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Carotid atherosclerosis: An independent risk factor for small fiber nerve dysfunction in patients with type 2 diabetes mellitus

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Keywords
Carotid atherosclerosis, Quantitative sensory testing, Small fiber neuropathy, Type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: To explore whether carotid atherosclerosis is an independent risk factor for small fiber nerve dysfunction in type 2 diabetes mellitus patients.

Materials and Methods: A total of 247 type 2 diabetes patients from Nanjing Drum Tower Hospital received carotid ultrasonography and quantitative sensory testing, including cold and warm detection thresholds, and some patients received cold and heat pain detection thresholds, respectively. According to the results of quantitative sensory testing, patients were divided into normal small fiber nerve function (NSF) and small fiber nerve dysfunction (SFD) group. Meanwhile, patients were divided into the non-carotid atherosclerosis group, carotid intimal thickening, unilateral carotid atherosclerosis and bilateral carotid atherosclerosis group. The correlation between carotid ultrasonography with quantitative sensory testing parameters was analyzed by SPSS 26.0.

Results: First, the incidence rate of SFD increased significantly in patients with carotid atherosclerosis (72.2%, $P < 0.001$) especially in bilateral carotid atherosclerosis (81.7%, $P < 0.001$). Second, compared with the NS group, the carotid intima-media thickness in SFD was thicker ($P = 0.018$) and the size of atherosclerotic plaque was larger ($P < 0.001$). In addition, the cold detection threshold decreased ($P < 0.001$), whereas the warm detection threshold ($P < 0.001$) and heat pain detection threshold ($P < 0.001$) increased as aggravation of carotid atherosclerosis. In the correlation analysis, the size of atherosclerotic plaque presented a positive correlation with the warm detection threshold ($r = 0.476$, $P < 0.001$) and heat pain detection threshold ($r = 0.213$, $P < 0.001$), but presented a negative correlation with the cold detection threshold ($r = –0.239$, $P < 0.01$). Furthermore, carotid atherosclerosis (odds ratio 2.326, $P = 0.017$), especially bilateral carotid atherosclerosis (odds ratio 5.042, $P = 0.001$), was an independent risk factor for SFD ($P < 0.05$).

Conclusions: Carotid atherosclerosis was significantly associated with quantitative sensory testing and found to be an independent risk factor for small fiber nerve dysfunction in type 2 diabetes patients.

INTRODUCTION

It is well known that macrovascular and microvascular complications are common causes of declining quality of life or even death among type 2 diabetes patients. Type 2 diabetes patients have already atherosclerotic disease and neuropathy as the initial diagnosis of type 2 diabetes, even in the prediabetes stage.[1]

As for microvascular complications, small fiber neuropathy (SFN) occurs in the very early stage of diabetes, with occult clinical manifestations that result in patients and clinicians being unaware. The most common clinical manifestations of SFN are symmetrical skin abnormalities, including burning sensation, crawling sensation, stabbing pain, electric shock, deep pain, numbness and pain relief or allergy. Patients can also suffer autonomic symptoms, such as obvious dryness of eyes or lips, arrhythmia, erect dizziness, gastroparesis, constipation or

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diarrhea, frequent urination, urgent urination or dysuria, sexual dysfunction, changes in sweating and skin color, and so on.[2] Some clinical studies have shown that the incidence of diabetic SFN is 7–54%[3,4] in type 2 diabetes patients, and that its prevalence also increases with age and the course of diabetes, which could develop to painful neuropathy, mixed neuropathy and diabetic foot or even amputation. Therefore, early evaluation of SFN is of the utmost importance in type 2 diabetes patients for improving their quality of life and lifespan. However, due to the hidden characteristics of SFN development, it is difficult to obtain the deep understanding and attention of patients and clinicians, and at present, the means for SFN screening has not been widely carried out.

Regarding macrovascular complications, atherosclerosis is a systemic disease, and severe atherosclerosis can lead to vascular stenosis, occlusion and tissue ischemia. These kinds of pathologies might occur in small vessels in central or peripheral small vessels and result in neural degeneration.[5,6] Clinically, there is no technology to accurately detect atherosclerosis in small vessels. However, as one of the most common forms of atherosclerosis, the study of Wagenknecht *et al.*[7] also observed an increased rate of progression of carotid atherosclerosis (CA) in diagnostic diabetes. At present, color Doppler ultrasound has become an important means to screen carotid atherosclerosis, which shows its advantages, including price concessions, simple non-invasive operation, and wide and mature clinical application.[8]

In fact, previous studies have found that parameters of small fiber neuropathy presented a correlation with impaired vascular endothelial function, which also leads to atherosclerotic disease in type 2 diabetes patients.[9] Although the relationship between SFN and atherosclerosis has been described, its correlation with carotid atherosclerosis has not been further confirmed or discussed as to whether the atherosclerosis parameters are able to assess the situation of small fiber dysfunction. Therefore, we aimed to explore the relationship between quantitative sensory testing and carotid atherosclerosis in type 2 diabetes patients, and hope to gain a better understanding of whether carotid atherosclerosis could be used as a new risk factor to assess small fiber nerve dysfunction, and further screening and treatment of complications of type 2 diabetes.

MATERIALS AND METHODS

Individuals aged between 18 and 70 years were recruited among inpatients with type 2 diabetes from the Endocrinology Department of Nanjing Drum Tower Hospital in Nanjong, China, from May 2020 to May 2021. Type 2 diabetes was diagnosed according to the 1999 World Health Organization criteria.[10] Patients with other types of diabetes mellitus, neuropathy other than diabetic neuropathy, severe acute and chronic complications, and a history of malignancies were excluded. None of the participants had a history of drinking alcohol or taking analgesics within 24 h before the test, and we ensured that no intelligence or mental factors led to failure to complete quantitative sensory testing (QST).

The demographic characteristics were collected, including sex, age, diabetes duration, body mass index, waist-to-hip ratio, smoking and drinking history, and blood pressure. Regarding the clinical history, we recorded the results of blood tests, including fasting blood glucose, fasting insulin, C-peptide, 120-min blood glucose, 120-min insulin and 120-min C-peptide of standard meal test to evaluate the islet function of patients (after at least 10 h of overnight fasting, patients were given the 100 g standard steamed bread meal test, which was made of 100 g of all-purpose grain flour containing 75 g of carbohydrate glucose, and a venous specimen was collected for the measurement of plasma glucose before and 120 min after taking the steamed bread, respectively. In addition, other points for attention in the standard meal test are consistent with the oral glucose tolerance test),[11,12] glycosylated hemoglobin (HbA1c), glycosylated albumin, creatinine (Scr), blood urea nitrogen, uric acid, estimated glomerular filtration rate, urinary albumin-to-creatinine ratio, total protein, albumin, cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, osteocalcin, beta C-terminal telopeptide of type I collagen (β-CTX); propeptide of type I procollagen, 25-(OH)-vitamin D, folic acid and vitamin B_{12}.

The study was approved by the Ethics Committee of Drum Tower Hospital Affiliated to Nanjing University Medical School, and informed consent was obtained from patients.

EXAMINATION METHODS

Quantitative sensory testing

All participants in both groups received quantitative sensory testing, including warm and cold threshold tests (WDT and CDT), and some patients also underwent heat pain threshold (HPT, n = 199) and cold pain threshold (CPT, n = 120) tests on the dorsum of both feet by the limit method. Thermal stimulation was evaluated by the Pathway Thermal Sensory Analyzer (MEDOC LTD ADVANCED MEDICAL SYSTEMS, Ramat Yishai, Israel) using a 30 × 30 mm probe, with ramp stimuli of 1°C/s from 32°C. In thermal pain tests, 50 and 0°C were used as stop points to protect the skin of the subjects. Each test was repeated 3–4 times, and the average value was taken as the final result.[13]

According to the normal range of different thermal tests in QST,[14] patients were assigned to two groups, including the normal small fiber nerve function group (NSF; n = 103) and, if WDT or CDT were abnormal, patients were assigned to the small fiber nerve dysfunction group (SFD; n = 144).[15,16]

Carotid ultrasonography

Ultrasound of both carotid arteries was carried out with the same color Doppler ultrasound diagnostic instrument by the same skilled and qualified Doppler ultrasound doctors to explore the common carotid artery, internal carotid artery and external carotid artery, respectively, and the methodology related to carotid artery ultrasound has previously been reported. In short, carotid intima-media thickness (cIMT) was defined as the distance

between the media–adventitia interface and the lumen–intima interface on the longitudinal ultrasound image of each artery, and normal results are considered to be cIMT <1.0 mm, carotid intimal thickening 1.0 mm ≤ cIMT < 1.5 mm and carotid plaque cIMT ≥1.5 mm, and the diameter of the greatest plaque was measured.[17] The carotid intimal thickness and carotid plaque size analyzed in the present study are the maximum carotid intimal thickness and the maximum carotid plaque diameter of patients.

According to the diagnostic criteria, patients were assigned to three groups, including the normal group (NCA; $n = 85$), carotid intimal thickening group (CIT; $n = 36$) and carotid atherosclerosis group (CA; $n = 126$), patients in the CA group were further grouped to the unilateral carotid atherosclerosis group (UCA; $n = 55$) and bilateral carotid atherosclerosis group (BCA; $n = 71$).

Statistical analysis

Blood test data, carotid ultrasonography parameters (cIMT and the size of atherosclerotic plaque) and thermal threshold test data were summarized by means and standard deviations. Independent *t*-tests, χ^2-tests and one-way ANOVA were used to compare the statistical significance of normally distributed variables. Mann–Whitney *U*-tests and Kruskal–Wallis H-tests were used to analyze abnormal distributions. The correlation between QST and carotid ultrasonography parameters was analyzed by the Spearman correlation test. Logistic regression was used to analyze the independent risk factors for small fiber nerve dysfunction. The general influencing factors, such as age, course of diabetes and HbA1c, were excluded in the logistic regression analysis. At the same time, the factors with differences between the two groups in Table 1 were also excluded, including renal function, bone metabolism index and folic acid. The Bonferroni test was used to avoid the multiple comparison problem. All statistical assessments were two-tailed, and $P < 0.05$ was considered statistically significant. All analyses were carried out using SPSS 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Demographic and biochemical differences

In total, 247 patients with type 2 diabetes were recruited for the present study. The demographic and biochemical results were preliminarily analyzed, as shown in Table 1. Compared with the NSF group, patients with SFD were older ($P < 0.001$), presented a longer diabetes duration ($P < 0.001$) and a higher rate of smoking ($P = 0.003$). However, there was no statistical difference in the glucose indexes between two groups. Furthermore, the renal function of SFD patients was worse, which was reflected by the higher Scr ($P = 0.013$) and lower estimated glomerular filtration rate ($P = 0.018$). Also, the SFD group presented a lower level of osteocalcin ($P = 0.012$), β-CTX ($P = 0.002$) and folic acid ($P = 0.001$), but there was no statistical difference in other indexes between these two groups. Regarding the carotid ultrasonography parameters in the SFD group, the cIMT was thicker ($P = 0.018$) and the size of atherosclerotic plaque size was greater ($P < 0.001$) than that in the NSF group. As for the prevalence of different groups of carotid atherosclerosis, it suggested that SFD presented a higher prevalence in UCA (22.9%; $P < 0.001$) and BCA (40.3%; $P < 0.001$), but lower prevalence in CIT (9.7%; $P = 0.017$) than NSF patients (UCA 21.4%, BCA 12.6%, CIT 21.4%). Furthermore, the QST parameters and the frequency of abnormal CDT or WDT are presented in Table 1.

The incidence rate of small fiber nerve dysfunction in different groups

To compare the incidence rate of small fiber nerve dysfunction in the non-CA and CA group, Table 1 shows that the rate of small fiber nerve dysfunction in the present study was 58.3% (NSF 41.7%). In Figure 1a, the incidence rate of SFD in patients without carotid atherosclerosis was 45.9% (NSF 54.1%), but it significantly increased to 72.2% (NSF 27.8%) in the CA group ($P < 0.001$). Regarding Figure 1b, it also showed that the incidence rate of SFD in the BCA group (NSF 18.3%, SFD 81.7%) was significantly higher than that in the UCA group (NSF 40.0%, SFD 60.0%; $P < 0.001$).

QST parameters differences among three groups

The differences in QST parameters among three groups, including the NCA, UCA and BCA groups, are shown in Figure 2, which showed that compared with the NCA group, CDT in the UCA ($P < 0.001$) or BCA ($P = 0.037$) group was lower, WDT in the UCA ($P < 0.001$) or BCA ($P < 0.001$) group was higher, CPT in the BCA group ($P = 0.020$) was lower and HPT in the UCA ($P = 0.006$) or BCA ($P = 0.256$) groups was higher. Compared with the UCA group, CDT in the BCA group ($P = 0.037$) was lower, and WDT in the BCA group ($P < 0.001$) was higher.

Correlation between thermal thresholds detection and carotid ultrasonography parameters

To confirm the relationship between IMT or the size of atherosclerotic plaque and QST parameters, the Spearman correlation test was applied. The results in Table 2 showed that the IMT correlated positively with WDT ($r = 0.237$, $P < 0.001$) or HPT ($r = 0.176$, $P < 0.01$), and negatively with CDT ($r = -0.178$, $P < 0.001$) and CPT ($r = -0.193$, $P < 0.01$), and the atherosclerotic plaque size correlated positively with WDT ($r = 0.646$, $P < 0.001$) and HPT ($r = 0.354$, $P < 0.001$), and negatively with CDT ($r = -0.557$, $P < 0.001$). After adjusting the influencing factors, including age, diabetes duration, HbA1c, Scr, osteocalcin, β-CTX and folic acid, a correlation between atherosclerotic plaque size with CDT was also presented ($r = -0.239$, $P < 0.001$), WDT ($r = 0.476$, $P < 0.001$) and HPT ($r = 0.213$, $P < 0.001$).

Independent risk factors for small fiber dysfunction

To understand whether carotid atherosclerosis was an independent risk factor of small fiber dysfunction in type 2 diabetes

Table 1 | Demographic and biochemical differences

	Total (n = 247)	NSF (n = 103, 41.7%)	SFD (n = 144, 58.3%)	P
Male	169 (68.4%)	64 (62.1%)	105 (72.9%)	0.072
Age (years)	51.03 ± 11.91	47.84 ± 11.29	53.31 ± 11.86	<0.001
Diabetes duration (years)	7.41 ± 7.00	5.32 ± 6.14	8.91 ± 7.22	<0.001
BMI (kg/m^2)	25.06 ± 3.92	25.39 ± 4.71	24.82 ± 3.23	0.658
WHR	0.94 ± 0.09	0.94 ± 0.11	0.93 ± 0.06	0.535
Smoking history	91 (36.8%)	27 (26.2%)	64 (44.4%)	0.003
Drinking history	28 (11.3%)	8 (7.8%)	20 (13.9%)	0.135
SP (mmHg)	133.52 ± 16.37	133.46 ± 16.84	133.57 ± 16.09	0.582
DP (mmHg)	84.40 ± 11.29	85.62 ± 11.76	83.52 ± 10.89	0.183
FBG (mmol/L)	8.53 ± 2.56	8.41 ± 2.41	8.62 ± 2.66	0.742
Fasting insulin (μU/mL)	9.24 ± 12.48	8.40 ± 8.39	9.85 ± 14.75	0.940
Fasting C-peptide (pmol/L)	614.07 ± 326.61	594.42 ± 327.55	628.20 ± 326.36	0.347
Standard meal (2 h) Glucose (mmol/L)	15.02 ± 4.10	15.09 ± 3.95	14.98 ± 4.21	0.978
Standard meal (2 h) Insulin (μU/mL)	30.51 ± 27.46	28.37 ± 23.59	32.04 ± 29.91	0.962
Standard meal (2 h) C-peptide (pmol/L)	1518.94 ± 892.05	1457.09 ± 724.05	1563.06 ± 994.88	0.900
HbA1c (%)	9.32 ± 2.25	9.34 ± 2.14	9.31 ± 2.33	0.787
Glycated albumin (%)	20.02 ± 8.68	19.68 ± 8.47	20.28 ± 8.85	0.724
Scr (mmol/L)	59.74 ± 15.79	57.56 ± 15.00	61.29 ± 16.20	0.013
BUN (mmol/L)	5.26 ± 1.34	5.11 ± 1.28	5.36 ± 1.37	0.172
UA (umol/L)	341.90 ± 97.97	338.17 ± 85.34	344.54 ± 106.23	0.620
eGFR (mL/min/1.73 m^2)	130.32 ± 39.25	133.11 ± 28.55	128.36 ± 45.27	0.018
ACR (mg/g)	61.98 ± 229.15	29.17 ± 58.12	84.55 ± 292.09	0.727
Total protein (g/L)	68.91 ± 4.95	69.63 ± 5.04	68.40 ± 4.84	0.117
Albumin (g/L)	43.03 ± 2.43	43.30 ± 2.42	42.83 ± 2.44	0.122
Triglyceride (mmol/L)	1.87 ± 1.62	1.71 ± 1.21	1.99 ± 1.86	0.486
Cholesterol (mmol/L)	4.87 ± 1.10	4.78 ± 1.07	4.94 ± 1.11	0.173
LDL (mmol/L)	2.90 ± 0.94	2.87 ± 0.89	2.92 ± 0.98	0.534
HDL (mmol/L)	1.19 ± 0.35	1.19 ± 0.31	1.18 ± 0.37	0.277
Osteocalcin (ng/mL)	13.70 ± 4.81	14.54 ± 5.09	13.11 ± 4.53	0.012
ß-CTX (ng/mL)	0.43 ± 0.22	0.48 ± 0.23	0.40 ± 0.21	0.002
PINP (ng/mL)	40.12 ± 17.03	42.08 ± 16.56	38.70 ± 17.27	0.065
25-(OH)-VitD (ng/mL)	19.42 ± 7.64	19.03 ± 6.04	19.70 ± 8.61	0.772
Folic acid (ng/mL)	12.80 ± 4.69	13.99 ± 4.15	11.90 ± 4.89	0.001
Vitamin B_{12} (pg/mL)	612.20 ± 294.71	604.72 ± 232.53	617.71 ± 334.08	0.516
cIMT (cm)	0.10 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	0.018
Atherosclerotic plaque size (cm)	0.55 ± 0.69	0.29 ± 0.48	0.74 ± 0.75	<0.001
NCA	85 (34.4%)	46 (44.7%)	39 (27.1%)	<0.001
UCA	55 (22.3%)	22 (21.4%)	33 (22.9%)	
BCA	71 (28.7%)	13 (12.6%)	58 (40.3%)	
CIT	36 (14.6%)	22 (21.4%)	14 (9.7%)	0.017
CDT	27.24 ± 2.99	28.71 ± 1.24	25.78 ± 4.30	<0.001
Abnormal CDT	69 (27.9%)	0	69 (47.9%)	<0.001
WDT	39.02 ± 3.65	36.06 ± 1.82	41.22 ± 3.75	<0.001
Abnormal WDT	90 (36.4%)	0	90 (62.5%)	<0.001
CPT	15.86 ± 9.15	18.48 ± 7.62	14.48 ± 9.63	0.161
HPT	44.20 ± 2.87	42.90 ± 7.62	45.05 ± 2.50	0.040

Data are shown as the mean ± standard deviation or as n. Independent t-tests and χ^2-tests were used to compare the normally distributed variables, and Kruskal–Wallis tests were used for analyses of abnormal distributions. ACR, albumin to creatinine ratio; BCA, bilateral carotid atherosclerosis; BMI, body mass index; BUN, blood urea nitrogen; CDT, cold detection threshold; cIMT, carotid intima-media thickness; CIT, carotid intimal thickening; DP, diastolic pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NCA, non-carotid atherosclerosis; PINP, propeptide of type I procollagen; Scr, serum creatinine concentration; SP, systolic pressure; β-CTX, beta C-terminal telopeptide of type I collagen; UCA, unilateral carotid atherosclerosis; WDT, warm detection threshold; WHR, waist-to-hip ratio.

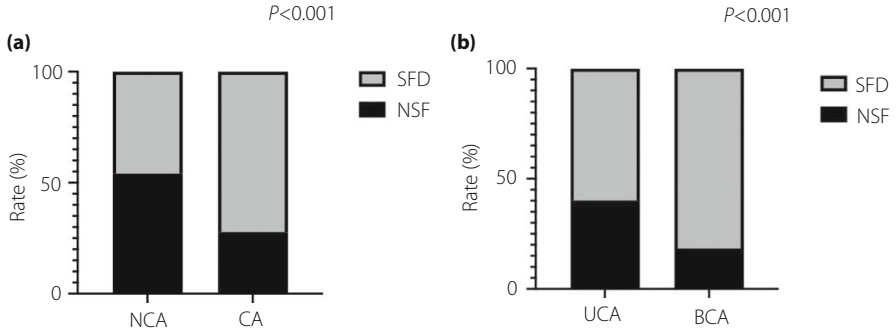

Figure 1 | The incidence rate of small fiber nerve dysfunction in different groups, (a) Incidence rate of small fiber nerve dysfunction group (SFD) and normal small fiber nerve function group (NSF) patients in carotid atherosclerosis (CA) group and non-carotid atherosclerosis (NCA) group. (b) Incidence rate of SFD and NSF patients in the unilateral carotid atherosclerosis (UCA) group and bilateral carotid atherosclerosis (BCA) group.

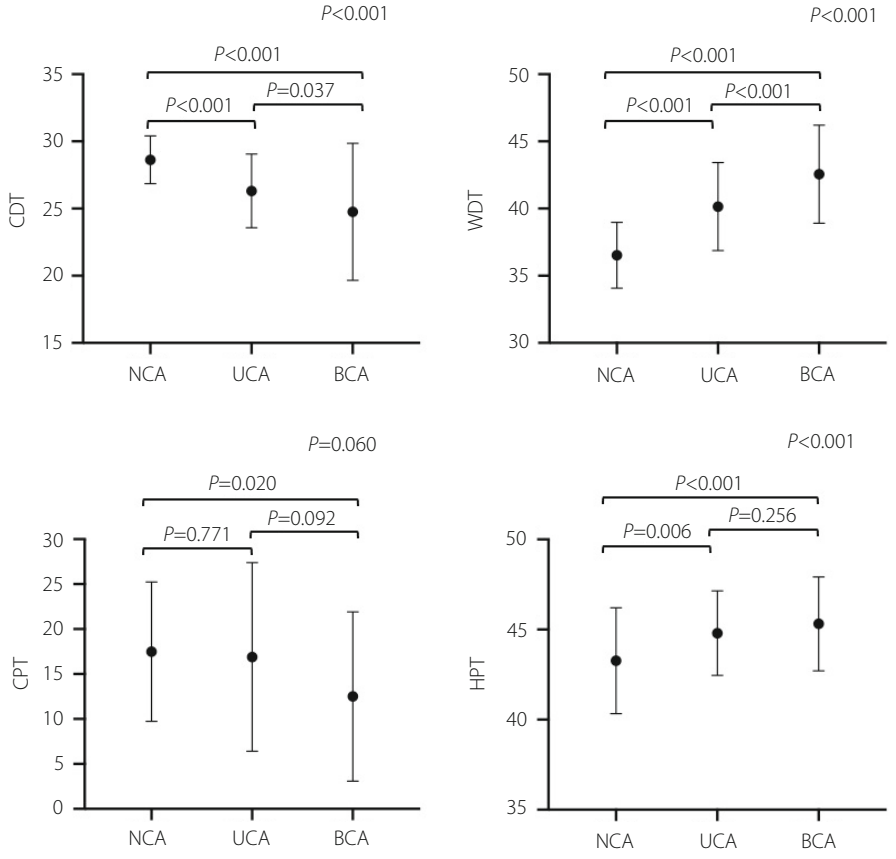

Figure 2 | Quantitative sensory testing parameters in different groups of carotid atherosclerosis. Independent *t*-tests and Mann–Whitney *U*-tests were used to analyze the difference between two groups. One-way ANOVA were used to compare the statistical significance of normally distributed variable and Kruskal–Wallis *H*-tests were used to analyze abnormal distributions among three groups. The Bonferroni test was used to avoid the multiple comparison problem. BCA, bilateral carotid atherosclerosis; CDT, cold detection threshold; CPT, cold pain detection threshold; HPT, heat pain detection threshold; NCA, non-carotid atherosclerosis; UCA, unilateral carotid atherosclerosis; WDT, warm detection threshold.

patients, both before or after adjusting some influence factors (age, diabetic duration, HbA1c, Scr, osteocalcin, β-CTX and folic acid), CA (before adjusting: odds ratio [OR] 3.336, 95% confideince interval [CI] 1.963–5.668, $P < 0.001$; after adjusting: OR 2.326, 95% CI 1.164–4.649, $P = 0.017$) and especially BCA (before adjusting: OR 5.724, 95% CI 2.841–11.533, $P < 0.001$; after adjusting: OR 5.042, 95% C: 1.977–12.861, $P = 0.001$) were found to be the independent risk factors for small fiber

Table 2 | Correlation between quantitative sensory testing and carotid ultrasonography parameters

r	CDT	WDT	CPT	HPT
cIMT	−0.178**	0.237**	−0.193*	0.176*
Atherosclerotic plaque size	−0.557**	0.646**	−0.218*	0.354**
cIMT (After adjusting)	0.028	0.043	0.033	0.063
Atherosclerotic plaque size (After adjusting)	−0.239**	0.476**	−0.089	0.213**

***P*-values <0.001, **P*-values <0.01.

Partial correlation analyze was used to obtain the correlation coefficient between quantitative sensory testing and carotid ultrasonography parameters after controlling the influence factors including age, diabetic duration, glycosylated hemoglobin, creatinine, osteocalcin, beta C-terminal telopeptide of type I collagen and folic acid.

CDT, cold detection threshold; cIMT, carotid intima-media thickness; CPT, cold pain threshold; HPT, heat pain threshold; WDT, warm detection threshold.

dysfunction. However, both models showed that CIT was not a risk factor for small fiber dysfunction (before adjusting: OR 0.397, 95% CI 0.192–0.819, $P = 0.012$; after adjusting: OR 0.435, 95% CI 0.180–1.051, $P = 0.064$; Figure 3).

DISCUSSION

SFN is a most common kind of peripheral neuropathy in type 2 diabetes patients. We calculated the incident rate of small fiber dysfunction in the present study (58.3%), and it significantly increased to 72.2% in patients with CA and 81.7% in BCA patients. Epidemiology showed that the prevalence of SFN increases with age (up to 44%) and diabetes duration (up to 65%).[18] Although the incidence rate of SFN has reached such a high level in patients with type 2 diabetes and could lead to serious consequences, such as mixed fiber neuropathy, diabetic foot or even amputation,[19] there is still a lack of international awareness and census methods, which leads to misdiagnosis and missed diagnosis of SFN. In addition, type 2 diabetes is characterized by a two- to fourfold increased risk of cardiovascular disease.[20] As a most common complication of cardiovascular disease in type 2 diabetes patients, CA also occurs at the beginning of the condition of blood glucose elevation. Previous studies also mentioned that coronary heart disease might be a risk factor for neuropathy,[21] but there is no in-depth research, especially into CA, at present. Furthermore, the screening and diagnosis of CA has been widely up till the present moment, and the carotid color Doppler ultrasonography has been widely used. Therefore, the present study aimed to explore the correlation between carotid atherosclerosis and quantitative sensory testing parameters, which reflexed the peripheral small fiber nerve function in type 2 diabetes patients, providing a new clue for the comprehensive evaluation of the occurrence and development of complications of small fiber neuropathy in type 2 diabetes patients, and timely intervention and treatment.

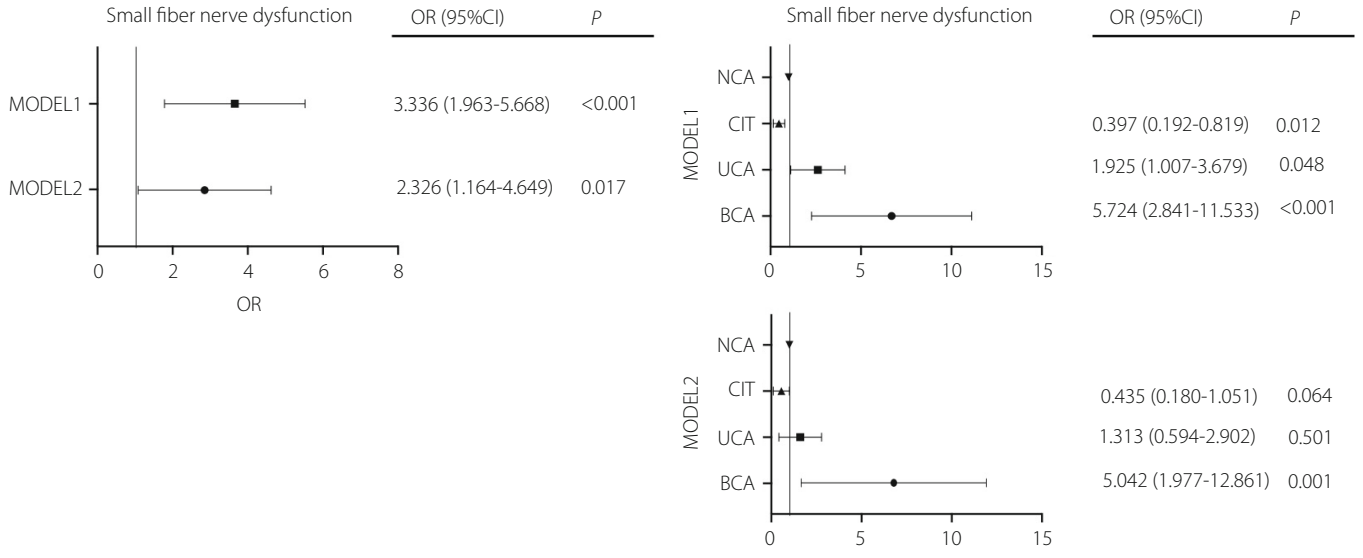

Figure 3 | Forest graph in searching that carotid atherosclerosis as the independent risk factors for small fiber dysfunction. Model 1: unadjusted; model 2: adjusted for age, diabetic duration, glycosylated hemoglobin, creatinine, osteocalcin, beta C-terminal telopeptide of type I collagen and folic acid. CI, confidence interval; OR, odds ratio. BCA, bilateral carotid atherosclerosis; CIT, carotid intimal thickening; NCA, non-carotid atherosclerosis; UCA, unilateral carotid atherosclerosis.

The study of Alhusaini *et al.*[22] showed that as CA deteriorated, cognitive function declined, which proved that there was a significant correlation between CA and central nervous system function. Regarding the peripheral nerve function, the present study first compared the carotid arthrosclerosis parameters between the NSF group and SFD group. The results showed that the carotid intima-media thickness in patients with SFD was thicker and the size of atherosclerotic plaque was larger. As atherosclerosis became aggravated, WDT and HPT increased, and CDT decreased, which suggested that patients with BCA develop worse small fiber nerve function than that with UCA. To explain this relationship, the situation of hyperglycemia could activate the process of insulin resistance, oxidative stress, inflammation, increased oxidative stress and endothelial dysfunction, which are the copathogenesis in atherosclerosis and neuropathy.[23] In addition, the results also confirmed that even after adjusting the influencing factors, CDT presented a negative correlation, but WDT and HPT presented a positive correlation with atherosclerotic plaque size. It suggested that in clinical practice, we should pay more attention to the size and changes of the carotid plaque, which can evaluate and compare the small fiber nerve function of patients. A series of follow-up studies on the size of carotid plaque and peripheral nerve function can be further carried out in the future.

There are many studies that proved that cardiovascular autonomic neuropathy, which belongs to small fiber neuropathy, presents a close relationship with carotid atherosclerosis.[23–25] Especially in Chan-Hee Jung's study, a total of 131 type 2 diabetes mellitus patients were grouped based on cIMT and the number of carotid plaques, and the results also suggested that cIMT and the number of carotid plaques both positively correlated with the cardiovascular autonomic neuropathy score.[26] But these results ignore the relationship between peripheral small fiber nerve and CA. In addition, a previous study found that the role of TRPV1 channels, reducing due to the small fiber neuropathy, is able to promote the development of atherosclerosis.[27] To confirm whether carotid arthrosclerosis presents the ability to assess the situation of SFD, this study first calculates the incidence rate of small fiber nerve dysfunction in patients without CA and with CA, and it showed that it significantly was increased in the CA group. Furthermore, both before or after adjusting for age, diabetes duration, HbA1c, Scr, estimated glomerular filtration rate, osteocalcin, β-CTX and folic acid, which were significantly different in the results of clinical data and regarded as the traditional influencing factors in SFN, the results of logistic regression suggested that CA and BCA are independent risk factors of SFD.

These results further support the idea of that SFN and CA might develop synchronously, and CA parameters, including intimal thickness, unilateral or bilateral condition and the size of carotid plaques, are helpful to evaluate the condition of small fiber nerve function. Notably, there was no significant association between CPT and carotid arthrosclerosis parameters in the present study, which might be due to there being no significant difference according to different individuals[28] and the small number patients who received CPT; a larger number of participants or perspective studies are required to explore the correlation between quantitative sensory testing, especially CPT and cardiovascular disease. At the same time, according to the incidence rate, correlation analysis results and risk factor results of carotid intimal thickening in the NSF and SFD groups, it can be concluded that there is no correlation between small fiber nerve function and arterial intimal thickness. However, this conclusion still requires a large number of patients and long-term follow up.

The present study had several advantages. To the best our knowledge, this is the first study to explore the relationship between CA and SFD at present, and proved that CA, especially BCA, was independent risk factor for SFD. On this basis, physicians could search the link between SFN and cardiovascular disease in a future study. However, the present study also had some limitations. First, this study was a cross-sectional analysis and did not discover whether carotid atherosclerosis parameters would change with the deterioration of small fiber nerve function. Second, for the influencing factors, such as as area and racial differences on QST, the number of participants needs to be expanded and different racial studies are required to verify the conclusion more convincingly, especially in CPT.

In the present study, the thermal detection thresholds in QST presented a close correlation with CA parameters, including the diagnosis of CA, intimal thickness, unilateral or bilateral condition and even the size of carotid plaques. WDT or HPT increased, and CDT decreased with the development of CA. Furthermore, after adjusting for influencing factors, CA, especially BCA, was an independent risk factor for small fiber nerve dysfunction. Therefore, it suggests that in clinical practice, for patients with type 2 diabetes or patients with cardiovascular complications, it is important to pay more attention to small fiber nerve function for the early prevention, detection and intervention of SFN. However, further prospective studies and possibly multicenter studies with wide racial and ethnicity participation are required to confirm these findings.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The study protocol and ethical consideration was approved by the Institutional Review Board of Drum Tower Hospital Affiliated to Nanjing University Medical School.

Informed consent: Written informed consent was obtained from all patients.

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