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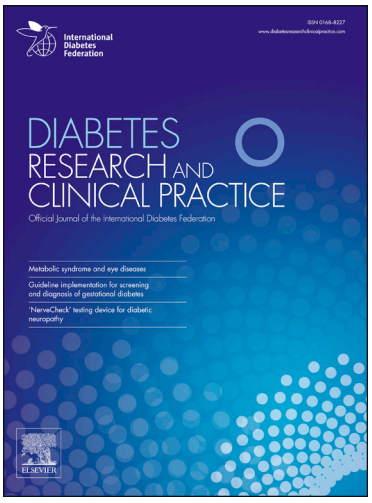

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Correlation of cardiac autonomic neuropathy with small and large peripheral nerve function in type 2 diabetes mellitus

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Abstract

Aims: To analyse the correlation of cardiac autonomic neuropathy (CAN), sympathetic and parasympathetic dysfunction with the different diagnostic tools for large and small peripheral nerve fibres in type 2 diabetes mellitus (T2DM).

Methods: We included 153 T2DM subjects (92 men) with mean age of 64.4 years. CAN, as well as sympathetic and parasympathetic dysfunction were diagnosed by the Ewing's cardiovascular reflex tests. Vibration perception threshold (VPT), monofilament, Ipswich Touch test, automated sural nerve conduction study and neuropathy disability score (NDS) evaluated large and small peripheral nerve fibre function.

Results: CAN (adjusted odds ratio [aOR]: 44.57), parasympathetic (aOR: 18.40) and sympathetic dysfunction (aOR: 5.50) correlated with measures of small fibre function evaluated by pinprick sensation and temperature perception. Among tools for large nerve fibres, positive correlation was shown between: 1) CAN and abnormal VPT (aOR: 16.78), 2) parasympathetic dysfunction and abnormal VPT (aOR: 39.47).

Conclusions: CAN and parasympathetic dysfunction correlate with peripheral neuropathy, especially when the latter is assessed through VPT and measures of small fibre function as evaluated by pinprick sensation and temperature perception. The latter additionally correlate with sympathetic nervous system impairment.

Key words: cardiac autonomic neuropathy; diabetic polyneuropathy; diagnosis; type 2 diabetes mellitus.

1. Introduction

Cardiovascular autonomic neuropathy (CAN) is a common complication of diabetes, associated with significant cardiovascular morbidity and mortality [1]. Predictors of CAN include age, diabetes duration, poor glycaemic control and microvascular disease [2]. Nonetheless, the degree of co-existence between CAN and diabetic peripheral neuropathy (DPN) still needs clarification. So far, several studies have provided evidence of a concomitant evolution [3-8], while others have shown lack of association [9-11].

In this context, we have previously demonstrated that CAN (diagnosed as 2 out of 4 abnormal cardiovascular autonomic function tests) did not correlate with presence or severity of DPN in both type 1 (T1DM) and type 2 diabetes mellitus subjects (T2DM). Evaluation of DPN was based on neuropathy disability score (NDS) [12]. Based on these findings, we then formulated two hypotheses: 1) parallel involvement could not be established, because the studies (including our own) examining this relationship did not attempt a separate analysis of the various components of small and large nerve fibre function; 2) parallel involvement could principally affect the more vulnerable large nerve fibres.

Indeed, autonomic function is regulated through small myelinated preganglionic (B fibres) and small unmyelinated (C) postganglionic fibres [13]. Sympathetic nervous system dysfunction is mainly driven by the small unmyelinated (C) nerve fibres [13], whereas the vagus nerve, the longest nerve of the autonomic nervous system is the major nerve of the parasympathetic nervous system [14, 15]. Conversely, A-alpha large myelinated nerve fibres regulate muscle contraction, while A-alpha/A-beta large myelinated nerve fibres convey information related to vibration perception and light touch. Among small peripheral nerve fibres, A-delta and unmyelinated C nerve fibres mediate pain and temperature perception [16].

Thus, the aim of the present study was to separately analyse the correlation of 1) CAN, 2) sympathetic and 3) parasympathetic dysfunction with the different diagnostic tools that evaluate large and small peripheral nerve fibres in T2DM subjects.

2. Subjects, Materials and Methods

2.1 Subjects

Over a period of 12 months, a total of 153 T2DM subjects (92 men, 61 women) with mean age of 64.4±7.8 years and median T2DM duration of 12 years (1-34 years) regularly attending the Diabetes Centre of the Second Department of Internal Medicine at Democritus University of Thrace, Greece, were randomly chosen and offered an examination for CAN [12]. The study was approved by the institutional ethics committee and participants gave their informed consent.

Inclusion criteria were: age above 18 years and T2DM. Exclusion criteria of the patients were: age ≥ 85 years, inability to undertake the examination, arrhythmia, severe illness, severe infection, severe hypoglycaemia, liver cirrhosis, overuse of alcohol (women 14 units per week and men 21 units per week), heart failure (New York Heart Association classification 3 and 4) and other causes of neuropathy [12, 17]. Patients with proliferative retinopathy were excluded from the Valsalva examination [1, 18].

2.2 Assessment of CAN

Smoking, food, caffeine-containing liquids and particular pharmacologic agents (antidepressants, neuroleptics) were prohibited 12 hours preceding the examination. Participants were further requested to avoid insulin and hypoglycaemic agents on the day of the procedure. CAN assessment was performed between 07:00 and 09:00 AM in a quiet examination room and at steady room temperature (22-24^{o}C).

After 5 minutes at supine resting position, all subjects were submitted to 4 standardised cardiovascular autonomic reflex function tests (CARTs) as described by Ewing in 1970 in the following order: deep breathing with a respiration frequency of 6 breaths per minute to assess expiration to inspiration (E/I) ratio (the mean of the longest R-R interval during expiration divided by the mean of the shortest R-R interval during inspiration); heart rate analysis response to changing from supine to standing position (the 30:15 ratio, namely the longest R-R interval during beats 20-40 after standing to the shortest R-R interval during beats 5–25 after standing); the Valsalva manoeuvre to determine the ratio of the longest R-R interval to the shortest R-R interval during forced expiration into the mouthpiece of a manometer against a fixed resistance of 40 mmHg for 15 seconds (Valsalva ratio); and the postural change of blood pressure [14, 18, 20]. Each test was separated by a 2-minute resting period.

The 3 heart rate CARTs were measured with the use of the computer-aided system Varia cardio TF5 (MIE Medical Research, Leeds, UK). Blood pressure was measured using an automatic device.

2.2.1 Definition of CAN, parasympathetic and sympathetic cardiovascular nervous system impairment

An E/I ratio above the age-related reference value, a Valsalva ratio ≥1.21, a posture ratio ≥1.04 and a systolic blood pressure reduction in response to standing ≤10 mmHg were considered normal [18, 19, 20]. An E/I ratio below the age-related values, a Valsalva ratio ≤1.10, a posture ratio ≤1.00 and a systolic blood pressure fall in response to standing ≥20 were considered abnormal. Each of the items was scored as 0 for normal, 1 for borderline, and 2 for abnormal. CAN was defined as ≥2 abnormal tests [18, 219, 20]. Parasympathetic nervous system impairment was determined as an

abnormal E/I ratio while sympathetic nervous system impairment was defined as abnormal orthostatic hypotension test [21-23].

2.3 Assessment of DPN: Large nerve fibres

2.3.1 Vibration perception threshold (VPT): VPT was measured at the hallux bilaterally with a neurothesiometer (Horwell Scientific Laboratory Supplies, London, UK). The traditional threshold of 25 Volts is associated with a low sensitivity for early detection of DPN [24], and so the lower threshold of ≥16 Volts was preferred [25].

2.3.2 10 g Semmes-Weinstein monofilament: Loss of protective sensation was assessed in a random manner at ten sites of each lower extremity: the distal part of the hallux, 3rd and 5th toe, 1st, 3rd and 5th metatarsal heads, medial foot, lateral foot, heel and dorsally between the hallux and the 2nd toe [26]. Sites with ulcer, callus or scar were avoided [27]. Inability to feel the monofilament in at least 2 sites in at least one lower extremity was defined as abnormal [26].

2.3.3 Ipswich touch test: This involved lightly touching with the index finger for approximately 1-2 seconds on the tips of the 1st, 3rd and 5th toe in both feet [28]. An abnormal result was defined as ≥2 insensate out of the six (overall for the two lower extremities) sites.

2.3.4 NC-stat®/DPNCheck ™ device (NeuroMetrix, Inc., Waltham, MA): [29] This was used for automated bilateral sural nerve conduction testing. The sural nerve was stimulated with stainless steel probes which were placed just anterior to Achilles tendon and posterior to lateral malleolus. Examination was considered abnormal when sural nerve amplitude was less than 4 microvolts and/or when sural conduction velocity was less than 40 meters/second in at least one of the two lower extremities [30].

2.3.5 Ankle reflexes and 128-Hz graded tuning fork: Achilles tendon reflexes and vibration perception at the apex of the hallux were examined separately, as both

reflect the large nerve fibre components of Neuropathy Disability Score (NDS) [31]. The test was considered positive for large fibre impairment when Achilles reflex was absent despite facilitation and/or vibration perception was abnormal in at least one foot.

2.4 Assessment of DPN: Small nerve fibres

2.4.1 Pinprick sensation and temperature perception (with a Tiptherm rod): These were assessed as the small nerve fibre components of NDS [32]. The test was characterised positive when at least one of the two parameters was abnormal in at least one lower extremity.

2.5 Overall DPN assessment: Both large and small nerve fibre investigation

NDS: This involved testing of sensory dysfunction and examination of ankle reflexes. Three sensory parameters (pinprick sensation, temperature perception and vibration perception threshold) were scored as normal (0) or abnormal (1), while ankle (Achilles) reflexes were scored as present (0), present with reinforcement (1) and absent (2) [33]. Thus, the maximum deficit score was 10. A score of ≥3 was considered diagnostic of DPN [33].

2.6 Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL), version 19.0. Normality of quantitative variables was tested with Kolmogorov-Smirnov test. Normally distributed quantitative variables were expressed as the mean ± standard deviation (SD), while non normally distributed quantitative variables were expressed as the median value and range (min to max). Categorical variables were expressed as frequencies (and percentages). Odds ratios (OR) and their 95% confidence interval (CI) were estimated as a measure of association between A) CAN and impairment of the parasympathetic and sympathetic nervous system and B) diagnostic tools implicated in assessment of DPN in patients

with T2DM. Multivariate logistic regression analysis was performed to explore the independent effect of diagnostic tools implicated in assessment of DPN on CAN and impairment of the parasympathetic and sympathetic nervous system: adjustment was made for gender, age, height, T2DM duration and haemoglobin A_{1c} (HbA_{1c}). All tests were two-tailed and significance was defined at the 5% level ($p< 0.05$).

3. Results

Table 1 shows the correlation between CAN and presence of large or small nerve fibre neuropathy. Abnormality in sural nerve conduction parameters as evaluated with the use of NC-stat®/DPNCheck™ device was associated with a threefold higher likelihood of CAN (Odds Ratio [OR]: 3.76, 95% Confidence Interval [CI]: 1.35-7.73). Subjects with an abnormal VPT examination (OR: 4.75, 95% CI: 1.96-11.51) and Ipswich touch test (OR: 3.55, 95% CI 1.40-9.00) also exhibited a higher likelihood of CAN. On the contrary, no association was observed between CAN and assessment of large nerve fibre function with the use of monofilament (OR: 1.86, 95% CI: 0.79-4.39) and with measures of large fibre function assessed by ankle reflexes and 128-Hz graded tuning fork (OR: 1.31, 95% CI: 0.54-3.16). However, these associations were modified after multivariate analysis (Table 2). Following adjustment for age, sex, height, HbA_{1c} and T2DM duration, only two correlations between CAN and large nerve fibre DPN remained significant: 1) with VPT: subjects with abnormal vibration perception had a sixteen times higher likelihood of CAN (adjusted Odds Ratio [aOR]: 16.78, 95% CI: 2.2-127.59); 2) with NDS (Achilles reflex and vibrations perception). In the latter case, detection of abnormality seemed to significantly reduce CAN risk (aOR: 0.05, 95% CI: 0.01-0.42).

Assessment of small nerve fibre function with the use of NDS (pinprick sensation, temperature perception) was further positively associated in both univariate (OR: 20.97, 95% CI: 5.91-74.35) and multivariate regression analysis (aOR: 44.57, 95% CI: 10.24-194.09) with a higher likelihood of CAN (Tables 1 and 2). Finally, subjects with abnormal NDS also exhibited a higher likelihood for CAN in univariate analysis (OR: 2.73, 95% CI: 1.16-6.45) (Table 1), but significance was lost after adjustment for confounding factors (Table 2).

Table 3 shows the association of parasympathetic cardiovascular nervous system impairment and large or small nerve fibre peripheral neuropathy. All DPN tools for large nerve fibres (NC-stat®/DPNCheck™, monofilament, VPT, Ipswich touch test and measures of large fibre function assessed by ankle reflexes and 128-Hz graded tuning fork *k*) and NDS significantly correlated with the presence of cardiovascular parasympathetic impairment. The strongest correlation was observed with VPT (OR: 12.83, 95% CI: 4.71-34.96). These correlations were modified in multiple regression analysis (Table 2). Following adjustment for confounders, two correlations pertaining to large nerve fibre DPN remained (Table 2): 1) with VPT (aOR: 39.47, 95% CI: 5.49-283.89); 2) with the two NDS parameters (Achilles reflex and vibrations perception) (aOR: 0.13, 95% CI: 0.02-0.95).

Measures of small fibre function assessed by pinprick sensation and temperature perception were further positively correlated with parasympathetic nervous system impairment (Table 3) (OR: 6.87, 95% CI: 2.68-17.63). This was further confirmed in multivariate analysis (Table 2).

Finally, we examined the correlation between cardiovascular sympathetic nervous system impairment and large or small nerve fibre dysfunction (Table 4). Only two DPN parameters yielded significant correlations in univariate analysis: 1) measures

of small fibre function evaluated by pinprick sensation and temperature perception (OR: 5.86, 95% CI: 2.27-15.18); 2) abnormal NDS (OR: 3.01, 95% CI: 1.26-7.24). However, in multiple regression analysis (Table 2), cardiovascular sympathetic impairment correlated only with measures of small fibre function assessed by pinprick sensation and temperature perception (aOR: 5.50, 95% CI: 2.09-14.51).

4. Discussion

The present study had 3 main findings: 1) CAN was correlated in both univariate and multivariate analysis with measures of small fibre function assessed by pinprick sensation and temperature perception (aOR: 44.57). Among DPN tools for large nerve fibres, positive correlation following correction for confounding factors was shown only with abnormal VPT (aOR: 16.78); 2) In multiple regression analysis (following adjustment for gender, age, height, T2DM duration and HbA_{1c}), cardiovascular parasympathetic nervous system impairment was correlated with small nerve fibre dysfunction as evaluated with the use of NDS and defined as abnormal pinprick sensation and/or temperature perception in at least one foot (aOR: 18.40) and abnormal VPT (aOR: 39.47); 3) After adjustment for confounders, cardiovascular sympathetic nervous system impairment correlated only with assessment of small nerve fibre function evaluated by pinprick sensation and temperature perception (aOR: 5.50).

Surprisingly, measures of large fibre function assessed by ankle reflexes and 128-Hz graded tuning fork significantly reduced the risk for both CAN (aOR: 0.05) and parasympathetic nervous system impairment (aOR: 0.13). This oxymoron could probably derive from statistical analysis and the so called Simpsons's paradox (Yule-Simpson effect) [34]. Indeed, in the field of statistical proof of correlation or not between two variables, Simpson's paradox is a phenomenon occurring when there is an

unrecognised confounding variable not included in multivariate analysis [34]. In our case, previous studies have shown that 128-Hz graded tuning fork as compared to quantitative testing, seems to overestimate reduction of vibration perception (Pearson correlation coefficient, r = 0.58) [35]. Similarly, compared with electrophysiology in individuals with diabetes mellitus (diabetes type not mentioned), evaluation of Achilles reflex was associated with only moderate sensitivity (72.22-78.95%) and low specificity (43.42-46.77%) [36]. Therefore, the paradoxical outcome of our study may be due to the fact that we did not regard as confounding factor the overestimation of large fibre dysfunction associated with a) assessment of Achilles reflex and b) the 128-Hz graded tuning fork.

The relationship between CAN and DPN has been thoroughly studied with a number of studies providing evidence of co-existence [3-8] and others showing lack of concomitant evolution [9-11]. Nonetheless, in aforementioned studies 1) CAN diagnosis was not based on a unanimous definition and 2) DPN diagnosis was based on different diagnostic tools. Taken together, the results of the present study and those of previous works seem to confirm the correlation between CAN and DPN, especially when the latter is diagnosed through NDS [3, 4, 6, 7] and VPT [5, 8, 10]. NDS evaluates both large and small nerve fibres [33]. However, as aforementioned, a tendency of overestimation of large nerve fibre impairment has been shown with Achilles reflexes and 128-Hz graded tuning fork examination [35, 36]. VPT mainly evaluates large nerve fibres [37, 38]. However, it was previously suggested that the combination of skin biopsy with the VPT (with a threshold ≥20.5 volts) and tuning fork increased sensitivity for the diagnosis of small nerve fibre DPN from 74% to 93%, as compared to skin biopsy alone [39]. In another study, VPT predicted small fibre DPN (as documented by the gold standard method of skin biopsy) with adequate sensitivity (71%) and

specificity (51%) [40]. Hence, it appears that CAN is accompanied by DPN; 1) with measures of small fibre function evaluated by pinprick sensation and temperature perception and 2) with large nerve fibre impairment, especially when assessed with VPT.

The present study provided further evidence of the correlation of cardiovascular parasympathetic nervous system impairment with measures of small fibre function evaluated by pinprick sensation and temperature perception and with VPT. Indeed, vagus nerve, the major nerve of the parasympathetic nervous system, is the longest nerve of the autonomic nervous system [14, 15]. Importantly, it was previously described that parallel development of CAN and DPN involves vulnerable large nerve fibres, with recent evidence pointing to an association between parasympathetic nervous system impairment and reduced peroneal motor nerve conduction velocity [41]. This comes in line with an earlier study by Valensi et al. [42]. The authors showed that DPN, evaluated with modalities for large nerve fibre function (conduction velocity, action potential amplitude, and Hoffman reflexes) did not appear to coexist with CAN, but showed a positive and significant correlation with function of the cardiovascular parasympathetic nervous system. These findings were further confirmed in a study of 89 T1DM subjects [43]. This revealed a positive association between low heart rate variability during deep breathing and large nerve fibre neuropathy, the latter documented with electrophysiology and clinical examination.

In this same study [43], as well as in our study, a further correlation was found between parasympathetic nervous system impairment and small fibre DPN. Indeed, it has been argued that heart rate variability during deep breathing also reflects small (type C) nerve fibre function [44]. Accordingly, deep breathing test positively correlates with

small and large nerve fibre function indices and may therefore be present in early or more advanced DPN.

Finally, sympathetic nervous system impairment, which is mediated by small type C nerve fibres [45], correlated in our study with measures of small fibre function evaluated by pinprick sensation and temperature perception. Recently, modalities assessing peripheral sympathetic nervous system function have been proposed for CAN diagnosis [46]. Such modalities include the non-invasive LDIflare technique, a novel test of C-fibre function on the dorsum of the foot and sudomotor function testing [47]. Thus, Yajnik et al. [48] attempted to evaluate the relationship between peripheral sympathetic nervous system activity and that of the cardiovascular sympathetic nervous system. The authors showed that Sudoscan, a device that quantitavely assesses sudomotor function, correlated with cardiovascular sympathetic nervous system activity in T2DM subjects (the latter assessed with heart rate variability frequency and time domain analysis) [47].

Nonetheless, we found no correlation between cardiovascular sympathetic nervous system impairment and large nerve fibre DPN. This was quite unexpected, especially since orthostatic hypotension is traditionally thought as a more advanced CAN manifestation [14, 15]. Therefore, we had anticipated a correlation between orthostatic hypotension and at least some diagnostic tools reflecting large nerve fibre DPN. However, as previously described, the reproducibility of orthostatic hypotension test is particularly poor, even after correction for multiple confounding factors (daily fluctuation, hormones, plasma volume, pharmacological agents) [48]. This suggests an inherent weakness of the orthostatic hypotension test to reliably reflect sympathetic activity. Indeed, very recently, Baker et al. [49] studied the clinical significance of heart rate variability in 165 healthy volunteers and patients with moderate (N = 25) and

severe (N = 34, of whom 5 persons with diabetes) autonomic dysfunction. Heart rate variability positively correlated with sympathetic nervous system activation [49]. Therefore, it appears that sympathetic activation or sympathetic nervous system impairment may be best studied through non-parametric heart rate variability spectral analysis, and this approach merits further exploration among subjects with diabetes mellitus.

The strength of this study is the separate examination of sympathetic *vs* parasympathetic component of the cardiovascular autonomic nervous system, and the attempt to correlate these with large and/or small nerve function in the somatic nervous system. Its limitations include the lack of prospective data and of electrophysiological measurements: these were beyond the scope of this work. A further limitation is that we did not include T1DM subjects. Finally, the tertiary health care setting suggests that some caution is required before extrapolating our results to the general diabetic population.

The practical implications of the present study may be outlined as follows. CAN correlates with DPN, especially when the latter is assessed through VPT and through measures of small fibre function evaluated by pinprick sensation and temperature perception. Similar correlations apply to cardiovascular parasympathetic nervous system impairment. Thus, it appears that both CAN and deep breathing test abnormality may be present in early or more advanced DPN. Furthermore, cardiovascular sympathetic nervous system impairment correlates with measures of small fibre function evaluated by pinprick sensation and temperature perception. It also seems plausible that an overestimation of large nerve fibre DPN occurs when the latter is assessed through Achilles reflex and the use of 128-Hz tuning fork. Of relevance,

orthostatic hypotension test may not accurately reflect sympathetic nervous system function.

In conclusion, this study has demonstrated that both CAN and parasympathetic nervous system impairment correlate with DPN, especially when the latter is assessed through VPT and through measures of small fibre function evaluated by pinprick sensation and temperature perception. Additionally, sympathetic nervous system impairment correlates with assessment of small nerve fibre function with the use of NDS (pinprick sensation, temperature perception). Our results add to the growing insight into CAN as a multifaceted complication of diabetes [50].

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Conflicts of interest: NP has been an advisory board member of Astra-Zeneca, Boehringer Ingelheim, MSD, Novo Nordisk, Pfizer, Takeda and TrigoCare International; has participated in sponsored studies by Astra-Zeneca, Eli-Lilly, GSK, MSD, Novo Nordisk, Novartis and Sanofi-Aventis; has received honoraria as a speaker for Astra-Zeneca, Boehringer Ingelheim, Eli-Lilly, Elpen, MSD, Mylan, Novo Nordisk, Pfizer, Sanofi-Aventis and Vianex; and attended conferences sponsored by TrigoCare International, Eli-Lilly, Galenica, Novo Nordisk, Pfizer and Sanofi-Aventis. The other authors report no conflicts of interest.

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LEGENDS TO THE TABLES

Table 1. Correlation of DPN tools with the presence or absence of CAN.

Table 2. Independent correlation of DPN tools with the presence or absence of CAN, parasympathetic and sympathetic nervous system impairment.

Table 3. Correlation of DPN tools with the presence or absence of parasympathetic nervous system impairment.

Table 4. Correlation of DPN tools with sympathetic nervous system injury.

Table 1. Correlation of DPN tools with the presence or absence of CAN.

	CAN	p value	OR (95% CI)
NC-stat		**0.006**	
Normal	13 (11.8)		
Abnormal	13 (30.2)		3.23 (1.35-7.73)
Monofilament		0.151	
Normal	14 (13.4)		
Abnormal	12 (23.1)		1.86 (0.79-4.39)
VPT		**<0.001**	
Normal	10 (9.5)		
Abnormal	16 (33.3)		4.75 (1.96-11.51)
Ipswich Touch		**0.005**	
Test	16 (12.9)		
Normal	10 (34.5)		3.55 (1.40-9.00)
Abnormal			
NDS SMALL		**<0.001**	
Normal	3 (3.1)		
Abnormal	23 (40.4)		20.97 (5.91-74.35)
NDS LARGE		0.548	
Normal	9 (14.8)		
Abnormal	17 (18.5)		1.31 (0.54-3.16)
NDS		**0.019**	
Normal	12 (11.9)		
Abnormal	14 (26.9)		2.73 (1.16-6.45)

CAN: cardiovascular autonomic neuropathy, 95% CI: 95% Confidence Interval, NDS: neuropathy disability score, OR: Odds Ratio, VPT: vibration perception threshold.

Table 2. Independent correlation of DPN tools with the presence or absence of CAN, parasympathetic and sympathetic nervous system impairment.

	CAN **aOR (95% Δ.E.)**	**p Value**	**Abnormal E/I ratio** **aOR (95% Δ.E.)**	**p Value**	**Abnormal Orthostatic Hypotension test** **aOR (95% Δ.E.)**	**p Value**
NC-stat		ns		ns		ns
Normal						
Abnormal						
Monofilament		ns		ns		ns
Normal						
Abnormal						
VPT		**0.006**		**<0.001**		ns
Normal						
Abnormal	16.78 (2.2-127.59)		39.47 (5.49-283.89)			
Ipswich Touch Test		ns		ns		ns
Normal						
Abnormal						
NDS SMALL		**<0.001**		**<0.001**		**0.001**
Normal						
Abnormal	44.57 (10.24-194.09)		18.40 (4.67-72.57)		5.50 (2.09-14.51)	
NDS LARGE		**0.005**		**0.045**		ns
Normal						
Abnormal	0.05 (0.01-0.42)		0.13 (0.02-0.95)			
NDS		ns		ns		ns
Normal						
Abnormal						

CAN: cardiovascular autonomic neuropathy, 95% CI: 95% Confidence Interval, E/I ratio: expiration to inspiration ratio, NDS: neuropathy disability score, aOR: adjusted Odds Ratio after adjustment for age, sex, height, glycated haemoglobin A1c and diabetes duration, VPT: vibration perception threshold.

Table 3. Correlation of DPN tools with the presence or absence of parasympathetic nervous system impairment.

	Abnormal E/I ratio	p Value	OR (95% CI)
NC-stat		**<0.001**	
Normal	10 (9.1)		
Abnormal	17 (39.5)		6.54 (2.68-15.96)
Monofilament		**0.002**	
Normal	11 (10.9)		
Abnormal	16 (30.8)		3.63 (1.54-8.59)
VPT		**<0.001**	
Normal	6 (5.7)		
Abnormal	21 (43.8)		12.83 (4.71-34.96)
Ipswich Touch Test		**<0.001**	
Normal	14 (11.3)		
Abnormal	13 (44.8)		6.38 (2.54-16.01)
NDS SMALL		**<0.001**	
Normal	7 (7.3)		
Abnormal	20 (35.1)		6.87 (2.68-17.63)
NDS LARGE		**0.013**	
Normal	5 (8.2)		
Abnormal	22 (23.9)		3.52 (1.25-9.87)
NDS		**<0.001**	

Normal	9 (8.9)	
Abnormal	18 (34.6)	5.41 (2.21-13.20)

CAN: cardiovascular autonomic neuropathy, 95% CI: 95% Confidence Interval, E/I ratio: expiration to inspiration ratio, NDS: neuropathy disability score, OR: Odds Ratio, VPT: vibration perception threshold.

Table 4. Correlation of DPN tools with sympathetic nervous system injury.

		Abnormal orthostatic hypotension test	p Value	OR (95% CI)
	NC-stat		0.636	
	Normal	17 (15.5)		
	Abnormal	8 (18.6)		1.25 (0.50-3.16)
	Monofilament		0.490	
	Normal	18 (17.8)		
	Abnormal	7 (13.5)		0.71 (0.28-1.84)
	VPT		0.309	
	Normal	15 (14.3)		
	Abnormal	10 (20.8)		1.58 (0.65-3.83)
Test	Ipswich Touch		0.207	
		18 (14.5)		
	Normal	7 (24.1)		1.87 (0.70-5.03)
	Abnormal			
	NDS SMALL		**<0.001**	
	Normal	7 (7.3)		
	Abnormal	18 (31.6)		5.86 (2.27-15.18)
	NDS LARGE		0.666	
	Normal	9 (14.8)		

Abnormal	16 (17.4)		1.21 (0.50-2.96)
NDS		**0.011**	
Normal	11 (10.9)		
Abnormal	14 (26.9)		3.01 (1.26-7.24)

CAN: cardiovascular autonomic neuropathy, 95% CI: 95% Confidence Interval, NDS: neuropathy disability score, OR: Odds Ratio, VPT: vibration perception threshold.