin A and produced mainly by the liver and adipose tissues. RBP4 levels were closely associated with obesity, particularly visceral adiposity in mice and humans[7-8]. The main finding of our study was that RBP4 concentrations were associated with the prevalence ofcarotid artery atherosclerosis in 1,076 samples of T2DM, which suggested a participation of RBP4 in modulation of atherosclerotic process and cardio- and cerebrovascular diseases. Our result is inconsistent with some previous studies which reported an inverse correlation between RBP4 levels and CIMT in postmenopausal women[9-10], which could attribute to different study populations and designs.

It is known that elevated RBP4 levels are associated with a clustering of components of metabolic syndrome in insulin-resistant subjects. In population-based studies, RBP4 levels were positively associated with the obesity index, high blood pressure and unfavorable lipid profiles[11-12]. Similar to our finding of higher RBP4 value with triglyceride level, previous studies have shown that RBP4 was correlated with serum triglycerides[13-15]. In the elderly, RBP4 concentrations were associated with metabolic syndrome and its components in both sexes and prior cerebrovascular disease in men. These findings are consistent with the hypothesis that circulating RBP4 could be a marker of metabolic complications and possibly also

Table 2 **Correlation between plasma RBP4 concentrations and various metabolic parameters**

	Spearman coefficient	*P*
Age	0.193	0.042
Duration	0.205	0.028
BMI	0.035	0.363
Weight	0.436	0.001
FPG	0.102	0.067
HbA1c	0.306	0.009
SBP	0.210	0.023
DBP	0.251	0.015
TG	0.427	0.002
TC	0.208	0.034
HDL-C	-0.138	0.062
LDL-C	0.239	0.027
APOA-1	-0.071	0.104
APOB	0.205	0.047
Lp(a)	0.113	0.035
HOMA-IR	0.301	0.007
HsCRP	0.472	0.001

Duration: duration of diabetes; BMI: body mass index; FPG: fasting plasma glucose; HbA1c: hemoglobin A1c; SBP: systolic pressure; DBP: diastolic pressure; TC: total cholesterol; TG: triglyceride.

Table 3 **Logistic regression analysis of carotid atherosclerosis risk factors**

Factors	B	S.E.	Wald	Sig	Exp(B)	95% C.I. for EXP(B)	
						Lower	Upper
RBP4	0.024	0.005	25.409	0.000	1.019	1.021	1.048
Duration	0.683	0.103	47.359	0.000	2.061	1.482	2.621
Waist	0.997	0.102	90.314	0.000	2.743	2.445	3.483
HsCRP	0.739	0.081	78.682	0.000	2.074	1.768	2.433
HOMA-IR	0.841	0.116	47.301	0.000	2.259	1.837	2.861
LDL-C	1.382	0.081	286.213	0.000	3.954	3.391	4.963
Lp(a)	0.107	0.153	0.571	0.482	1.015	0.827	1.491
DBP	0.190	0.189	0.952	0.324	1.210	0.741	1.439
HbA1c	0.564	0.097	37.197	0.000	1.780	1.426	2.031

RBP4: retinol-binding protein 4;Duration: duration of diabetes; HsCRP: high sensitivity C reactive protein; HOMA-IR: homeostasis model assessment insulin resistance index; LDL-C: low-density lipoprotein cholesterol; Lp(a): lipoprotein (a); DBP: diastolic blood pressure; HbA1c: hemoglobin A1c.

atherosclerosis and overt CVD[16]. Our study showed that plasma RBP4 levels were significantly higher in patients with carotid artery plaque, and plasma RBP4 levels showed significant correlation with cardiovascular risks in T2DM patients. Some studies reported that RBP4 levels could play an important role in lipid metabolism in morbid obesity, increasing triglyceride levels and contributing to the formation of small HDL[17-18]. In animal models, the overexpression of human RBP4 or the injection of recombinant RBP4 induced insulin resistance in mice, whereas *RBP4* knockout mice showed enhanced insulin sensitivity. Since evidence showing relationship of RBP4 with cardiometabolic risk in humans is inconsistent, there is still an argument on whether elevated RBP4 levels contribute to the pathogenesis of abnormal glucose homeostasis or insulin resistance. RBP4 can down-regulate glucose transporter4 (GLUT4) selectively in adipocytes and the insulin-activated glucose transporter is responsible for translocation of glucose into both muscle and fat cells. Studies reported that the serum level of RBP4 was highly negatively correlated with obesity or insulin sensitivity[19-21]. However, Janke *et al.* reported discrepancy of relationship of RBP4 with glucose homeostasis and insulin resistance between rodents and humans. Our results suggested a direct relationship between circulating insulin and RBP4 levels, which indicated that this protein might contribute to the development of muscle insulin resistance[22-23]. A retrospective cohort study provided evidence that RBP4 may be a mechanism through which obesity influenced insulin resistance and hypertriglyceridemia in overweight postpubertal black youth and suggested utility of RBP4 as a biomarker of risk[24]. In mice, overexpression of RBP4 has been shown to cause insulin resistance presumably by enhanced expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase and impairment of muscle insulin action[18]. More data are needed to clarify the potential role of RBP4 in abnormal metabolic consequences.

Balagopal *et al.* reported that RBP4 was positively associated with hsCRP in a small group of obese children[25]. However, Liu Y *et al.* showed that plasma RBP4 levels were associated with an adverse profile of oxidative stress and inflammatory markers in a middle-aged and elderly Chinese population and the association was independent of conventional risk factors[26]. It is well known that the risk of atherosclerosis is much higher in diabetic patients and the endogenous defense of the vascular endothelium begins to break down in response to diabetes. However, previous studies on whether RBP4 was involved in insulin-induced proliferation of vascular smooth muscle cells and inflammation leading to atherosclerosis in humans have shown conflicting findings[5]. In this study, correlation analysis showed that the plasma HsCRP level was significantly correlated with carotid atherosclerosis, which is consistent with the hypothesis that circulating HsCRP could be used as early biochemical markers of cardiovascular diseases.

This study has some limitations. Firstly, the cross-sectional nature of the study design cannot be translated into a clear cause-effect inference. Prospective studies and randomized clinical trials are needed. Secondly, the key threat to any observational study is bias of unmeasured or residual confounding that cannot be ruled out; however, the multivariate models adjusted for a wide range of risk factors that have been implicated in the development of cardiovascular diseases in patients with T2DM. Thirdly, the study did not consider the impact of smoking and drinking on RBP4, in addition, only inpatient patients took part in the study and duration of diabetes was more than five years. These factors may contribute to some bias.

In summary, in patients with type 2 diabetes, serum RBP4 is positively correlated with carotid atherosclerosis, and may cause carotid artery atherosclerosis through the influence of insulin sensitivity, lipid metabolism, and the body oxygen oxidative stress. RBP4 can be used as an early predictor of cardiovascular disease risk factor in T2DM patients. Compared to vascular ultrasound, RBP4 detection is more convenient and inexpensive, and could be defined as a routine test item to help early detection and intervention of vascular complications.

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