Clinical Neurophysiology 130 (2019) 1160–1165

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Close relationship between cardiovagal function and sural sensory nerve action potential in type 2 diabetes

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ARTICLE INFO

Article history:
Accepted 31 March 2019
Available online 8 May 2019

Keywords:
Cardiac autonomic neuropathy (CAN)
Diabetic distal symmetrical polyneuropathy (DSPN)
Heart rate response to deep breathing (HR_DB)
Sensory nerve action potentials (SNAPs)
Valsalva ratio (VR)

HIGHLIGHTS

- Sural SNAP was positively correlated with parameters of HR_DB and Valsalva ratio.
- A longer duration of diabetes had both higher severity and a higher percentage of CAN and DSPN.
- Multiple linear regression show sural SNAPs were independently associated with HR_DB.

ABSTRACT

Objective: Both diabetic distal symmetrical polyneuropathy (DSPN) and cardiac autonomic neuropathy (CAN) indicate the length-dependent pattern of disease. Decreased parasympathetic activity has been found in the early phase of CAN and sural sensory nerve action potential (SNAP) imply axonal loss in DSPN.
Method: All patients with type 2 diabetes underwent cardiovascular autonomic function and nerve conduction studies (NCS). We constructed modified composite autonomic scoring scale (CASS) and composite score of NCS to measure the severity of CAN and DSPN, respectively.
Results: Patients with a longer duration of diabetes had a lower heart rate response to deep breathing (HR_DB), Valsalva ratio (VR), and baroreflex sensitivity (BRS), higher CASS, a higher percentage of CAN, lower sural SNAP, higher composite score of NCS, and a higher percentage of DSPN. Multiple linear regression analysis showed that only sural SNAPs were independently associated with mean HR_DB.
Conclusion: Sural SNAP was closely correlated with parameters of cardiovagal functions in patients with different durations of diabetes. The percentage and severity of CAN and DSPN increase with longer duration of diabetes.
Significance: The independent association of sural sensory nerve action potential amplitude and heart rate response to deep breathing with type 2 diabetes is important because combined testing increases diagnostic sensitivity and specificity.

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https://doi.org/10.1016/j.clinph.2019.03.036
1388-2457/

1. Introduction

Both diabetic distal symmetrical polyneuropathy (DSPN) and cardiac autonomic neuropathy (CAN) indicate the length-dependent pattern of disease (Papanas and Ziegler, 2015). The vagus nerve, responsible for approximately 75% of the parasympathetic activity of humans, is the longest nerve of the autonomic nervous system and it can be damaged in the early phase of CAN (Ewing et al., 1985), with decreased parasympathetic activity contributing to sympathetic predominance. As the diseases progress, sympathetic denervation occurs in the late stage of CAN. Sural SNAP and sural nerve conduction velocity (SNCV) may imply that the amount of peripheral sensory nerve axon is important in the diagnosis of axonal loss in DSPN (Binns-Hall et al., 2018) and sural SNAP, and SNCV is highly correlated to the morphological severity of DSPN as assessed by biopsy (Veves et al., 1991).

For research on the relationship between CAN and DSPN in diabetes, most previous studies were small series studies and classified patient groups according to clinical phenotypes (Young et al., 1986, Tackmann et al., 1981). The results and conclusions from these studies were inclusive. Electrodiagnostic criteria for diagnosis and estimating the severity of DSPN and CAN is also a formidable challenge for clinicians. Until recently, clinical studies have assessed the criteria of nerve conduction abnormalities in DSPN more accurately and provided a method for estimating the severity of DSPN and CAN (England et al., 2009, Dyck et al., 2011a).

To our knowledge, no clinical study has investigated the relationship between parameters of CAN and DSPN in type 2 diabetes in patients with different durations of diabetes. In this study, we test the hypothesis that there is a close relationship between parameters of cardiovagal functions and sural SNAP in patients with different durations of type 2 diabetes. The successful translation of these approaches to the clinic offers the promise of reducing microvascular complications and improving quality of life in patients with type 2 diabetes.

2. Patients and methods

2.1. Patients

Patients with type 2 diabetes who visited the outpatient diabetes clinic at Kaohsiung Chang Gung Memorial Hospital in Taiwan were recruited. All these patients had follow-up sessions for more than 6 months at the neurology outpatient clinic. Patients were excluded if they: (1) suffered from moderate-to-severe heart failure (NYHA class III and IV); (2) had any type of arrhythmia that prevent analysis of heart rate variability, or pacemaker implantation due to any cause; (3) had neoplastic disorders; (4) had degenerative disorders known to affect the autonomic system, such as Parkinson's disease, multiple system atrophy, and pure autonomic failure; or (5) had history of major stroke and (6) any other causes of peripheral neuropathy not related to diabetes. Thus, we enrolled 238 participants were enrolled in the study. The study was approved by the Ethics Committee of Chang Gung Memorial Hospital Institutional Review Board (201701243B0 and 201800388B0C501).

2.2. Baseline clinical and laboratory measurements

All patients underwent complete neurologic and physical examinations upon enrollment, and at subsequent follow-up sessions at the outpatient clinic. A detailed medical history regarding the prior use of medications was obtained from patients and their families using standardized questions. Demographic data, including age, sex, duration of diabetes (years), body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP), microvascular complications of diabetes (retinopathy and proteinuria), and laboratory parameters were obtained at baseline levels.

2.3. Assessment and scoring of cardiovascular autonomic functions

All subjects underwent a standardized evaluation of the cardiovascular autonomic function, as described by Low (Low, 2003) and the test battery consisted of the heart rate response to deep breathing (HR_DB), Valsalva ratio (VR), and baroreflex sensitivity (BRS). We constructed a Composite Autonomic Scoring Scale (CASS) to measure the severity of cardiovascular autonomic neuropathy (CAN) (Low, 1993). The severity of CAN was assessed using the cardiovagal and adrenergic sub-scores of the CASS (Low, 1993). However, the scale was modified in the adrenergic sub-score since 5-minute head-up tilt test was not done in the current study. Thus, CASS version used here allotted 3 points instead of 4 for adrenergic domain (Table 1). The detailed methodology can be found in our previous study (Huang et al., 2016).

2.4. Assessment and scoring of nerve conduction studies

Confirmed DSPN was diagnosed in patients displaying both the presence of neurological symptoms/signs and abnormalities in nerve conduction evaluations, and fulfilling the diagnostic criteria of DSPN from the clinical research report of the American Academy of Neurology (Feldman et al., 1994, England et al., 2005). Our nerve conduction studies (NCS) were performed using Nicolet Viking machines. For each patient, data of sural SNAP and sensory nerve conduction velocity (SNCV) were included. Sural sensory NCS was recorded at the lateral malleous and stimulated at the lateral calf at a distance of 14 cm. The onset latency was the time from stimulus to initial negative deflection from baseline for biphasic sensory nerve action potentials (SNAP), or to initial positive peak for triphasic SNAP. Amplitude was measured from baseline to negative peak (Huang et al., 2009). The detailed methodology and reference values can be found in our previous study (Huang et al., 2009).

To improve assessment of the attributes of a nerve conduction composite score, we further constructed a composite score of NCS to measure the severity of peripheral neuropathy for compound muscle action potential amplitudes (CMAP) (ulnar, peroneal, and tibial nerves) and SNAP (ulnar and sural nerves) (Dyck et al., 1997, 2011b, Suanprasert et al., 2014). Additionally, these values were expressed using the calculated percentile values (e.g., N5th = 0 points; $\leq$5th–N1st = 1 point and $\leq$1st = 2 points; and similarly when the abnormality is in the upper tail of the normal distribution) with maximum scores of: Σ5 NCs = 10 points.

Table 1
Modified composite autonomic scoring scale.

Cardio-vagal sub-score	
0	Normal
1	HR_DB mildly reduced but > 50% of minimum
2	HR_DB reduced to < 50% of minimum, or HR_DB and VR both reduced
3	Both HR_DB and VR reduced to < 50% of minimum
Adrenergic sub-score	
0	Normal
1	Early phase II reduction between 20–40 mmHg MBP (30–40 mmHg for those older than 50 years) Late phase II does not return to baseline Pulse pressure reduction to $\leq$ 50% of baseline
2	Early phase II reduction > 40 mmHg MBP
3	Early phase II reduction > 40 mmHg, with absent late phase II and phase IV

Slightly modified from Huang et al. (2016).
Abbreviations: HR_DB, heart rate response to deep breathing; VR, Valsalva ratio; MBP, mean blood pressure.

2.5. Statistical analysis

Data are expressed as mean ± SD or median (interquartile range). Categorical variables were compared using chi-square or Fisher's exact tests. Continuous variables that were not normally distributed were logarithmically transformed to improve normality prior to analysis. Four separate statistical analyses were performed. First, patients with Type 2 diabetes were divided into four groups stratified by duration of diabetes and compared using one-way analysis of variance (ANOVA) or chi-square or Fisher's exact tests. Second, correlation analysis was used to explore the relationship between parameters of CAN and sural SNAP and SNCV, and variables, including diabetes duration, age, BMI, composite score of NCS, and CASS. Third, two stepwise models of multiple linear regression analysis were performed to evaluate the influence of independent variables on mean HR_DB according to correlation analysis. The factors which significantly correlated with mean HR_DB were assessed by model 1 of multiple linear regression analysis. Subsequently, results from model 1 were further analyzed by model 2 of multiple linear regression analysis. Finally, Receiver Operating Characteristic (ROC) curves were generated for sural SNAP, HR_DB, VR, and BRS in the presence of CAN. The areas under the ROC curves were calculated and compared. All statistical analyses were conducted using the SAS software package, version 9.1 (2002, SAS Statistical Institute, Cary, North Carolina).

3. Results

3.1. General characteristics of patients with diabetes

Patient characteristics and microvascular complication rates at their last assessment are presented in Table 2, stratified by duration of diabetes and divided into four groups. Those with a longer duration of diabetes had a higher percentage of proteinuria ($P = 0.001$) and were older ($P < 0.0001$).

3.2. Baseline NCS and cardiovascular autonomic study stratified by ascending quartiles of duration of diabetes

Baseline NCS and cardiovascular autonomic study in patients with type 2 diabetes stratified by duration of diabetes, are listed in Table 3. Patients with a longer duration of diabetes had lower HR-DB, VR and BRS ($P = 0.013$, $P < 0.0001$, and $P = 0.003$, respectively), and higher CASS ($P = 0.022$). The percentage of CAN was 28.6% (12/42) and 22.9% (16/70), 45% (27/60), and 50% (31/62), stratified by ascending quartiles of duration of diabetes, respectively ($p = 0.007$). Patients with a longer duration of diabetes had lower sural SNAP ($P < 0.0001$), and a higher composite score of NCS ($P < 0.0001$). The percentage of DSPN was 38.1% (16/42), 45.7% (32/70), 51.7% (31/60), and 78.8% (52/66), stratified by ascending quartiles of duration of diabetes, respectively ($P < 0.0001$).

3.3. Correlation analysis of parameters of cardiovagal functions in patients

The correlation analysis used to test the influence of variables including diabetes duration, age, BMI, sural SNAP and SNCV, and composite score of NCS and CASS nerve conduction study on parameters of cardiovagal functions, are listed in Table 4. The statistical significant results (correlation coefficient, *P*-value) between HR_DB and parameters were as follows: sural SNAP ($r = 0.432$, $P < 0.0001$), composite score of NCS ($r = -0.426$, $P < 0.0001$) and CASS, age ($r = -0.217$, $P < 0.0001$), and diabetes duration ($r = -0.217$, $P < 0.0001$) (Fig. 1). The statistical significant results (correlation coefficient, *P*-value) between VR and parameters were as follows: sural SNAP ($r = 0.353$, $P < 0.0001$), composite score of NCS ($r = -0.303$, $P < 0.0001$) and CASS, age ($r = -0.281$, $P < 0.0001$), and diabetes duration ($r = -0.302$, $P < 0.0001$). The statistical significant results (correlation coefficient, *P*-value) between BRS and parameters were as follows: sural SNAP ($r = 0.341$, $P < 0.0001$), composite score of NCS ($r = -0.343$, $P < 0.0001$) and CASS ($r = -0.465$, $P < 0.0001$), and diabetes duration ($r = -0.249$, $P = 0.001$). Further, partial correlation analysis between sural SNAPs and parameters of cardiovagal functions including HR_DB, VR and BRS after controlling for age, sex, BMI, and diabetes duration were as follows: HR_DB and SNAPs ($r = 0.465$, $P < 0.0001$), VR and SNAPs ($r = 0.433$, $P < 0.0001$) and BRS and SNAPs ($r = 0.168$, $P = 0.138$).

3.4. HR_DB is independently associated with sural SNAPs

The factors which significant correlated with HR_DB except CASS and the composite score of NCS were chosen to be included in the multiple linear regression analysis (Table 5). The result of multiple linear regression analysis showed that only sural SNAPs

Table 2
Baseline characteristics of patients with Type 2 diabetes stratified by ascending Quartiles of duration of diabetes.

	1st duration Quartiles ($n = 42$)	2nd duration Quartiles ($n = 70$)	3rd duration Quartiles ($n = 60$)	4th duration Quartiles ($n = 66$)	P-value for trend
Age (year)	57.8 ± 10.6	61.8 ± 8.8	62.6 ± 9.9	66.1 ± 8.4	<0.0001**
Sex (female/male)	13/29	19/51	27/33	28/38	0.108
Diabetes duration (year) (range)	1–4	5–9	10–15	16–23	
Body mass index	25.6 ± 3.9	26.9 ± 4.3	25.8 ± 4.0	25.7 ± 3.2	0.152
SBP (mmHg)	136.6 ± 18.7	137.5 ± 19.0	141.5 ± 19.5	140.6 ± 19.9	0.475
DBP(mmHg)	76.0 ± 10.9	74.2 ± 9.9	74.3 ± 11.0	74.6 ± 12.0	0.852
Retinopathy, *n* (%)	8 (19.0)	14 (20.0)	19 (31.6)	20 (30.3)	0.271
Proteinuria, *n* (%)	13 (30.9)	38 (54.3)	39 (65.0)	43 (65.2)	0.001*
HbA1c (%)	6.9 ± 0.9	7.4 ± 0.9	7.3 ± 1.1	7.4 ± 1.3	0.171
Total cholesterol (mmol/L)	166.0 ± 32.4	155.3 ± 34.1	152.9 ± 23.7	155.3 ± 29.4	0.161
Triglyceride (mmol/L)	134.8 ± 80.9	144.8 ± 91.6	132.9 ± 71.4	136.7 ± 74.3	0.852
HDL-C (mmol/L)	49.9 ± 11.3	51.9 ± 15.1	53.0 ± 13.8	52.6 ± 14.8	0.719

Data are presented as means ± standard deviations or *n* (%).
Abbreviations: *n*, number of cases; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycohemoglobin.
* Indicates that *p* value < 0.005.
** Indicates that *p* value < 0.001.

Table 3
Baseline nerve conduction study and cardiovascular autonomic study with Type 2 diabetes stratified by ascending quartiles of duration of diabetes.

	1st duration Quartiles (n = 42)	2nd duration Quartiles (n = 70)	3rd duration Quartiles (n = 60)	4th duration Quartiles (n = 66)	P-value for trend
Cardiovascular autonomic study					
Composite autonomic scoring scale	1.4 ± 0.8	1.1 ± 1.0	1.9 ± 1.5	1.8 ± 1.1	0.022*
HR_DB (beats/min)	8.6 ± 5.1	8.4 ± 4.8	6.9 ± 5.0	6.1 ± 4.1	0.013*
Valsalva ratio	1.4 ± 0.3	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.1	<0.0001***
Baroreflex sensitivity	1.9 ± 1.3	1.8 ± 1.2	1.3 ± 0.8	1.2 ± 0.9	0.003**
Percentage of CAN, n (%)	12 (28.6)	16 (22.9)	27 (45.0)	31 (50.0)	0.007*
Nerve conduction study					
Composite score of nerve conduction	3.0 ± 2.1	4.1 ± 3.0	4.4 ± 3.0	6.5 ± 2.9	<0.0001***
Sural nerve SNCV (m/s)	47.6 ± 7.7	47.5 ± 5.2	47.4 ± 6.4	46.0 ± 7.2	0.664
Sural nerve SNAP (μV)	10.1 ± 7.6	7.3 ± 5.5	6.9 ± 5.8	4.0 ± 2.9	<0.0001***
Percentage of DSPN, n (%)	16 (38.1)	32 (45.7)	31 (51.7)	52 (78.8)	<0.0001***

Data are presented as means ± standard deviations or n (%).
Abbreviations: n, number of cases; SNAP = sensory nerve action potential; SNCV = sensory nerve conduction velocity; HR_DB, heart rate response to deep breathing; DSPN, diabetic distal symmetrical polyneuropathy; CAN, cardiac autonomic neuropathy.
* Indicates that p value < 0.05.
** Indicates that p value < 0.005.
*** Indicates that p value < 0.0001.

Table 4
Correlation analysis of parameters of cardiovagal functions in patients with type 2 diabetes.

Variables	HR_DB		Valsalva ratio		Baroreflex sensitivity	
	r	P value	r	P value	r	P value
Diabetes duration (year)	−0.217	0.001**	−0.302	<0.0001***	−0.249	0.001**
Age	−0.147	0.027*	−0.281	<0.0001***	−0.119	0.114
BMI	0.007	0.913	−0.016	0.138	0.076	0.315
Composite autonomic scoring scale	−0.684	<0.0001***	−0.481	<0.0001***	−0.465	<0.0001***
Sural nerve SNCV (m/s)	0.122	0.12	0.163	0.062	0.118	0.173
Sural nerve SNAP (μV)	0.432	<0.0001***	0.353	<0.0001***	0.341	<0.0001***
Composite score of nerve conduction study	−0.426	<0.0001***	−0.303	<0.0001***	−0.343	<0.0001***

r: correlation coefficient.
Abbreviations: SNAP, sensory nerve action potential; n, number of cases; SNAP = sensory nerve action potential; SNCV = sensory nerve conduction velocity; HR_DB, heart rate response to deep breathing.
* Indicates that p value < 0.05.
** Indicates that p value < 0.001.
*** Indicates that p value < 0.0001.

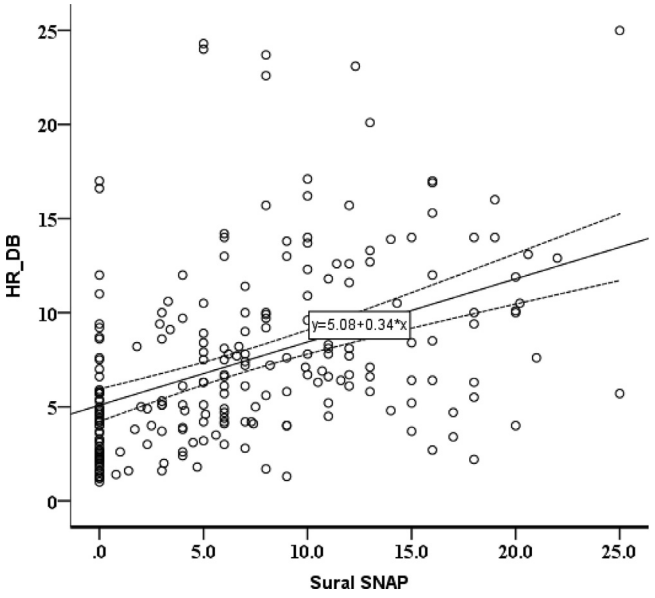

Fig. 1. Relationship between sural nerve action potential and heart rate to deep breathing in patients with Type 2 diabetes after adjustment for age, gender, BMI and diabetes duration.

were independently associated with mean HR_DB. The multiple linear regression formula was calculated as follows: HR_DB = 5.082 + 0.336× (Sural nerve SNAPs).

3.5. Sensitivity, specificity, and area under the curve for sural SNAP, HR-DB, VR and BRS in diagnosing CAN, by ROC curve analysis

The area under the ROC curve for sural SNAP, HR-DB, VR and BRS values in the presence of CAN were 0.737 (P < 0.0001, 95% CI = 0.658–0.817), 0.866 (P = 0.002, 95% CI = 0.81–0.922), 0.764 (P = 0.001, 95% CI = 0.689–0.938), and 0.72 (P < 0.0001, 95% CI = 0.638–0.801), respectively (Table 6). The cut-off value of sural SNAP, HR-DB, VR and BRS values in the presence of CAN were 5.5 (sensitivity 71.3% and specificity 69.4%), 6.0 (sensitivity 80.2% and specificity 72.6%), 1.23 (sensitivity 72.3% and specificity 64.5%), and 1.15 (sensitivity 63.4% and specificity 62.9%), respectively (Fig. 2). In the first quartiles of duration of diabetes, the cut-off value of sural SNAP in the presence of DSPN and CAN were 6.5 (sensitivity 100% and specificity 93.7%) and 7.65 (sensitivity 75.9% and specificity 753%, P < 0.0001), respectively.

4. Discussion

4.1. Major findings of our study

Our study confirms the hypothesis that sural SNAP was closely correlated with parameters of cardiovagal functions in patients with different durations of diabetes.

We examined the relationship between sural SNAP and parameters of cardiovagal functions in patients with different durations of diabetes and discovered five major findings. First, those with a longer duration of diabetes had a higher percentage of proteinuria

Table 5
Multiple regression analysis for association of heart rate response to deep breathing with the sural nerve SNAP and the variables on in patients with type 2 diabetes according to correlation analysis.

	Model 1			Model 2		
	Regression coefficient	Standard error	P value	Regression coefficient	Standard error	P value
Constant	8.39	2.03	<0.0001	5.082	0.438	<0.0001
Sural nerve SNAP (μV)	0.313	0.05	<0.0001*	0.336	0.447	<0.0001*
Diabetes duration (year)	−0.039	0.041	0.348			
Age	−0.043	0.032	0.183			

Regression coefficient for each individual variable. Abbreviations: SNAP = sensory nerve action potential.
* Indicates that p value < 0.0001.

Table 6
Sensitivity, specificity and areas under the curves for Sural SNAP, HR-DB, VR and BRS in diagnosing CAN, by ROC curve analysis.

	AUC (95% CI)	Cut-off value	Sensitivity (%)	Specificity (%)	*P*-value
Sural nerve SNAP§	0.737 (0.658–0.817)	5.5	71.3%	69.4%	<0.0001
HR_DB (beats/min)	0.866 (0.81–0.922)	6.0	80.2%	72.6%	<0.0001
Valsalva ratio	0.764 (0.689–0.938)	1.23	72.3%	64.5%	<0.0001
Baroreflex sensitivity	0.72 (0.638–0.801)	1.15	63.4%	62.9%	<0.0001

Data are presented as means ± standard deviations or n (%). Abbreviations: n, number of cases; AUC, area under the curve; SNAP=sensory nerve action potential; ; HR_DB, heart rate response to deep breathing; VR, Valsalva ratio; BRS, Baroreflex sensitivity; CAN, cardiac autonomic neuropathy; § = In the first quartiles of duration of diabetes, the cut-off value of sural SNAP on presence of DSPN and CAN was 6.5 (sensitivity 100% and specificity 93.7%) and 7.65 (sensitivity 75.9% and specificity 753%, $P < 0.0001$), respectively.

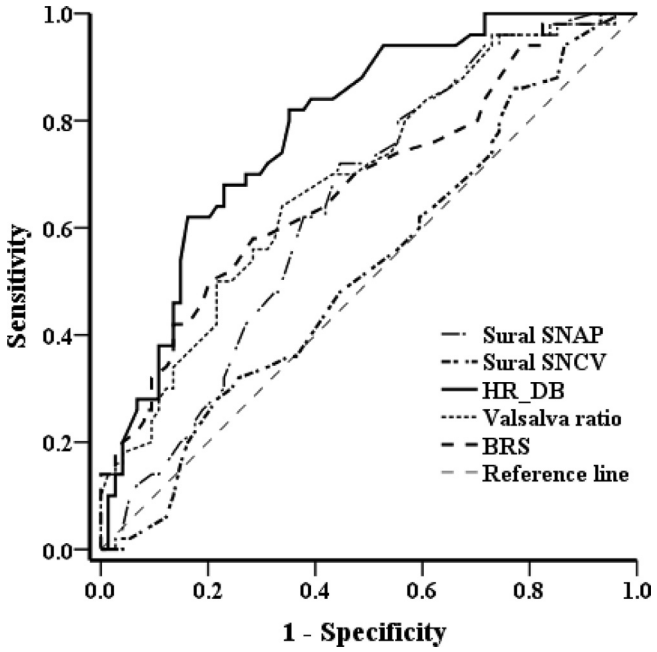

Fig. 2. The receiver operator characteristic curve for presence of cardiac autonomic neuropathy in type 2 diabetes.

and were older. Second, those patients with a longer duration of diabetes had lower values of parameters of cardiovagal functions including HR-DB, VR and BRS, and a higher severity and percentage of CAN. Third, those patients with a longer duration of diabetes had lower sural SNAP, and a higher severity and percentage of DSPN. Fourth, sural SNAP was positively correlated with parameters of cardiovagal functions including HR_DB and VR after controlling for age, sex, BMI, and diabetes duration. Finally, multiple linear regression analysis showed that only sural SNAPs were independently associated with mean HR_DB and the formula was calculated as follow: HR_DB = 5.082 + 0.336× (Sural nerve SNAPs).

The independent association between Sural SNAP amplitudes and HR_DB results in patients from earlier to longer diabetes duration is the most novel finding of the study. Besides the natural course of diabetes duration, the increased pathology load could also be a result of treatment paradigms. Aggressively control blood glucose to an acceptable range and avoid blood glucose fluctuations by individualized treatment to prevent further nerve damage (Perkins et al., 2001; Su et al., 2018).

4.2. Does CAN precede DSPN in type 2 diabetes, or is it the other way around?

One important issue that should be addressed in DSPN and CAN is whether CAN precedes DSPN in type 2 diabetes, or if it happens the other way around. In the first quartiles of duration of diabetes, the cut-off value of sural SNAP in the presence of DSPN and CAN were 6.5 (sensitivity 100% and specificity 93.7%) and 7.65 (sensitivity 75.9% and specificity 75.3%, $P < 0.0001$), respectively. The cut-off value of sural SNAP in the presence of CAN is higher than those of DSPN in the first quartiles of the duration of diabetes; therefore, CAN may precede DSPN in type 2 diabetes in its early stages according to ROC analysis. The results of ROC analysis can be influenced by the diagnostic criteria of DSPN and CAN, the sensitivity and specificity and the study cases; therefore, it cannot draw a conclusion based on the study results.

4.3. The efficiency and limitations of cardiovagal function parameters in cardiac arrhythmia

Another issue that should be addressed concerns the validity of cardiovagal function parameters in predicting CAN in those patients who have cardiac arrhythmia. Heart rate variability (HRV) is considered to represent a noninvasive tool to assess cardiac autonomic tone. It can be assessed either by calculation of indices, which are based on statistical operations on RR intervals (time-domain analysis) or spectral analysis of an array of RR intervals (frequency-domain analysis). With respect to the clinical application of HRV regarding arrhythmia risk stratification, both parameters of HRV appear to provide similar information. However, power spectrum analyses are extremely sensitive to the presence of arrhythmias, need very stable recording conditions and usually cope with only relatively short segments of recording (Hohnloser et al., 1997, Zuanetti et al., 1991). Our study demonstrated that a close relationship between HR_DB and sural SNAP

may be an alternative sign to the diagnosis of CAN, especially for those patients whose HR_DB cannot be a valid tool (e.g., for those with persistent cardiac arrhythmia or cardiac pacemaker rhythm).

4.4. Study limitations

This study has several limitations. First, although the value of sural SNAP is closely correlated with parameters of cardiovagal functions in patients with different durations of diabetes in this cross-sectional observational study, it is unclear whether the role of the association is causal. A prospective longitudinal study following a standard pattern and temporal relationship is necessary to evaluate the relationship between parameters of cardiovagal functions and sural SNAP. Second, both autonomic nerve dysfunction and cardiomyopathy might result in a reduced HR_DB. We excluded those patients who had a moderate-to-severe heart failure (NYHA class III and IV) and any type of arrhythmia that prevent analysis of heart rate variability, or pacemaker implantation due to any cause, in accordance with our study protocols. Thus, continued uncertainty was present in assessing the incidence of CAN in non-selected patients of diabetes. Third, one important issue that should be addressed in DSPN and CAN is whether CAN precedes DSPN in type 2 diabetes, or whether it happens the other way around. Evaluating the feasibility of a one-stop microvascular screening service for the early diagnosis of DSPN and CAN at a diabetes outpatient clinic, should be taken into account for future studies.

5. Conclusion

Our study confirms the hypothesis that sural SNAP was closely correlated with parameters of cardiovagal functions in patients with different durations of diabetes. Those patients with a longer duration of diabetes had both a higher severity and percentage of CAN and DSPN. The independent association between Sural SNAP amplitudes and HR_DB results is the most novel finding of the study. If a patient's SNAP value is below 5.5, we should be cautious about the presence of CAN, especially for those patients whose HR_DB cannot be a valid tool (e.g., for those with a persistent cardiac arrhythmia or cardiac pacemaker rhythm).

Acknowledgements

The authors thank all the subjects who participated in this study.

Competing interests

The authors declare that they have no competing interests.

Ethical approval and consent to participate

This study conformed to the guidelines of the Declaration of Helsinki, and the study has been approved by the Institutional Review Board of Chang Gung Memorial Hospital (201701243B0 and 201800388B0C501).

Funding

This work was supported by grants from Chang Gung Memorial Hospital (Chang Gung Medical Research Project CMRPG8H0501).

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