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Original Article

Changes of serum CypA and CNP levels in type 2 diabetes mellitus patients with vascular lesions and its clinical prognostic significance

Tianyi Huang[1], Shang Ju[1], Xi Li[1], Gang Wang[1], Bohua Yang[1], Ning Wang[2], Chengcheng Yan[2]

[1]Department of Peripheral Vascular, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing City, China; [2]Beijing University of Chinese Medicine, Beijing City, China

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Abstract: Objective: To investigate the changes of serum cyclophilin A (CypA) and C-type natriuretic peptide (CNP) levels in patients with type 2 diabetes mellitus (T2DM) with vascular lesions and evaluate their clinical prognostic value. Methods: A total of 86 patients with T2DM who were treated in Dongzhimen Hospital, Beijing University of Chinese Medicine between July 2016 and May 2017 were selected, of which 41 were T2DM patients (group A) and 45 were T2DM patients with vascular lesions (group B). An automatic chemiluminescence analysis system was used to determine the levels of fasting blood glucose (FBG), total cholesterol, triglyceride (TG), glycosylated hemoglobin (HbA1c), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C). Serum CypA, CNP, nuclear transcription factor-κB (NF-κB), and c-reactive protein (CRP) levels were determined by ELISA. The Pearson correlation analysis was employed to determine the correlation between CypA and CNP, and other general characteristics. Multivariate logistic regression analysis was performed to determine the influencing factors of vascular lesions in T2DM patients. Results: Between the two groups, a significant difference was observed in age, course of disease, and levels of CRP, HbA1c, FBG, NF-κB, and TG (all $P<0.05$). In addition, the Pearson correlation analysis indicated that there was a positive correlation between CypA and CRP, HDL-C, HbA1c, FBG, and NF-κB (all $P<0.05$). Moreover, CNP negatively correlated with CRP, LDL-LDL-C, HbA1c, and NF-κB (all $P<0.05$). Multivariate Logistic regression analysis showed that age, CypA, and CNP were influencing factors of T2DM with vascular lesions (all $P<0.05$). Conclusion: T2DM patients with vascular lesions present with a higher serum level of CypA and a lower serum level of CNP, which are risk factors for T2DM.

Keywords: Type 2 diabetes mellitus, cyclophilin A, C-type natriuretic peptide, vascular lesion

Introduction

Type 2 diabetes mellitus (T2DM) is a common non-communicable disease. Most patients diagnosed with T2DM show signs of various cardiovascular and cerebrovascular diseases, such as coronary heart disease, myocardial infarction, and cerebral thrombosis [1]. Vascular lesion is an important pathological issue for various complications in T2DM patients [2]. A study suggested that hyperglycemia would cause abnormal glucose and lipid metabolism in T2DM patients, thereby resulting in vascular endothelial cell damage and changes in blood rheology that might eventually lead to vascular disease [3]. Since the occurrence of vascular lesions in patients with T2DM is latent, when patients present with coronary heart disease, hypertension or other complications, the degree of vascular lesions has become more serious [4]. Therefore, it is of great clinical importance to investigate and identify the influencing factors of vascular lesions in patients with T2DM.

In previous studies, it was shown that the serum level of cyclophilin A (CypA) was higher in T2DM patients with vascular lesions, which could induce unstable plaque formation in blood vessels, leading to acute cardiovascular events [5]. C-type natriuretic peptide (CNP) is a 22-amino acid vasoactive polypeptide that acts on vascular smooth muscle cells (VSMCs) in an autocrine and paracrine manner, then

activating guanylate cyclase upon binding to the natriuretic peptide receptor B to inhibit abnormal proliferation of VSMCs and alleviate vascular lesions [6].

Currently, clinical studies on serum CypA and CNP in patients with T2DM with vascular lesions are limited. In the current study, we investigated the effects of CypA and CNP on vascular lesions in T2DM patients to provide a clinical basis for the treatment of T2DM.

Materials and methods

General information

A total of 86 T2DM patients treated in Dongzhimen Hospital, Beijing University of Chinese Medicine between July 2016 and May 2017 were selected for this study, of which 47 patients were males and 39 patients were females, aged 34-76 years with an average age of 53.36±6.72 years. Among them, 41 cases were diagnosed as T2DM and included in group A; 45 cases were diagnosed as T2DM with vascular lesions and included in group B.

Inclusion criteria: After diagnosis of T2DM, patients were screened for cardiovascular complications, such as coronary heart disease, carotid artery disease, or vascular disease of the lower extremities.

Exclusion criteria: Patients with Type 1 diabetes, gestational diabetes and other types of diabetes; patients with liver, kidney, or other type of organ failure; patients with a malignant tumor, hyperthyroidism, acute and chronic inflammation; and patients who were treated with hormonal and diuretic medicine.

This study was approved by the Ethics Committee of Dongzhimen Hospital, Beijing University of Chinese Medicine and all patients signed the informed consent prior to the start of the study.

Methods

A total of 6 mL of fasting venous blood was collected from every subject. After standing at 4°C for 1 h, blood was centrifuged at 1,200 rpm for 10 min at 4°C. Serum was collected and stored at -20°C. Serum total cholesterol (TC) and triglyceride levels were measured by the oxidase method; serum high-density lipoprotein-C (HDL-C) by a chemically-modified enzymatic method [2]; serum low-density lipoprotein-C (LDL-C) by a polyethylene sulfate chemical precipitation method [4]; fasting blood glucose (FBG) by the glucose oxidase method [6]; glycosylated hemoglobin (HbA1c) by high performance liquid chromatography [7]. Serum CypA, CNP, nuclear transcription factor-κB (NF-κB), and c-reactive protein (CRP) levels were determined by ELISA. CypA and CNP test kits were purchased from Shanghai Yu-Bo Biological Technology Co., Ltd. (Shanghai, China), NF-κB and CRP test kits were purchased from Beijing BioLab Technology Co., Ltd. (Beijing, China). Standard wells, negative control wells and sample wells were set according to the ELISA kit instructions. After the ELISA plate was washed, 100 μL of antigen coating solution was added to each well and the plate was incubated at 37°C for 2 h. After drying the liquid, 100 μL of 5% fetal calf serum was added to each well and wells were blocked for 2 h at 37°C. After the plate was washed, 30 μL of the test sample was added and incubated at 37°C for 1 h. After the plate was washed, enzyme-labeled working solution was added and incubated at 37°C for 1 h. Then, 20 μL substrate solution was added, and the reaction was terminated after 0.5 h. The OD value was measured using a microplate reader at a wavelength of 450 nm and a standard curve was created.

Observation indicators

Primary observation indicators: (1) Blood glucose related indicators, including FPG, and HbA1c; (2) Lipid-related indicators, including TC, TG, HDL-C, LDL-C; (3) CypA, CNP, NF-κB, and CRP.

Secondary observation indicators: Basic clinical data of study subjects, including age, gender, course of disease, body mass index (BMI), and systolic and diastolic blood pressure.

Statistical analysis

SPSS19.0 was used to analyze the data. Measurement data was expressed as the mean ± standard deviation ($\overline{x} \pm sd$). Measurement data tallied with normal distribution was analyzed by

Table 1. Comparison of general data between the two groups of patients

Group	Group A (n=41)	Group B (n=45)	t/χ^2	P
Gender (male/female)	23/18	24/21	3.274	0.064
Age	49.24±4.86	55.26±5.64	5.323	0.037
Course of disease (years)	6.43±2.16	7.18±2.62	5.167	0.041
BMI (kg/m^2)	25.15±3.27	25.42±3.39	2.549	0.083
Systolic pressure (mmHg)	127.48±18.24	124.62±17.28	3.427	0.062
Diastolic pressure (mmHg)	82.41±9.26	84.37±7.49	2.143	0.092

Note: BMI, body mass index.

Table 2. Comparison of laboratory test indexes

Index	Group A (n=41)	Group B (n=45)	t	P
CRP (mg/L)	2.47±0.51	4.86±0.94	5.348	0.037
LDL-C (mmol/L)	4.19±1.28	4.37±1.39	2.437	0.074
HDL-C (mmol/L)	2.48±0.39	2.36±0.42	3.143	0.061
HbA1c (%)	8.42±3.75	11.37±3.27	5.472	0.041
NF-κB (ng/L)	76.67±14.17	94.27±16.25	7.428	0.023
FBG (mmol/L)	8.13±2.74	9.27±1.86	6.273	0.036
TG (mmol/L)	2.61±0.28	3.21±0.43	5.237	0.043
TC (mmol/L)	6.24±1.31	6.36±1.24	2.516	0.068

Note: CRP, c-reactive protein; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; NF-κB, nuclear transcription factor-κB; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol.

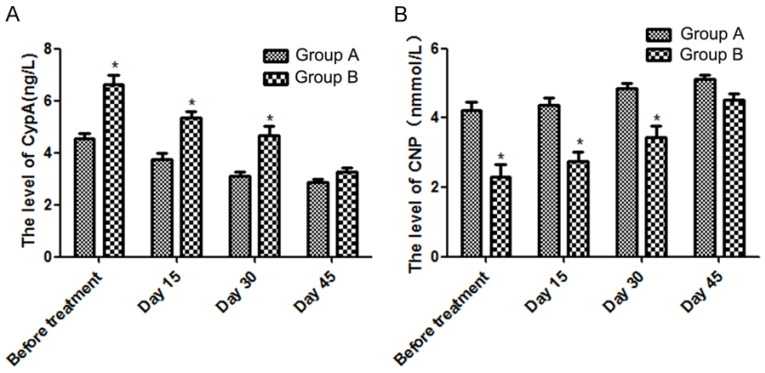

Figure 1. Changes of serum CypA and CNP levels after treatment in both groups. A: Serum CypA levels in both groups; B: Serum CNP levels in both groups. Compared with the group A, *P<0.05. CypA, cyclophilin A; CNP, C-type natriuretic peptide.

t-test, expressed by t. Counting data comparison was conducted with χ^2-test, which was expressed by chi-square. Pearson correlation was used to analyze data correlation and multivariate logistic regression was used to analyze the influencing factors of T2DM with vascular lesions. P<0.05 is considered statistically significant.

Results

Comparison of general data between the two groups of patients

No significant differences were observed between group A and group B in gender, BMI, systolic pressure, and diastolic pressure (all P>0.05). However, a significant difference was found in age and course of disease between the two groups (both P<0.05). See **Table 1**.

Analysis of laboratory test indexes of two groups of patients

There were no significant differences between the two groups in LDL-C, HDL-C and TC (all P>0.05). However, significant differences between the two groups were found in CRP, HbA1c, FBG, NF-κB, and TG (all P<0.05). See **Table 2**.

Changes in serum CypA and CNP levels after treatment in both groups

Prior to treatment, the serum level of CypA in group A was significantly lower than group B, whereas that of CNP was obviously higher compared to group B (both P<0.05). At 15 and 30 days after treatment, serum levels of CypA in group A were significantly lower compared to that in group B, and serum CNP levels were significantly higher when compared to those in group B (all P<0.05). After 45 days of treatment, no differences were observed in serum levels of CypA and CNP between two groups (both P>0.05). See **Figure 1**.

Correlation analysis of serum levels of CypA and CNP with general data

Pearson correlation analysis showed that there was a positive correlation between CypA and

Table 3. Correlative analysis of serum CypA and CNP with general data

Index	CypA			CNP		
	r	t	P	r	t	P
Age	0.243	1.248	0.126	0.146	1.462	0.148
Course of disease	0.143	1.569	0.114	0.241	1.437	0.159
BMI	0.243	3.261	0.086	0.146	1.243	0.241
Systolic pressure	0.167	3.582	0.072	0.213	1.458	0.149
Diastolic pressure	0.235	3.274	0.084	0.247	1.436	0.174
CRP	0.341	4.849	0.043	-0.126	4.964	0.042
LDL-C	0.425	4.246	0.058	-0.243	5.329	0.036
HDL-C	0.328	4.856	0.041	0.153	4.268	0.053
HbA1c	0.357	4.895	0.039	-0.264	5.316	0.039
FBG	0.358	5.318	0.034	0.312	4.417	0.067
NF-κB	0.523	5.627	0.026	-0.246	5.374	0.037

Note: BMI, body mass index; CRP, c-reactive protein; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; FBG, fasting blood glucose; NF-κB, nuclear transcription factor-κB; CypA, cyclophilin A; CNP, C-type natriuretic peptide.

Table 4. Influencing factor analysis of vascular lesions in T2DM patients

Variable	β	SE	Wald value	OR	95% CI	P
Age	0.243	0.036	4.462	0.646	0.349-0.876	0.032
CypA	0.157	0.016	5.284	1.529	1.246-1.823	0.027
CNP	-0.359	0.043	5.467	0.426	0.248-0.759	0.024

Note: CypA, cyclophilin A; CNP, C-type natriuretic peptide.

CRP, HDL-C, HbA1c, FBG, and NF-κB (all P< 0.05). CNP levels negatively correlated with CRP, LDL-C, HbA1c, and NF-κB (all P<0.05). See **Table 3**.

Influencing factors analysis of vascular lesions in T2DM patients

T2DM patients with vascular lesions were used as the dependent variable when multivariate logistic regression analysis was employed on patient clinical data. The results demonstrated that age, CypA, and CNP were influencing factors of vascular lesions in T2DM patients. See **Table 4**.

Discussion

CypA is a pro-inflammatory cytokine secreted by VSMCs and endothelial cells when activated by reactive oxygen species (ROS). In addition, CypA can exert chemotactic effects on neutrophils and monocytes, thus promoting the development of vascular lesions [7]. A study indicated that CypA could activate the Akt/AK signaling pathway to promote endothelial cells to produce inflammatory cytokines, thereby leading to endothelial cell damage in patients [8]. NF-κB is an important regulator of inflammatory responses, and a common channel for activation and cascade amplification of various inflammatory cytokines. CRP is an index that reflects the degree of inflammation *in vivo*. In this study, we observed significant differences in levels of CRP, HbA1c, FBG, CypA, NF-κB, and CNP between the two groups. Pearson correlation analysis indicated a linear positive correlation between CypA and HbA1c, NF-κB, and CRP, indicating that CypA was closely related to the degree of inflammation in T2DM patients. Wong et al. showed that CypA promoted activation of NF-κB signaling pathway in cells, leading to the secretion of more NF-κB and CRP inflammatory response factors *in vivo*, thus aggravating the degree of inflammation *in vivo*, which resulted in abnormalities of arterial intima and middle layer structures and promoted the occurrence of vascular lesions [9].

CNP can inhibit the proliferation and migration of VSMCs and reduce the degree of inflammatory reactions in patients with T2DM [10-12]. Real et al. showed that serum levels of CNP in rats with T2DM and vascular lesions were lower, and the lumen of arteries increased, serum levels of CRP decreased after exogenous CNP intervention [13]. Previous studies showed that CNP stimulated the expression of nitric oxide synthase, which in turn increased nitric oxide levels *in vivo*, exerted anti-inflammatory effects, inhibited the formation of neointima, and reduced vascular lesions in patients [14, 15]. In the current study, we found that serum levels of CNP levels were lower in group B when compared to that in group A, suggesting that reduced serum CNP levels might stimulate the occurrence of vascular lesions, which was consistent with the findings presented in previous studies [16]. Pearson correlation analysis indicated a negative correlation between CNP and CRP, LDL-C, HbA1c, and NF-κB (all P<0.05). Dymkowska demonstrated that the serum level of CNP was lower in patients with T2DM and

vascular lesions, and negatively correlated with serum levels of CRP, LDL-C, and HbA1c, which was consistent with the results described in this study [17].

Logistic multivariate regression analysis revealed that age, CypA and CNP were influencing factors of vascular lesions in T2DM patients. Older patients with decreased cardiovascular function were susceptible to disturbances of the glucose and lipid metabolism. In addition, Striepe et al. demonstrated that T2DM patients with vascular lesions were older, which was consistent with the findings presented in this study [18]. Rooney et al. found that hyperglycemia could stimulate the increase of ROS and result in the secretion of CypA by monocytes and macrophages, which increased the incidence of cardiovascular disease in patients [19]. This was consistent with our observations. Malik et al. showed that T2DM patients without vascular lesions had a relatively high serum level of serum CNP, exerting a protective effect on cardiovascular function, which was consistent with our findings [20]. However, the sample size of our study was relatively small, therefore it is warranted to perform a large sample and multi-center clinical study to collect additional data and explore the clinical relevance of serum changes in CypA and CNP in T2DM patients with vascular lesions.

In conclusion, serum levels of CypA increased and serum levels of CNP decreased in patients with T2DM with vascular lesions, which were both risk factors of vascular disease in patients with T2DM. Thus, our findings provide some clinical guidance for the treatment of T2DM patients.

Disclosure of conflict of interest

None.

Address correspondence to: Shang Ju, Department of Peripheral Vascular, Dongzhimen Hospital, Beijing University of Chinese Medicine, No.5 Haiyuncang, Dongcheng District, Beijing City 100700, China. Tel: +86-010-84013151; E-mail: jushang150m@163.com

References

[1] Nozue T, Fukui K, Koyama Y, Fujii H, Kunishima T, Hikita H, Hibi K, Miyazawa A, Michishita I and Investigators FT. Effects of sitagliptin on coronary atherosclerosis in patients with type 2 diabetes-A serial integrated backscatter-intravascular ultrasound study. Am J Cardiovasc Dis 2016; 6: 153-162.

[2] Bakarev MA, Karpova AA, Pichigin VI, Nikityuk DB and Lushnikova EL. Involvement of the coronary bed in patients with coronary heart disease against the background of primarily coronary and generalized atherosclerosis. Bull Exp Biol Med 2016; 162: 283-287.

[3] Berezin AE, Samura TA, Kremzer AA, Berezina TA, Martovitskaya YV and Gromenko EA. An association of serum vistafin level and number of circulating endothelial progenitor cells in type 2 diabetes mellitus patients. Diabetes Metab Syndr 2016; 10: 205-212.

[4] Choudhury RP, Birks JS, Mani V, Biasiolli L, Robson MD, L'Allier PL, Gingras MA, Alie N, McLaughlin MA, Basson CT, Schecter AD, Svensson EC, Zhang Y, Yates D, Tardif JC and Fayad ZA. Arterial effects of canakinumab in patients with atherosclerosis and type 2 diabetes or glucose intolerance. J Am Coll Cardiol 2016; 68: 1769-1780.

[5] Kang SH, Park GM, Lee SW, Yun SC, Kim YH, Cho YR, Park HW, Suh J, Yang DH, Kang JW, Lim TH, Jung CH, Koh EH, Lee WJ, Kim MS, Lee KU and Park JY. Long-term prognostic value of coronary CT angiography in asymptomatic type 2 diabetes mellitus. JACC Cardiovasc Imaging 2016; 9: 1292-1300.

[6] Sitnik D, Santos IS, Goulart AC, Staniak HL, Manson JE, Lotufo PA and Bensenor IM. Fasting glucose levels, incident diabetes, subclinical atherosclerosis and cardiovascular events in apparently healthy adults: a 12-year longitudinal study. Diab Vasc Dis Res 2016; 13: 429-437.

[7] Kim BJ, Cho SH, Cho KI, Kim HS, Heo JH and Cha TJ. The combined impact of neutrophil-to-lymphocyte ratio and type 2 diabetic mellitus on significant coronary artery disease and carotid artery atherosclerosis. J Cardiovasc Ultrasound 2016; 24: 115-122.

[8] de Boer SA, Hovinga-de Boer MC, Heerspink HJ, Lefrandt JD, van Roon AM, Lutgers HL, Glaudemans AW, Kamphuisen PW, Slart RH and Mulder DJ. Arterial stiffness is positively associated with 18F-fluorodeoxyglucose positron emission tomography-assessed subclinical vascular inflammation in people with early type 2 diabetes. Diabetes Care 2016; 39: 1440-1447.

[9] Wong ND, Zhao Y, Patel R, Patao C, Malik S, Bertoni AG, Correa A, Folsom AR, Kachroo S, Mukherjee J, Taylor H and Selvin E. Cardiovascular risk factor targets and cardiovascular disease event risk in diabetes: a pooling project of

the atherosclerosis risk in communities study, multi-ethnic study of atherosclerosis, and Jackson heart study. Diabetes Care 2016; 39: 668-676.

[10] Borowska M, Dworacka M, Winiarska H and Krzyzagorska E. Homocysteine as a non-classical risk factor for atherosclerosis in relation to pharmacotherapy of type 2 diabetes mellitus. Acta Biochim Pol 2017; 64: 603-607.

[11] Sharma A, Green JB, Dunning A, Lokhnygina Y, Al-Khatib SM, Lopes RD, Buse JB, Lachin JM, Van de Werf F, Armstrong PW, Kaufman KD, Standl E, Chan JCN, Distiller LA, Scott R, Peterson ED and Holman RR. Causes of death in a contemporary cohort of patients with type 2 diabetes and atherosclerotic cardiovascular disease: insights from the TECOS trial. Diabetes Care 2017; 40: 1763-1770.

[12] Mita T, Katakami N, Shiraiwa T, Yoshii H, Gosho M, Shimomura I and Watada H. Dose-dependent effect of sitagliptin on carotid atherosclerosis in patients with type 2 diabetes mellitus receiving insulin treatment: a post hoc analysis. Diabetes Ther 2017; 8: 1135-1146.

[13] Real JT, Folgado J, Molina Mendez M, Martinez-Hervas S, Peiro M and Ascaso JF. Plasma homocysteine, Lp(a), and oxidative stress markers in peripheral macroangiopathy in patients with type 2 diabetes mellitus. Clin Investig Arterioscler 2016; 28: 188-194.

[14] Kimura T, Kaneto H, Kanda-Kimura Y, Shimoda M, Kamei S, Anno T, Kawasaki F, Hashiramoto M, Matsuki M, Mune T and Kaku K. Seven-year observational study on the association between glycemic control and the new onset of macroangiopathy in Japanese subjects with type 2 diabetes. Intern Med 2016; 55: 1419-1424.

[15] Preil SA, Kristensen LP, Beck HC, Jensen PS, Nielsen PS, Steiniche T, Bjorling-Poulsen M, Larsen MR, Hansen ML and Rasmussen LM. Quantitative proteome analysis reveals increased content of basement membrane proteins in arteries from patients with type 2 diabetes mellitus and lower levels among metformin users. Circ Cardiovasc Genet 2015; 8: 727-735.

[16] Rigalleau V, Cougnard-Gregoire A, Nov S, Gonzalez C, Maury E, Lorrain S, Gin H and Barberger-Gateau P. Association of advanced glycation end products and chronic kidney disease with macroangiopathy in type 2 diabetes. J Diabetes Complications 2015; 29: 270-274.

[17] Dymkowska D. Oxidative damage of the vascular endothelium in type 2 diabetes - the role of mitochondria and NAD(P)H oxidase. Postepy Biochem 2016; 62: 116-126.

[18] Striepe K, Jumar A, Ott C, Karg MV, Schneider MP, Kannenkeril D and Schmieder RE. Effects of the selective sodium-glucose cotransporter 2 inhibitor empagliflozin on vascular function and central hemodynamics in patients with type 2 diabetes mellitus. Circulation 2017; 136: 1167-1169.

[19] Rooney MR, Pankow JS, Sibley SD, Selvin E, Reis JP, Michos ED and Lutsey PL. Serum calcium and incident type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study. Am J Clin Nutr 2016; 104: 1023-1029.

[20] Malik S, Zhao Y, Budoff M, Nasir K, Blumenthal RS, Bertoni AG and Wong ND. Coronary artery calcium score for long-term risk classification in individuals with type 2 diabetes and metabolic syndrome from the multi-ethnic study of atherosclerosis. JAMA Cardiol 2017; 2: 1332-1340.