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A Study of Association of C-Peptide with Diabetic Peripheral Neuropathy

Authors
Dr Prasad Potdukhe[1], Dr Pranay Gandhi[2], Dr Snehal Potdukhe[3], Dr Bhushan Naitam[4]
[1]Associate Professor in Medicine, GMC Chandrapur
[2]Assistant Professor in Community Medicine, GMCH, Chandrapur
[3]Cosmetologist and Dermatologist, Chandrapur
[4]Assistant Professor in Pathology, GMCH, Chandrapur
Corresponding Author
Pranay Gandhi
C/O Dr. Shailendra Gandhi, Near CDCC Bank, Civil Lines, Chandrapur 442401
Email: *dr.pranaygandhi@yahoo.co.in, Phone: 9028696449*

ABSTRACT
Background: *The aim of our study was to evaluate the relationship between C-peptide and DPN in community-based patients with type 2 diabetes.*
Methods: *In total, 220 consecutive type 2 diabetic patients treated by our regional medical consortium were enrolled. DPN was assessed by clinical symptoms, signs, and electromyography.*
Results: *Fasting C-peptide, 2-h postprandial C-peptide and ΔC-peptide (i.e., 2-h postprandial C-peptide minus the fasting C-peptide) serum concentrations in the non-DPN group were significantly higher than those in the clinical DPN group (all $P \leq 0.040$) and the confirmed DPN group (all $P < 0.002$). The three C-peptide parameters were independently associated with DPN (all $P < 0.05$) after adjusting for age, sex, diabetes duration, smoking status, systolic pressure, body mass index, angiotensin-converting enzyme inhibitors/ angiotensin receptor blocker use, fasting plasma glucose, HbA1c, triglyceride and estimated glomerular filtration rate. Compared with the ΔC-peptide quartile 1 (reference), patients in quartile 3 (odds ratio [OR], 0.110; 95% confidence interval [CI] 0.026–0.466; $P = 0.003$) and quartile 4 (OR, 0.012; 95% CI 0.026–0.559; $P = 0.007$) had a lower risk of DPN after adjusting for the confounders.*
Conclusion: *C-peptide was negatively associated with DPN in community-based type 2 diabetic patients.*
Keywords: *Diabetic peripheral neuropathy, C-peptide, Beta-cell function, Community-based.*

Background

Diabetic peripheral neuropathy (DPN) is a well-known microvascular complication of type 2 diabetes mellitus, which leads to further infections and increases the risk of foot ulcers, non-traumatic amputations and mortality[1, 2]. Although hyperglycemia plays an important role in the development of DPN, intensive glucose control does not eliminate the risk of developing DPN in patients with type 2 diabetes, suggesting that other factors may be involved in DPN development [3]. C-peptide levels in the peripheral blood are widely accepted as the most appropriate evaluation of insulin secretion, and are not eliminated in the first-pass metabolism through the liver [4,5]. Previously considered to be an inactive by-product of insulin synthesis, C-peptide is a hormonally active peptide [5,6]. In type 1 diabetes,

an Italian study with a large clinical cohort demonstrated a significant association between C-peptide and microvascular complications, including neuropathy [7]. Several studies in animal models of diabetes and in patients with type 1 diabetes have demonstrated beneficial effects of C-peptide replacement on both peripheral and autonomic nerve function in diabetes [8–10].

In contrast to the data collected in patients with type 1 diabetes, studies on the relationship between C-peptide and in type 2 diabetes mellitus-related complications were conflicting. Whereas some studies showed that residual insulin secretion, evaluated by serum C-peptide concentrations, has a protective effect on diabetic neuropathy [11–13], others either did not find such an effect [14], or concluded a contrary relationship [15]. Moreover, none of the studies focused on a community-based population [13]. Therefore, the aim of this study was to evaluate the relationship between C-peptide levels and DPN, independent of glycemic control and other risk factors in community-based type 2 diabetic patients.

Methods

Subjects

In total, 220 consecutive type 2 diabetic patients receiving care from a medical consortium with completed demographic information and neurological examination were enrolled in this study. World Health Organization diagnostic criteria were used for type 2 diabetes diagnoses [16]. Patients with acute complications of diabetes, renal dysfunction (estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2), acute cerebral infarction, vitamin B12 deficiency, alcohol abuse and asymmetrical neuropathy of the trunk or proximal lower limbs and chronic infection were excluded from this study. All the patients signed the consent form for allowing their information to be used for research.

Anthropometric measurements

Body weight was assessed with the patients wearing light clothing and no footwear before breakfast; all heights measurements were taken using the same wall-mounted stadiometer. Body mass index (BMI) was calculated as body weight (in kg) divided by the square of the height (in m). Diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured three times with 1-min intervals after 10 min of using a standard mercury sphygmomanometer and then averaged.

Laboratory measurements

Glucose and C-peptide concentrations were measured at baseline and 2 h following oral administration of a 100-g steamed bread meal test (equal to 75 g of glucose). ΔC-peptide levels were calculated as the 2-h postprandial serum C-peptide level minus the fasting C-peptide level. Although not a standard test in diabetes research or care, the 100 g steamed bread meal test was selected because it avoids severe glucose fluctuation in patients with type 2 diabetes mellitus [17]. Fasting blood samples were collected in a sodium fluoride anticoagulant tube, an EDTA anticoagulant tube and a coagulating tube. Glucose, glycosylated hemoglobin A1c (HbA1c) and biochemical indicators including serum creatinine (Cr), and lipid profiles including total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) were measured. 2-h postprandial blood samples were also collected for glucose and C-peptide measurements.

Examination of neurological symptoms and signs

Neurological symptoms and signs based on the neuropathy symptom score (NSS) and the neuropathy disability scores (NDS) were evaluated [1], Neurological symptoms included burning, numbness, tingling, fatigue, cramping or aching, and neurological signs included vibration sense, pain, temperature sensation and ankle reflex. The symptoms and signs abnormalities assessed for DPN were in a glove/stoking distribution.

Nerve conduction velocity tests

NCV studies of bilateral median, ulnar, tibial, common peroneal and superficial peroneal on

each subject were conducted. Subjects stayed calm and relaxed, and the local skin temperatures were kept at 32–33 °C throughout the examination. A decrease in NCV was set according to the NCV reference value for the Chinese population [19].

Diagnosis of DPN

DPN was diagnosed according to the modified Toronto Expert Consensus [3] as follows: (1) non-DPN, all neurological symptoms/signs and NCV were normal; (2) clinical DPN, at least two abnormal results among neurological symptoms/ signs, or ankle reflex in accordance with a distal symmetrical polyneuropathy and normal NCV; (3) confirmed DPN, at least one abnormal nerve parameter (of NCV, amplitude, latency, and F-wave) in two or more nerves among the median, peroneal, and sural nerves, regardless of neurological signs and symptoms.

Statistical Analysis

Data are presented as the mean ± standard deviation or percent of individuals. Variance homogeneity was assessed by the Levene test. Differences in C-peptide within the three groups were assessed by one-way ANOVA. The least significant difference and Dunnett tests were used to compare differences between groups with continuous variables, and a Chi square test was used to assess differences between categorical variables. Spearman's correlation analysis was used to examine the correlation of serum C-peptide concentrations with clinical variables. Multiple logistic regression analysis was performed to evaluate the association of DPN and C-peptide quartiles after adjusting for other clinical and biochemical variables. Analysis was performed using SPSS 17.0. A *P* value of <0.05 was considered to be statistically significant.

Results

The clinical characteristics of the patients in all three groups are summarized in Table 1. Compared to patients in the non-DPN group, those in the confirmed DPN groups had higher SBP ($P = 0.036$), FPG ($P = 0.033$), 2 h-PG ($P = 0.012$) and HbA1c ($P = 0.007$) as well as lower HDL-C ($P = 0.018$), NCV and nerve conduction amplitude (NCA) of median, titial and sural (all $P < 0.05$). However, no significant differences in age, sex, diabetes duration, smoking status, BMI, DBP, TC, TG, LDL-C, GA, Cr and eGFR were found among the three groups. The use of anti-diabetic drugs (e.g., metformin, sulfonylureas and insulin) and anti-hypertension drugs [angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blocker (ARB)] were also not significantly different among the three groups.

Multiple logistic regression analyses were performed to evaluate the risk factors associated with DPN (including both clinical and confirmed DPN) (Table 2). After adjusting for age, sex, diabetes duration, smoking status, BMI, SBP, ACEI/ARB use, FPG, HbA1c, TG and eGFR (model 2), the association between serum C-peptide levels and DPN remained statistically significant (odds ratio [OR], 0.329; 95% confidence interval [CI]), 0.107–0.903 for fasting C-peptide, $P = 0.028$; OR, 0.712; 95% CI 0.556–0.913 for 2-hpostprandial C-peptide, $P = 0.007$ and OR, 0.717; 95% CI 0.548–0.939 for ΔC-peptide, $P = 0.016$].

Compared with the ΔC-peptide concentrations in quartile 1 (reference), patients in quartile 3 (OR 0.110; 95% CI 0.026–0.466; $P = 0.003$) and quartile 4 (OR 0.012; 95% CI 0.026–0.559; $P = 0.007$) had a lower risk of DPN (including both clinical and confirmed DPN) after adjusting for age, sex, diabetes duration, smoking status, BMI, SBP, ACEI/ARB use, FPG, HbA1c, TG and eGFR. Trend test analysis showed a statistical difference in the prevalence of DPN among quartiles 2, 3 and 4, compared to quartile 1 ($P < 0.001$; Table 3). However, the same analysis of fasting C-peptide and 2-h postprandial C-peptide did not show a significant difference among the quartiles.

Table 1 showing characteristics of patients.

	Non-DPN (128)	P_{1V3}	Clinically-DPN (35)	P_{1V2}	Comfirmed DPN (57)	P_{2V3}
Age (year)	62.3 ± 8.4	0.449	63.3 ± 10.7	0.527	64.5 ± 8.8	0.726
Sex (male %)	41%	0.568	58.40%	0.634	46%	0.62
Diabetes duration (year)	8.5 ± 7.2	0.084	9.7 ± 7.7	0.435	12.6 ± 6.9	0.228
Smoking	12 (9.4%)	0.736	4 (11.4%)	0.543	6 (10.5) %	0.612
BMI (kg/cm^2)	25.1 ± 3.8	0.393	25.8 ± 4.7	0.421	26.9 ± 1.3	0.167
SBP (mmHg)	133 ± 16	0.036	135 ± 16	0.501	141 ± 13	0.056
DBP (mmHg)	78 ± 8	0.239	77 ± 11	0.431	83 ± 9	0.068
FPG (mmol/L)	8.40 ± 2.43	0.033	8.89 ± 2.33	0.523	9.37 ± 2.60	0.465
2 h-PG (mmol/L)	15.79 ± 4.81	0.012	16.47 ± 4.63	0.607	18.14 ± 4.81	0.263
TC (mmol/L)	5.30 ± 1.02	0.356	5.19 ± 1.06	0.737	5.10 ± 1.26	0.823
TG (mmol/L)	2.11 ± 1.17	0.742	2.38 ± 0.82	0.448	2.05 ± 1.12	0.383
HDL-C (mmol/L)	1.33 ± 0.45	0.018	1.18 ± 0.18	0.217	1.14 ± 0.22	0.788
LDL-C (mmol/L)	2.96 ± 0.77	0.906	3.02 ± 0.83	0.804	2.98 ± 1.09	0.878
HBA1c (%)	7.4 ± 1.4	0.007	7.6 ± 1.4	0.929	8.5 ± 1.9	0.229
GA (%)	18.7 ± 3.7	0.091	20.4 ± 5.2	0.417	22.8 ± 6.1	0.086
Cr (μmol/L)	61.54 ± 15.35	0.276	63.94 ± 17.11	0.63	67.6 ± 17.30	0.278
eGFR ($mL/min/1.73\ m^2$)	104.35 ± 31.32	0.857	106.34 ± 26.13	0.826	105.45 ± 30.86	0.93
Median MNCV	53.7 ± 3.1	<0.001	51.2 ± 5.7	0.033	46.5 ± 3.4	0.005
Median MNCA	8.9 ± 2.3	0.014	7.8 ± 2.8	0.398	6.9 ± 2.4	0.516
Median SNCV	51.8 ± 8.6	0.001	48.7 ± 8.8	0.086	41.9 ± 8.3	0.028
Median SNCA	21.4 ± 6.8	<0.001	17.0 ± 8.2	0.03	9.6 ± 5.4	<0.001
Tibial MNCV	44.5 ± 3.2	0.012	40.8 ± 5.89	0.007	35.8 ± 6.0	0.15
Tibial MNCA	8.7 ± 3.1	<0.001	8.2 ± 3.7	0.649	5.2 ± 3.5	0.02
Sural SNCV	50.3 ± 8.5	<0.001	47.5 ± 6.3	0.114	36.3 ± 3.9	0.002
Sural SNCA	13.2 ± 5.6	<0.001	10.4 ± 3.2	0.114	6.1 ± 3.1	<0.001
Insulin	16 (12.5%)	0.347	7 (20%)	0.576	13 (22.4%)	0.747
Sulfonylureas	32 (25.0%)	0.415	10 (28.6%)	0.589	21 (36.8%)	0.467
Metformin	46 (35.9%)	0.423	14 (40%)	0.71	25 (43.8%)	0.745
ACEI or ARB	33 (25.8%)	0.768	12 (34.3%)	0.896	19 (33.3%)	0.824

Results expressed as mean (standard deviation) or percentage

Table 2 Multiple regression analyses with the dependent variable of DPN and independent variable of C-peptide.

	Model[1]		Model[2]	
	Odds ratio (95% confidence interval)	*P*	Odds ratio (95% confidence interval)	*P*
Fasting C-peptide	0.457 (0.234–0.892)	0.022	0.392 (0.170–0.903)	0.028
2 h-postprandial C-peptide	0.749 (0.621–0.904)	0.003	0.712 (0.556–0.913)	0.007
ΔC-peptide	0.733 (0.592–0.908)	0.004	0.717 (0.548–0.939)	0.016

Table 3. DPN risk in different ΔC-peptide quartiles

	Q1 (≤0.22 nmol/L)	Q2 (0.23–0.59 nmol/L)	Q3 (0.60–1.15 nmol/L)	Q4 (>1.16 nmol/L)	*P* trend
Odds ratio (95% confidence interval)	–	0.645 (0.191–2.175)	0.110 (0.026–0.466)	0.012 (0.026–0.559)	<0.001
P	–	0.479	0.003	0.007	

Multiple regression analyses and trend test analysis were used. The regression was adjusted for age, sex and diabetes duration, smoking, systolic blood pressure, body mass index, angiotensin-converting enzyme inhibitors/angiotensin receptor blocker, fasting plasma glucose, glycosylated hemoglobin A1c, triglyceride, estimated glomerular filtration rate. ΔC-peptide, 2-hpostprandial C-peptide minus fasting C-peptide.

Discussion

This study suggested a close relationship between the serum C-peptide concentrations and DPN in community-based type 2 diabetes patients. The decrease in ΔC-peptide was strongly associated with the prevalence of DPN after adjusting for other variables.

Some studies showed that serum C-peptide concentration had a protective effect on neuropathy. In a large clinic-based cohort of 471 type 1 diabetic patients, higher values conferred a protective effect (OR 0.59; 95% CI 0.37–0.94) on diabetes microvascular complications including autonomic neuropathy, compared to C-peptide values in the lowest tertile (<0.06 nmol/L) [7]. However, the role of C-peptide concentrations for DPN in type 2 patients was still controversial. A study in Korea showed that the risk for diabetic neuropathy was associated with the lower fasting serum C-peptide quartile and lower ΔC-peptide quartile in type 2 diabetic patients after adjusting for multiple confounding factors[11]. A retrospective cohort study with a median follow-up of 14 years showed that the risks for incident neuropathy were negatively associated with the highest C-peptide tertile (OR 0.39; 95% CI 0.25–0.61)[12]. In a Chinese study that included hospitalized patients with type 2 diabetes, Zhao et al. [13] concluded that a higher level of area under the curve of C-peptide [AUC (C-pep)] was inversely associated with the prevalence of neuropathy. However, Sari et al.[14] demonstrated that C-peptide did not correlate with sensorial neuropathy. Moreover, another study found that patients with parasympathetic neuropathy had elevated fasting plasma C-peptide ($P < 0.001$) [15]. Our study focused on the relationship between C-peptide concentration and DPN in community-based patients, and the results suggested that the fasting C-peptide, 2-h postprandial C-peptide and ΔC-peptide concentrations were negatively associated with DPN after adjusting for multiple confounders.

In our study, DPN was associated with poor glycemic control as reflected by HbA1c, old age and longer diabetes duration. With increased diabetes duration, the islet function diminishes gradually, resulting in reduced C-peptide and insulin levels and the prevalence of DPN increases. Therefore, we conducted a different analysis to eliminate the effect of age and disease duration on the results. For example, under the circumstances of no obvious difference of age and diabetes duration among the three groups, the C-peptide differed significantly. Furthermore, multiple logistic regression analysis showed a strong relationship between C-peptide and DPN even after adjustment for confounding factors including age, sex, diabetes duration, smoking status, BMI, SBP, ACEI/ARB use, FPG, HbA1c, TG and eGFR, indicating that C-peptide was independently associated with DPN. These results are consistent with other studies that included patients with lower HbA1c levels or shorter disease duration or younger age [10–12].

The beneficial effects of C-peptide on the prevention of diabetes complications in type 1 diabetes patients have been confirmed by various studies [8,9]. In contrast, the role of C-peptide is not well-defined in type 2 diabetes. Experimental studies in type 1 diabetes showed that C-peptide specifically bound to cell surfaces, acting via a G-protein-related receptor; it also led to autophosphorylation of the insulin receptor in the presence of insulin [20]. Moreover, C-peptide stimulated p38 MAP-kinase and PI-3 kinase activities, and diminished the activation of JNK phosphorylation with subsequent effects on Na^+/K^+-ATPase activity and nitric oxide (NO) [21,22]. C-peptide also ameliorated the altered expression of insulin-like growth factor-1, nerve growth factor and neurotrophin-3 and their respective receptors, which corrected neurofilament (NF) and tubulin mRNA, and protein expression, as well as normalized the aberrant phosphorylation of NFs [23]. C-peptide also stabilized the attachment of the α-Na^+-channels at the nodal axolemma, and furthermore, it prevented a breach of the paranodal ion-channel barrier. These results correlate with the corrected effects of C-peptide on nodal and paranodal structural integrity [24,25]. In addition to the direct effect of the C-peptide on DPN, the residual beta cell function as represented by C-peptide concentrations also plays an important role. This highlighted the importance of some treatment strategies, such as avoiding drugs that overstimulate beta cells and initiating insulin therapy at an appropriate time to preserve endogenous beta cell activity in the progression of type 2 diabetes.

This study has some limitations that should be taken into account. First, due to its cross-sectional nature, we could not determine the causal relationship between the serum C-peptide levels and DPN. Thus, prospective studies are needed to confirm the protective effects of C-peptide on DPN. Second, the relationship between C-peptide levels and severity of DPN was not analyzed. Third, the clinical confirmed DPN should be validated by small fibre examinations, such as corneal confocal microscopy. Finally, the relationship between C-peptide levels and other microvascular complications was not investigated. This relationship will be evaluated in further studies.

Conclusion

Serum C-peptide levels were significantly associated with DPN in community-based type 2 diabetic patients

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