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Original Article

Relationship between postprandial C-peptide level and diabetic peripheral neuropathy in Chinese type 2 diabetes patients

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Abstract: Objectives: We conducted this study in order to assess the relationship between serum C-peptide levels and DPN in Chinese type 2 diabetes patients. Material and methods: A retrospective study of 1131 Chinese type 2 diabetic patients was conducted. The association between serum postprandial C-peptide (PCP) and other diabetic clinical parameters was assessed using Spearman correlation analysis. The relationship between DPN and each parameter was assessed using logistic regression analysis. Results: There were 397 (35.1%) patients with DPN among the subjects. There were significant differences in age, diabetes duration, BUN, serum creatinine (Cr), HbA1c, GA, 30-min and 120-min PCP between non-DPN and DPN groups. Diabetes duration, UA, HbA1c, and 120-min PCP had been shown to be correlated with DPN. Serum level of 120-min PCP was an independent risk factor for DPN. Conclusion: Patients with DPN showed relatively older age, longer diabetes duration, higher BUN, HbA1c, and GA levels, whereas, much lower serum PCP levels. Serum level of 120-min PCP might be a potential biomarker and a promising therapeutic target in the treatment of DPN, especially in longer diabetes duration subjects.

Keywords: Diabetic peripheral neuropathy, nerve conduction velocity, postprandial C-peptide, type 2 diabetes, diabetic complications

Introduction

Diabetic peripheral neuropathy (DPN) is defined as signs and symptoms of peripheral nerve dysfunction in a patient with diabetes mellitus (DM) in whom other causes of peripheral nerve dysfunction have been excluded. DPN is one of the most common long-term complications accompanying both type 1 and type 2 diabetes and progresses rapidly [1, 2]. Diabetic peripheral neuropathy is probably the most common but least understood of the microvascular complications of diabetes [3]. It is clear that impaired blood flow and endoneurial hypoxia play a major role in causing DPN in human and animal models [4]. Vascular changes due to microvascular disease have been shown to be associated with the development of T2DM-related complications such as peripheral neuropathy. The United Kingdom Prospective Diabetes Study revealed that more than 11% of patients had DPN at the time of diagnosis with diabetes [5]. However, the diagnosis of DPN is often delayed due to neglect of latent clinical onset or absence of obvious subjective symptoms. Additionally, the lack of the objective evaluation indexes also makes the quantitative diagnosis and screening of DPN quite difficult. The peripheral nerve conduction velocity (NCV) examination is well known as the most reliable method among many assessment methods for diabetic peripheral neuropathy [6, 7]. Serum C-peptide level provides an indirect measure of the insulin secretory reserve [8]. Studies have revealed the relationship between hyperinsulinemia and diabetic complications. However, the relationship between C peptide and diabetes complications is still unclear. Residual insulin secretion has been shown to have a protective effect on diabetes complications in some studies [9, 10]. This study aimed to evaluate the prevalence of DPN in type 2 diabetes patients using NCV

Table 1. Characteristics of the type 2 diabetes patients with diabetic peripheral neuropathy (DPN) and without (Non-DPN). Data are given as median (interquartile range), mean (standard deviation) or percentages

	Non-DPN (n=734)	DPN (n=397)	*P* Value
Age (years)	59.21±11.95	63.15±11.34	0.000
Diabetes duration (years)	7.68±6.55	9.49±7.28	0.000
BMI (Kg/m^2)	24.94±3.89	24.79±4.15	0.538
SBP (mm Hg)	130.83±16.98	132.32±17.95	0.167
DBP (mm Hg)	79.72±9.49	79.07±9.49	0.272
WC (cm)	88.00±10.49	89.14±11.38	0.100
HC (cm)	96.80±7.87	97.52±7.89	0.168
BUN (mmol/l)	5.59±2.64	6.03±2.70	0.010
Cr (μmol/l)	68.77±20.06	72.85±34.66	0.031
SUA (μmol/l)	314.54±90.87	320.05±99.40	0.355
HbA1c (%)	8.99±2.36	9.40±2.56	0.010
GA (%)	25.50±8.40	27.60±10.04	0.000
FPG (mmol/L)	8.15±2.68	8.35±3.27	0.269
120-min PPG (mmol/L)	13.79±4.87	13.84±4.84	0.883
TC (mmol/l)	4.76±1.11	4.78±1.13	0.757
TG (mmol/l)	1.85±1.58	1.81±1.41	0.656
HDL (mmol/l)	1.11±0.31	1.14±0.36	0.092
LDL (mmol/l)	3.12±0.98	3.10±0.96	0.814
FCP (ng/mL)	1.82±1.40	1.72±1.19	0.303
30-min PCP (ng/mL)	2.54±1.70	2.21±1.50	0.003
120-min PCP (ng/mL)	3.87±2.07	3.44±2.44	0.003

BMI: body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; WC: waist circumference; HC: hip circumference; BUN: blood uric nitrogen; Cr: creatinine; SUA: serum uric acid; HbA1c: glycosylated hemoglobin A1c; GA: glycosylated albumin; FPG: fasting plasma glucose; 120-min PPG: 120-min postprandial plasma glucose; TC: total cholesterol; TG: total triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FCP: fasting C-peptide; 30-min PCP: 30-min postprandial C-peptide; 120-min PCP: 120-min postprandial C-peptide.

measurements and to investigate the relationship between serum C-peptide level and DPN in the same population.

Subjects and methods

Subjects

A total of 1131 type 2 diabetes patients were recruited from the Department of Endocrinology, Shanghai Ninth People's Hospital, in the period January 2009-July 2013. The study was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University, School of Medicine, China. All subjects provided informed consent to participate in the study. Subject inclusion criteria were as follows: (1) age above 10-year-old; (2) male or female; (3) diagnosis of type 2 diabetes Mellitus according to World Health Organization 1999 criteria. The patients diagnosed with DPN were defined as DPN group, while the others were classified as non-DPN group; (4) no active foot disease such as ulceration and infection; (5) willingness to sign informed consent form. Exclusion criteria were (1) impaired glucose tolerance; (2) diagnosis in tertiary hospitals with cerebral vascular disease, other secondary peripheral nerve diseases or other motility-disabling disease. Subgroups in patients were further divided according to duration of diabetes (0-10 years, 11-20 years, 21-30 years, and above 30 years). All studies and procedures were approved by the Ethics Committees on Human Samples (Shanghai Ninth People's Hospital, Shanghai Jiao Tong University).

Data collection and biochemical measurement

All participants first answered a questionnaire regarding gender, age, medical history of diabetes, diabetic chronic complications and related diseases. The relevant data for diabetes included body weight, height, BMI, WC, HC, systolic blood pressure (SBP) and DBP of each patient were all recorded. Venous blood was collected from each subject to determine HbA1c levels (using high-pressure liquid chromatography), GA (using liquid enzymatic method), FPG, 2 h PPG, insulin, C-peptide (fasting, 30-min, and 120-min), blood lipid, BUN, Cr and SUA.

Diabetic peripheral neuropathy screening

The skillful, trained investigators performed the screen in a quiet, warm, relaxed environment. All studies of nerve conduction velocity (NCV) were done at room temperature (between 22°C and 24°C). Three parameters, including sensory nerve conduction velocity (SCV) or motor nerve conduction velocity (MCV), latent period

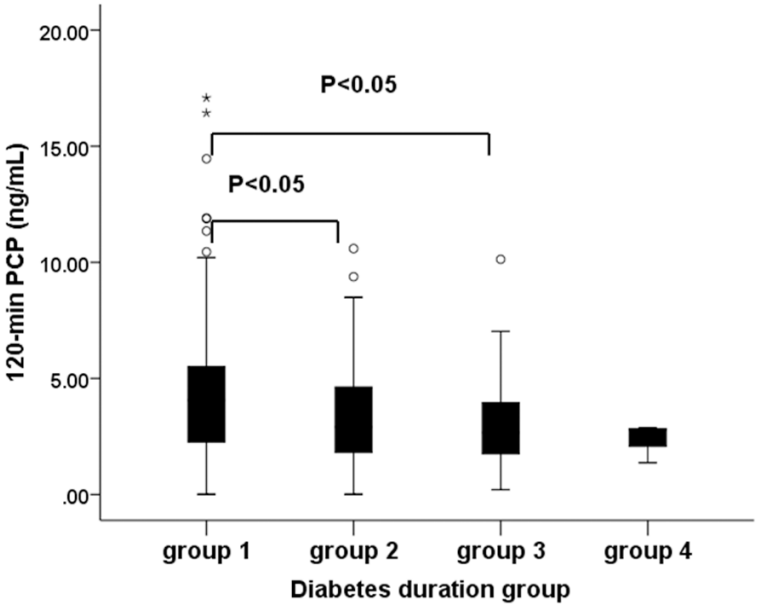

Figure 1. Serum 120-min PCP levels in subgroups classified by diabetes duration. The data were presented as mean ± SD. Comparisons among each group were perform χ^2 tested usingwith LSD method. *P*<0.05 (two-sided) was considered statistically significant.

and amplitude of median nerve, ulnar nerve, tibial nerve, common peroneal nerve and superficial peroneal nerve were studied simultaneously. Measurements of NCV were performed with a Nihon Kohden Neuropack Four Mini electromyography (Nihon Kohden, Tokyo, Japan). In this study, DPN was diagnosed according to NCV results. Other neuropathy caused by neurological or orthopedic diseases, such as cervical spondylosis, lumbar spondylosis, and Guillain-Barre syndrome, were all excluded.

Statistical analysis

All data were analyzed by SPSS (Windows version 20.0; SPSS, Chicago, IL, USA). All numerical results were presented as mean ± SD. The data were first tested for normal distribution by Kolmogorov-Smirnov test, and the variables with skewed distributions were further analyzed only after logarithmic transformation and analyzed by Kruskal-Wallis Rank-Sum test. Comparisons χ^2 test among each group were performed using with LSD method. Spearman correlation was used to assess the relationship between two variables. The risk factors for diabetic peripheral neuropathy were analyzed by multivariate logistic regression analysis, and relative risk [odds ratio (OR), 95% confidence interval (CI)] was reported; P<0.05 (two-sided) was considered statistically significant.

Results

Anthropometric characteristics and metabolic profiles of enrolled diabetes patients

In this study population, 397 (35.1%) diabetes subjects were diagnosed with DPN according to NCV results. Compared with the non-DPN group (n=734, 64.9%), the DPN group showed significantly older age, higher BUN, HbA1c, Cr and GA levels, longer diabetes duration, and lower 30-min and 120-min PCP levels (**Table 1**). There were no differences as for other values.

Serum levels of C-peptide showed a gradually decreased tendency with diabetes duration in type 2 diabetes patients

The type 2 diabetes patients were divided into four groups on the basis of diabetes duration: group 1 (0-10 years, n=781), group 2 (11-20 years, n=306), group 3 (21-30 years, n=39), and group 4 (>30 years, n=5). As shown in **Figures 1** and **3**, group 2 and group 3 showed much more lower FCP and 120-min PCP in contrast to group 1. Additionally, group 2 had lower 30-min PCP levels than those of group 1, as well (**Figure 2**). It indicated that Serum levels of C-peptide showed a gradually decreased tendency with diabetes duration.

Serum levels of 120-min PCP were correlations with metabolic

As shown in **Table 2**, Spearman correlation analysis indicated that serum levels of 120-min PCP were positively correlated with BMI, WC, HC, DBP, Cr, SUA, TG, LDL, 30-min and 120-min PPG; whereas, reversely correlated with diabetes duration, FPG, HbA1c, GA, HDL, and BUN.

Age, GA and 120-min PCP independently associated with DPN in all subjects

After adjustment of all parameters (age, diabetes duration, BUN, HbA1c, GA, 30-min and

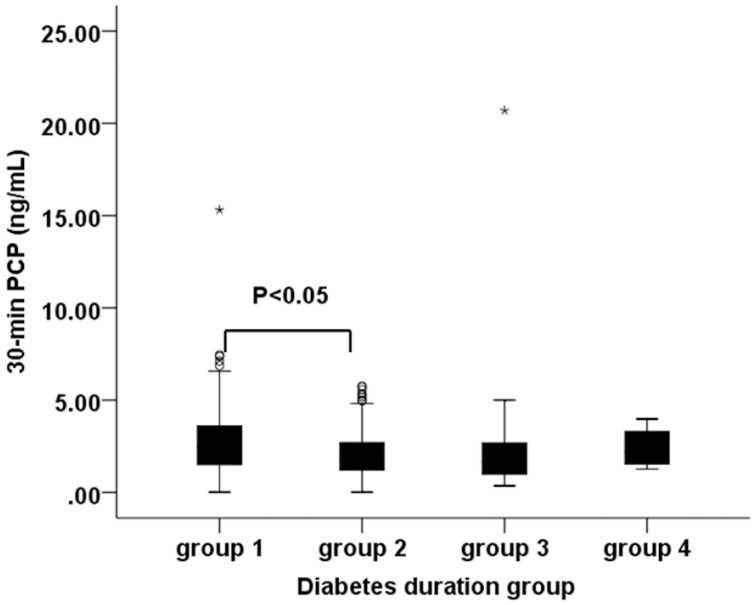

Figure 2. Serum 30-min PCP levels in subgroups classified by diabetes duration. The data were presented as mean ± SD. Comparisons among each group were performed using χ^2 test with LSD method. *P*<0.05 (two-sided) was considered statistically significant.

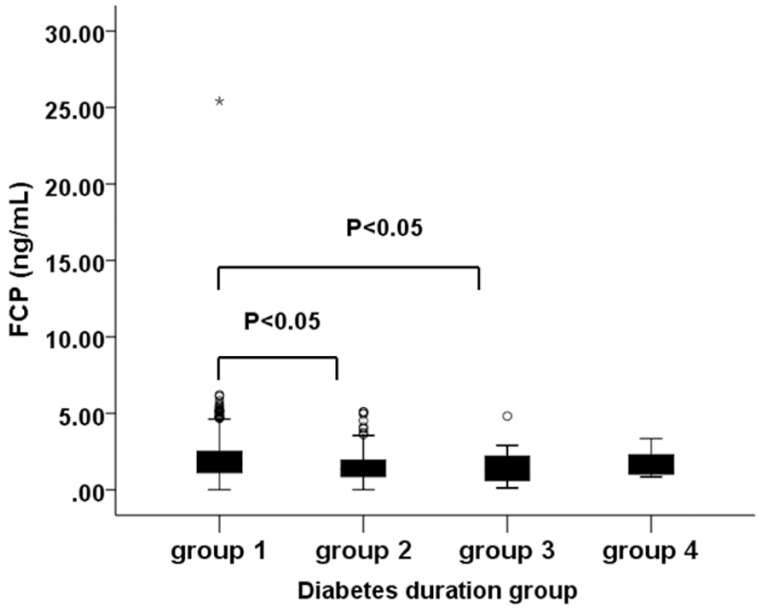

Figure 3. Serum FCP levels in subgroups classified by diabetes duration. The data were presented as mean ± SD. Comparisons among each group were performed using χ^2 test with LSD method. *P*<0.05 (two-sided) was considered statistically significant.

120-min PCP), Binary logistic regression analysis implicated diabetes duration (β=0.044, P=0.005, OR=1.045), UA (β=0.003, P=0.021, OR=1.003), HbA1c (β=0.036, P=0.003, OR=1.012), and 120-min PCP levels (β=-0.156, P=0.010, OR=0.760) as independent factors associated with the presence of DPN in all subjects (**Table 3**).

Discussion

Diabetic polyneuropathy (DPN), due to microvascular lesions, is the most common late diabetic complication, and is more frequent and severe in insulin-deficient conditions, such as type 1 diabetes and advanced type 2 diabetes [11]. It accounts for hospitalization more frequently than other complications of diabetes and also is the most frequent cause of non-traumatic amputation. According to an estimate, two thirds of diabetic patients have clinical or subclinical neuropathy [12]. All types of diabetic patients, insulin dependent diabetes mellitus (type 1 diabetes), non-insulin dependent diabetes mellitus (type 2 diabetes), and secondary diabetic patients, all can develop neuropathy. The prevalence of neuropathy increases with the duration of diabetes mellitus. In a study, the incidence of neuropathy increased from 7.5% on admission to 50% at 25 years follow up [13]. Some reports showed that the prevalence of DPN in patients with diabetes duration greater than 10 years was above 50% [13]. In this study, the prevalence is 34.3% in all subjects, and 35.1% in type 2 diabetes patients, which was accordance with those data.

The severity of DPN is related to the diabetes duration and the patient's level of glycemic control. This study found that DPN group subjects

Table 2. Correlation between serum levels of 120-min PCP and metabolic parameters

	Pearson correlation	*P* Value
Diabetes duration (years)	-0.163	0.000
BMI (Kg/m^2)	0.163	0.000
DBP (mm Hg)	0.063	0.032
WC (cm)	0.231	0.000
HC (cm)	0.245	0.000
FPG (mmol/L)	-0.148	0.000
HbA1c (%)	-0.438	0.000
GA (%)	-0.458	0.000
FCP (ng/mL)	0.752	0.000
30-min PCP (ng/mL)	0.856	0.000
TG (mmol/l)	0.113	0.000
HDL (mmol/l)	-0.132	0.000
Cr (μmol/l)	0.069	0.019
SUA (μmol/l)	0.173	0.000

Table 3. Adjusted odds ratios for DPN

	β	SE	Wald χ^2	*P* Value	Odds ratio (95% confidence interval)
Diabetes duration	0.044	0.016	7.831	0.005	1.045 (1.013-1.077)
UA	0.003	0.001	5.304	0.021	1.003 (1.000-1.005)
HbA1c	0.036	0.012	8.706	0.003	1.037 (1.012-1.062)
120-min PCP	-0.156	0.061	6.584	0.01	0.856 (0.760-0.964)
Constant	-1.346	0.79	2.905	0.088	0.26

SE, standard error.

had longer diabetes duration and higher HbA1c. It indicated that long-term glucose metabolic disturbance status might facilitate the onset and development of DPN. However, the pathogenesis of DPN remains largely unknown. Four major pathways of glucose metabolism (polyol, advanced glycation end product, protein kinase C, and hexosamine) are believed to be involved in the development of DPN [14]. It was initially thought to be unrelated mechanisms, new evidence suggests there is a connection in that each of these pathways all contributes to the formation of reactive oxygen species (ROS), which leads to cellular oxidative stress and in turn results to metabolic and vascular imbalances that initiate and promote DPN. Additionally, the reduced availability of neurotrophic growth factors contributes to the pathogenesis of DPN, as well [15].

As a matter of fact, the severity of DPN is not parallel to the blood glucose control status. Although substantial numbers of patients even strictly control the blood glucose levels, DNP still progresses. Furthermore, several studies have demonstrated that it is possible to retard the progression of diabetic complications by intensified insulin treatment and improved metabolic control, but development of neuropathy cannot be prevented [16-18]. Thus, these facts suggested that hyperglycemia might not be the only cause of DPN. Recent studies reported that C-peptide deficiency was likely to have important effects on diabetes complications, include DPN [11].

C-peptide, in contrast to previous belief, has been presented to possess the characteristics of a bioactive peptide. C-peptide binds specifically to various cell membranes, including endothelial, renal and nerve cells [19], with subsequent activation of an intracellular signaling cascade resulting in stimulation of endothelial nitric oxide synthase (eNOS) and Na^+, K+-ATPase [20, 21]. Recent data demonstrated that C-peptide stimulates several transcriptional factors, as well as neurotrophic factors [22]. In case of type 2 diabetes, after several years, patients often become insulin and C-peptide deficient, and at this stage it is presenting with characteristics similar to that of type 1 diabetes. Thus, it has been suggested that exogenous administration of C-peptide to patients lacking endogenous C-peptide may improve nerve function in both type 1 and type 2 diabetes patients [23, 24].

Our data demonstrated that DPN group subjects showed much lower levels of 120-min PCP, in contrast to non-DPN group ones. However, there were no significant differences in FCP levels (**Table 1**). Furthermore, logistic regression analyses showed that 120-min PCP level was an independent factors associated with the presence of DPN in all subjects. All data suggested that serum 120-min PCP level was closely related to the pathogenesis of DNP, other than FCP level. As 120-min PCP is belie-

ved to be a better indirect measure of the insulin secretory reserve than FCP. In addition to traditional intensified insulin replacement therapy, the beneficial effects on nerve function following C-peptide replacement therapy may indicate a new potential treatment paradigm, even though extended clinical trials will be needed to finally elucidate its usefulness.

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Disclosure of conflict of interest

None.

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