Original Article

Age and Disease Duration Independent Cardiac Autonomic Neuropathy in Patients with Diabetic Foot Complications: Case-Control Study

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Abstract

Introduction: Cardiac autonomic neuropathy (CAN) in people with diabetes is associated with high mortality. We aimed to study age and disease duration, independent prevalence of CAN in people with diabetic foot complications. **Methods:** 530 patients with diabetes were screened to undergo CAN assessment (automated CANS-analyser). CAN was defined as "early", "definite," or "severe" according to the Toronto consensus. History pertaining to autonomic symptoms, prior cardiovascular events (CVE), and assessment for peripheral neuropathy was done. Participants were grouped into those with diabetic foot complication (group A, $n = 82$) [Charcot foot ($n = 42$), diabetic foot ulcer ($n = 40$)]; with DPN without foot complications (group B, $n = 82$); and without DPN or foot complications (group C, $n = 82$). **Results:** Symptoms of autonomic dysfunction were prominent in people with foot complications than the other groups. Resting heart rate was significantly greater in those with foot complications [99.89 ± 26.71 (group A) vs. 86.99 ± 22.24 (group B) vs. 88.32 ± 17.08 (group C); $P = 0.001$]. The prevalence of CAN was 75.6% in group A (51.2% early, 12.2% definite, 12.2% severe), 57.2% in group B (45.1% early, 12.2% severe) and 58.5% in group C (43.9% early, 1.2% definite, 13.4% severe) ($P = 0.002$). Patients with foot complications were more likely to have CAN (75.6% vs. 57.9%, $P < 0.001$). Charcot foot had higher prevalence of CAN (78.6%) as compared with those with DFU (72.5%) or without DFU or DPN (57.9%), $P < 0.001$. **Conclusion:** CAN is present in more than two-third of patients with diabetes and foot complications with highest prevalence in Charcot neuroarthropathy.

Keywords: Cardiac autonomic neuropathy, Charcot neuroarthropathy, diabetic foot, diabetic foot ulcer, diabetic peripheral neuropathy, mortality

Introduction

Diabetes is one of the most prevalent noncommunicable diseases with significant health implications. Diabetes also significantly contributes to mortality predominantly secondary to cardiovascular complications. Many patients with diabetes have a silent myocardial ischemia that might go unnoticed and contribute to mortality. One of the most common reasons for silent MI in these individuals is the presence of cardiac autonomic neuropathy (CAN). The presence of CAN is considered as a predictor of cardiovascular morbidity and mortality in both type 1 and type 2 diabetes.[1,2] CAN provides additional prognostic information for death and/or cardiac events that might not be captured by perfusion imaging or other invasive investigative modalities.[3] A metaanalysis of 15 studies found relative mortality risk of 3.65 in patients of diabetes with CAN.[4] CAN may also be associated with recurrent cardiovascular events (CVE) and stroke in patients of type 2 DM. There is also an association between cardiac autonomic neuropathy and nephropathy progression in type 2 DM.[5]

The symptoms related to CAN are nonspecific including palpitations, light-headedness, dizziness, blurred vision that

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contribute to under evaluation in busy clinics. In addition, tests and definition for diagnosing CAN have not been uniformly used along with a considerable heterogeneity related to age, duration of diabetes, prevalent microvascular complications amongst the included participants. Overall, the prevalence of CAN has been variably reported from 10%–80%.[6-10] Most of the studies are consistent regarding close association of diabetic peripheral neuropathy and CAN. There is being an increasingly recognized association between peripheral neuropathy and CAN in patients of type 2 DM.[11] It is seen that all cases of type 2 DM having distal peripheral neuropathy (DPN) had different grades of CAN and more than 50% of patients had severe autonomic neuropathy.[11,12]

Patients of diabetes with DPN are prone for foot complications including diabetic foot ulcer (DFU) and Charcot neuroarthropathy (CN) of foot that is also associated with high morbidity and mortality.[13-15] We identified that diabetic patients with CN have a 1.62-fold higher risk of mortality compared with individuals with diabetes but not CN.[13] Cardiovascular events were the most common cause of death (30%) followed by renal failure (25.6%). A significant number were unexplained deaths that could possibly be associated with CAN as amongst the patients who died during follow up; peripheral neuropathy was found to be more prevalent in patients with CN than controls. Thus, it is prudent to assess the prevalence of CAN in patients with foot complications considering the nonspecific presentation of CAN and an increased risk of mortality in patients with CAN and/or DFU or Charcot foot. Studies pertaining to prevalence of CAN amongst diabetic patients with foot complications matched for age and duration of diabetes compared with those without foot complications are sparse.

The present study was planned to study the prevalence of CAN in patients with diabetes mellitus having foot complications including neuropathic foot ulcers or CN and corroborate the high prevalence of CAN amongst these individuals compared with those without foot complications or peripheral neuropathy.

Methods

This is a cross-sectional study conducted amongst patients with diabetes mellitus attending outpatient facility at tertiary care center in North India. Patients who were able to understand and provide an informed signed consent and perform maneuvers like Valsalva and hand grip required for CAN assessment were invited to participate in the study. Patients with prior amputations, features of overt hypothyroidism or hyperthyroidism, active congestive heart failure (CHF), recent acute coronary syndrome (ACS), hospitalized for CHF (<3 months), h/o rhythmic disturbances of electrical activity of heart, (e.g., atrial fibrillation, undergone PCI, TPI, or PPI insertion), LVAD placement within last 3 months, undergone cardiac resynchronization therapy in past, ankle brachial index (ABI <0.9), alcohol consumers (intake >80 mgs/day), anemia, (hemoglobin <10 gm/dL), other illness, or autoimmune disease that may affect autonomic nerve fibers like systemic lupus erythematosus, coexisting degenerative disease, (e.g., Parkinson disease or multiple system atrophy), h/o panic attacks, on medications that could affect heart rate such as beta blockers, verapamil, diltiazem, amiodarone, or nitrates, chemotherapeutic agents, chronic obstructive pulmonary diseases, pregnant and lactating women were excluded from the study. A written and informed consent was obtained from all the participants and the study protocol was approved by the Institute Ethics Committee on 9th November 2020, reference number NK/6055/MD/832.

Study population was subsequently divided into three groups namely those having Charcot's foot and/or neuropathic foot ulcer with distal symmetric peripheral neuropathy (DSPN) (group A), those with DSPN but no foot complications (group B), and those having neither DSPN or foot complication who were matched for the age and duration of diabetes (group C). Relevant history regarding autonomic symptoms, presence of any other comorbidities, microvascular complications of diabetes, treatment history and compliance was obtained. Before objective assessment of diabetic neuropathy and CAN, a questionnaire-based composite Autonomic Symptom Score 31 (COMPASS 31) score was administered for assessing symptoms severity in all the participants. The COMPASS-31 is modified version of expanded COMPASS. The COMPASS-31 consists of six domains (orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, pupillomotor) with 31 items (questions) and provides a total score (range 0–100).[16] A thorough neurological examination including foot examination was performed. Ankle Brachial Index (ABI) was measured by automated noninvasive vascular screening device (BP—203RPE111), obtaining simultaneous recordings of blood pressure in all 4 limbs based on oscillometric method.

All included participants underwent cardiac autonomic function testing using CANS (Cardiac Autonomic Neuropathy System Analyzer, CANS 0504, Diabetik Foot India Ltd, Chennai, India). The instrument uses automated non-invasive blood pressure measurement system. The instrument was regularly calibrated after every 10 tests as per protocol. Participants were advised to avoid tea/coffee, heavy meal intake prior to 8 h before testing and avoid alcohol intake the night before the test. Participants lied down supine with eyes closed for 15 min before the start of procedure.

Procedures

- Baseline blood pressure (BP), heart rate (HR), and heart rate variability was obtained with 60 s ECG in supine position.
- HR response to standing: Patient was asked to stand for 60 s. R–R interval was recorded at 15th s and 30th s after standing. R–R interval of 30:15 s ratio was calculated. (Parasympathetic function)
- HR variation with deep breathing: Patient was instructed to breath slowly and deeply, at a rate of about six breaths per minute—5 s each for inspiration and

expiration. Maximum and minimum R–R interval are calculated as E: I ratio and E–I difference was calculated. (Parasympathetic function)
- HR response to Valsalva maneuver: Participants were asked to exhale forcefully against open glottis into the manometer with closed nostrils (with help of nose clips) to maintain a pressure of minimum 40 mmHg for 5 s. ECG was recorded from 30 s before till 60 s after the procedure (Parasympathetic function).
- Systolic BP variation during standing: Patient was asked to stand for 120 s. Systolic BP was measured after the above period in standing position (Sympathetic function).
- BP variability after sustained isometric hand grip: We asked the patient to maintain 30% of maximum hand grip from manufacture supplied hand dynamometer for a period of 60 s. BP was recorded after sustained handgrip in contra lateral arm with an expected response being an increase in diastolic BP (DBP) more than 15 mmHg (Sympathetic function).

Interpretation of the tests

- Resting Heart rate—Resting HR of more than 100 beats per minute was considered abnormal.
- Heart rate response to standing: Ratio of >1.04 was considered normal and <1 is as abnormal
- Heart rate variation with deep breathing: Maximum of 6 HR differences was taken as deep breathing difference. Difference in heart rate of more than 15 beats per min is normal and <10 beats per minute is abnormal. E: I ratio is calculated; >1.21 is normal and <1.1 is abnormal.
- HR response to Valsalva maneuver: Healthy subjects develop tachycardia and peripheral vasoconstriction during strain and bradycardia with rise in BP after stoppage of maneuver. The ratio of longest to shortest R–R interval was obtained and the ratio >1.21 was considered normal and <1.21 as abnormal.
- SBP variation with standing—Fall in systolic BP up to 10 mm is expected in a normal person. Fall in SBP of more than 20 mmHg is abnormal.
- DBP response to isometric hand grip—Difference in DBP after release & before beginning of handgrip. An increase in DBP >16 mmHg was considered normal

Definitions: CAN was labelled according to the Toronto Consensus Panel on Diabetic Neuropathy as "early CAN" if any one of parasympathetic tests was abnormal or two of them being borderline, "definite CAN" in the presence of two abnormal parasympathetic tests and "severe CAN" in those participants with definite CAN and an additional postural fall in blood pressure or abnormal dynamic grip using Ewing's criteria.[17] However, "extended criteria for CAN" was also considered that included the resting heart rate >100/min in addition to the above Ewing's criteria.

Statistical analysis

Assuming an overall effect size of 20% in three groups with alpha value of 5% and power of 80%, total sample size calculated was 246 which was divided in the three groups (82 each). This is calculated by using G* Power 3.1.42 software. Data was entered in an excel spreadsheet. Quantitative variables are expressed as means ± SD, or median (inter quartile range), as appropriate. All the categorical variables are expressed in the form of proportions or percentages. Kolmogrov–Smironov test was applied to test the normality assumption for continuous variables. ONE way ANOVA was applied for between group comparisons for normally distributed variables and Kruskal–Wallis test for nonnormally distributed variables. Consequently, post hoc comparisons were done as applicable. Categorical variables were analyzed using Chi χ^2 or Fisher exact test, as applicable. Univariate and multivariate logistic regression analysis was applied to assess the risk factors depending on the baseline characteristics (co-variates) like duration of diabetes, HbA1C, smoking, hypertension, hypertriglyceridemia, presence of DPN that could affect the presence of CAN. Statistical analysis was done using SPSS software (v22.0, SPSS Inc: USA), and *P* value less than 0.05 was considered as statistically significant.

Results

A total of 530 patients of diabetes were screened, out of which 246 patients (82 patients in each of the three groups) were included in the study. The mean age of the participants was 53.8 ± 12 years and duration of diabetes 11.5 ± 7.3 years. Among patients with diabetic foot complications, 42 participants had (51.2%) chronic CN and 40 participants had prior or active DFU. Symptoms pertinent to CAN were found in 14.6% (postural dizziness) and 24% (palpitations) patients of foot complications. However, symptoms suggestive of autonomic neuropathy were prominent in people with foot complications including palpitations ($P < 0.001$), postural dizziness ($P < 0.001$), heart burn ($P = 0.046$), urinary incontinence ($P = 0.011$) or loss of sweating ($P < 0.001$) and gustatory sweating ($P = 0.005$) than the other two group [Table 1]. Patients of group A had higher COMPASS-31 score compared to the other groups ($P < 0.01$). Resting heart rate was significantly greater in group A compared with other groups [99.89 ± 26.71 (group A) vs. 86.99 ± 22.24 (group B) vs. 88.32 ± 17.08 (group C); $P = 0.001$]. Palpitations, postural dizziness and gustatory sweating were more common in participants with DFU or CN compared with complications without foot. The individual parameters of objective CAN testing in the three group are shown in supplementary Table S1. Parasympathetic dysfunction (resting tachycardia) was the most common abnormality noticed in those with CAN followed by abnormal RR ratio on breathing. Abnormal response of DBP on hand grip test was the most common sympathetic abnormality noticed on CAN testing. A sub group analysis of comparison of baseline parameters, symptoms and objective CAN assessment in those with CN compared to DFU or without foot complications is provided in Table S2.

Overall, the prevalence of CAN by standardized Ewing's battery was 59.8% in group A (51.2% early, 3.7% definite,

Table 1: Baseline characteristics of the study population

Parameters	Group			*P*
	Diabetic Foot Complications (*n*=82)	Diabetes With Peripheral Neuropathy (*n*=82)	Diabetes Without Peripheral Neuropathy (*n*=82)	
Age (Years)	55.06±8.42	57.29±9.92	56.09±14.19	<0.083
BMI (kg/m²)	26.18±3.40	26.9±3.19	27.01±4.12	0.171
Gender				0.363
Male	56 (68.3%)	55 (67.1%)	48 (58.5%)	
Female	26 (31.7%)	27 (32.9%)	34 (41.5%)	
History: CVA (Present)	0 (0.0%)	4 (4.9%)	0 (0.0%)	0.035
History: CHF (Present)	4 (4.9%)	7 (8.5%)	1 (1.2%)	0.104
History: CAD (Present)	10 (12.2%)	2 (2.4%)	1 (1.2%)	0.006
History: Hypothyroidism (Present)	8 (9.8%)	15 (18.3%)	15 (18.3%)	0.218
History: Hypertension (Present)	54 (65.9%)	64 (78.0%)	38 (46.3%)	<0.001
History: Alcohol Use (Present)	20 (24.4%)	26 (31.7%)	18 (22.0%)	0.333
History: Smoking (Present)	8 (9.8%)	5 (6.1%)	3 (3.7%)	0.281
Palpitations (Present)	20 (24.4%)	4 (4.9%)	4 (4.9%)	<0.001
Postural Dizziness (Present)	12 (14.6%)	0 (0.0%)	2 (2.4%)	<0.001
Epigastric fullness (Present)	14 (17.1%)	11 (13.4%)	4 (4.9%)	0.046
Diarrhea (Present)	5 (6.1%)	7 (8.5%)	3 (3.7%)	0.427
Early Satiety (Present)	11 (13.4%)	13 (15.9%)	8 (9.8%)	0.505
Bloating (Present)	26 (31.7%)	28 (34.1%)	15 (18.3%)	0.052
Abdominal Pain (Present)	5 (6.1%)	6 (7.3%)	3 (3.7%)	0.696
Increased Frequency of Urination (Present)	22 (26.8%)	30 (36.6%)	18 (22.0%)	0.107
Urinary Incontinence (Present)	5 (6.1%)	0 (0.0%)	0 (0.0%)	0.011
Erectile Dysfunction (Present)	4 (7.0%)	1 (1.8%)	2 (3.8%)	0.402
Dysuria (Present)	8 (9.8%)	11 (13.4%)	8 (9.8%)	0.688
Loss of Sweating (Present)	44 (53.7%)	22 (26.8%)	10 (12.2%)	<0.001
Gustatory Sweating (Present)	21 (25.6%)	12 (14.6%)	6 (7.3%)	0.005
Glossy Skin (Present)	0 (0.0%)	3 (3.7%)	1 (1.2%)	0.328
Systolic BP (mmHg)	143.23±23.55	142.74±18.99	131.01±14.17	<0.001
Diastolic BP (mmHg)	83.51±7.93	82.46±9.93	79.20±8.57	0.003
Pulse (BPM)	91.20±13.08	85.32±16.24	86.49±14.26	0.006
Respiratory Rate (CPM)	14.11±1.21	15.91±1.65	16.20±1.27	<0.001
Temperature (F)	98.44±0.12	98.37±0.22	98.37±0.20	0.036
COMPASS 31	22.12±7.80	18.31±6.98	10.43±4.12	<0.01
Hemoglobin (g/dL)	11.89±1.15	12.03±0.86	12.48±1.18	0.054
FPG (mg/dL)	145.65±56.58	140.12±46.37	130.45±35.37	0.300
PPG (mg/dL)	219.40±71.82	218.71±79.14	192.89±56.03	0.019
HbA1c (%)	8.85±1.90	8.31±1.66	8.00±1.81	0.002
Vitamin D3 (ng/mL)	22.79±15.38	20.84±17.51	18.83±7.80	0.918
T3 (ng/dL)	1.00±0.21	1.93±1.84	1.97±1.40	0.065
T4 (µg/dL)	7.53±1.51	7.24±1.86	9.05±9.02	0.083
TSH (µIU/mL)	3.16±1.87	4.89±5.86	3.96±4.05	0.058
HDL (mg/dL)	42.86±9.06	43.76±9.34	44.88±9.92	0.164
LDL (mg/dL)	81.06±27.66	76.04±31.54	82.54±29.25	0.144
TG (mg/dL)	138.85±64.84	151.95±58.35	144.88±73.12	0.065
Blood Urea (mg/dL)	35.25±11.83	34.38±11.82	29.13±7.72	0.001
Creatinine (mg/dL)	1.01±0.29	1.46±3.87	0.82±0.23	<0.001
AST (IU/L)	24.67±12.64	26.10±12.71	26.64±10.75	0.098
ALT (IU/L)	27.41±23.39	29.81±16.73	30.98±19.12	0.100
ALP (IU/L)	112.47±44.61	107.22±49.17	100.19±34.91	0.144
S. Bilirubin (mg/dL)	0.53±0.25	0.78±0.76	0.66±0.26	0.071
24 Hr Urinary Protein (mg)	923.3 (203.1-1367.9)	713.62 (213.8-1032.1)	119.4 (43.29-208.3)	<0.001
Duration of DM (Years)	13.59±6.97	13.65±7.89	11.30±5.03	<0.067
eGFR (mL/min/1.73 m²)	79.95±23.84	80.48±24.82	92.71±40.01	<0.001

Contd...

Table 1: Contd...

Parameters	Group			*P*
	Diabetic Foot Complications (*n*=82)	Diabetes With Peripheral Neuropathy (*n*=82)	Diabetes Without Peripheral Neuropathy (*n*=82)	
S.Albumin (g/dL)	3.83±0.47	3.92±0.43	3.98±0.39	0.081
VPT (Right)	42.94±12.26	31.20±12.66	10.72±2.09	<0.001
VPT (Left)	43.40±12.11	30.27±12.79	10.59±2.01	<0.001
ABI (Right)	1.14±0.10	1.16±0.04	1.18±0.04	<0.001
ABI (Left)	1.12±0.09	1.15±0.08	1.19±0.04	<0.001

P<0.05 considered significant. ABI: Ankle CAD: Coronary artery Disease; CHF: Congestive Heart Failure; CVA: Cerebral vascular accident; eGFR: estimated glomerular filtration rate; FPG: fasting plasma glucose; HDLc: High density lipoprotein cholesterol; LDLc: Low density lipoprotein cholesterol; PPG: Post-prandial plasma glucose TG: triglycerides; VPT: Vibration perception threshold

4.9% severe), 45.1% in group B (40.2% early, 4.9% severe), and 48.8% in group C (42.7% early, 2.4% definite, 3.7% severe) (P = 0.380). However, when resting heart rate was included with Ewing's battery (extended criteria), the prevalence of CAN was 75.6% in group A (51.2% early, 12.2% definite, 12.2% severe), 57.2% in group B (45.1% early, 12.2% severe), and 58.5% in group C (43.9% early, 1.2% definite, 13.4% severe) (P = 0.002). [Figure 1] Participants with foot complications had higher prevalence of definite or severe CAN using extended criteria (24.4% vs. 12.2% and 14.6% in three groups, respectively; P = 0.012) or Ewing's battery (8.5% vs. 4.9% vs. 6.1% in three groups, respectively, P = 0.042) [Figure 2]. Amongst all participants with definite CAN, 90.9% (10/11) had diabetic foot complications. However, early CAN was more commonly observed in those without foot complications [63.5% (73/115); P < 0.001]. Parasympathetic function of resting heart rate and sympathetic function parameter of postural fall in SBP were more likely to be abnormal in those with foot complications than those without foot complications [Table S2].

Correlation of early and definite CAN with baseline parameters

A history of diabetic foot complications including charcot foot or prior foot ulcer (P < 0.001), and presence of urinary incontinence (P = 0.007) was significantly associated with the presence of CAN. Examination findings of higher resting pulse rate (P = 0.007) and laboratory parameter of higher urinary protein excretion (P = 0.045) corelated with presence of CAN as shown in Table 2.

Regression analysis for predictors of CAN

The prevalent HbA1c [OR 1.24 (1.01–1.53), P = 0.044] and the presence of abnormal vibration perception threshold [OR 1.17 (1.01–1.35), P = 0.035] were identified as predictors of CAN amongst patients with diabetes. The age of the participant and the duration of diabetes were not shown to be significantly associated with CAN as shown in Table 3.

Sub-group analysis of prevalence of CAN

We observed that patients with foot complications were more likely to have CAN [75.6% vs. 57.9%, P < 0.001) including definite and severe CAN (24.4% vs. 13.4%, P < 0.001)] than diabetic patients without foot complications, respectively.

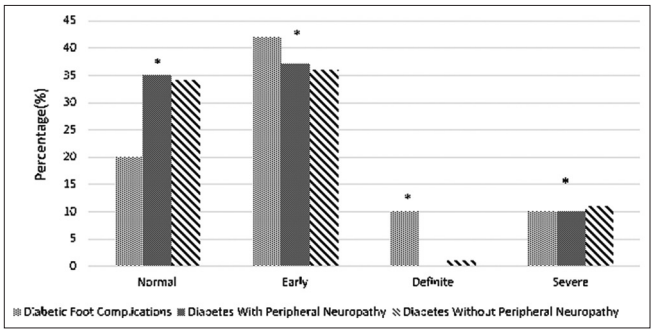

Figure 1: Prevalence of cardiac autonomic neuropathy in three groups. * P < 0.05 (intergroup)

Participants with Charcot foot were more likely to have CAN (78.6%) as compared to those with DFU (72.5%) or without DFU or DPN (57.9%), P < 0.001 using the extended criteria [Figure 3] [Table S2]. Definite CAN was observed in 16.7% of participants with CN compared to 7.5% with DFU or 0.6% without foot complications (P < 0.001). Amongst various CAN parameters, those with CN had higher resting heart rate (parasympathetic) and significant postural fall in SBP (sympathetic) compared to those with or without DFU (P = 0.005). There was no difference in prevalence of other abnormal CAN tests in patients with or without CN [Table S2].

Discussion

The present observational cohort study found that more than three-fourth of patients with diabetic foot complications and peripheral neuropathy have CAN as defined by extended Ewing's criteria that included resting heart rate. Patients with Charcot neuroarthropathy were more likely to have underlying CAN particularly definite or severe CAN as compared to those with or without diabetic peripheral neuropathy. Interestingly, more than half of patients without foot complications also had CAN when matched for age and duration of diabetes suggesting the need of routine evaluation for CAN in people with diabetes.

The clinical manifestations of CAN are myriad and occult that results in limited evaluation for CAN in diabetic patients. A careful history may be helpful in eliciting symptoms

Table 2: Correlation of early or definite CAN (Extended Ewing's criteria) with demographic and baseline parameters

Parameters	CANS			*P*
	Normal (*n*=89)	Early (*n*=115)	Definite (*n*=11)	
Age (Years)	53.04±12.94	54.47±11.31	55.55±4.16	0.873
Age				0.574
≤20 Years	2 (2.2%)	2 (1.7%)	0 (0.0%)	
21-30 Years	3 (3.4%)	0 (0.0%)	0 (0.0%)	
31-40 Years	9 (10.1%)	8 (7.0%)	0 (0.0%)	
41-50 Years	20 (22.5%)	28 (24.3%)	1 (9.1%)	
51-60 Years	29 (32.6%)	46 (40.0%)	8 (72.7%)	
61-70 Years	20 (22.5%)	24 (20.9%)	2 (18.2%)	
71-80 Years	5 (5.6%)	6 (5.2%)	0 (0.0%)	
81-90 Years	1 (1.1%)	0 (0.0%)	0 (0.0%)	
>90 Years	0 (0.0%)	1 (0.9%)	0 (0.0%)	
Gender				0.091
Male	56 (62.9%)	79 (68.7%)	4 (36.4%)	
Female	33 (37.1%)	36 (31.3%)	7 (63.6%)	
History of CVA (Present)	0 (0.0%)	4 (3.5%)	0 (0.0%)	0.299
History of CHF (Present)	4 (4.5%)	8 (7.0%)	0 (0.0%)	0.768
History of CAD (Present)	4 (4.5%)	5 (4.3%)	1 (9.1%)	0.613
Hypothyroidism (Present)	17 (19.1%)	12 (10.4%)	2 (18.2%)	0.203
Hypertension (Present)	55 (61.8%)	75 (65.2%)	7 (63.6%)	0.881
Current alcohol user	21 (23.6%)	33 (28.7%)	4 (36.4%)	0.554
Current smoking	4 (4.5%)	6 (5.2%)	1 (9.1%)	0.643
Diabetic Foot Complication (Present)	20 (22.5%)	42 (36.5%)	10 (90.9%)	<0.001*
Foot Complications				<0.001*
Charcots Foot	9 (10.1%)	22 (19.1%)	7 (63.6%)	
Diabetic Foot Ulcer	11 (12.4%)	20 (17.4%)	3 (27.3%)	
None	69 (77.5%)	73 (63.5%)	1 (9.1%)	
Palpitations (Present)	6 (6.7%)	16 (13.9%)	2 (18.2%)	0.204
Postural Dizziness (Present)	3 (3.4%)	8 (7.0%)	1 (9.1%)	0.316
Heart Burn (Present)	10 (11.2%)	14 (12.2%)	1 (9.1%)	0.944
Recurrent diarrhea (Present)	6 (6.7%)	7 (6.1%)	0 (0.0%)	0.676
Early Satiety (Present)	14 (15.7%)	15 (13.0%)	1 (9.1%)	0.767
Bloating (Present)	27 (30.3%)	28 (24.3%)	3 (27.3%)	0.633
Increased frequency of urination (Present)	27 (30.3%)	32 (27.8%)	5 (45.5%)	0.469
Urinary incontinence (Present)	0 (0.0%)	2 (1.7%)	2 (18.2%)	0.007
Erectile dysfunction (Present)	5 (8.5%)	2 (2.4%)	0 (0.0%)	0.321
H/O Sudomotor dysfunction: Loss of Sweating (Present)	19 (21.3%)	41 (35.7%)	4 (36.4%)	0.076
Gustatory Sweating (Present)	15 (16.9%)	17 (14.8%)	2 (18.2%)	0.900
Systolic BP (mmHg)	138.70±20.67	139.06±20.19	147.27±15.96	0.249
Diastolic BP (mmHg)	81.17±8.94	82.15±9.15	82.64±7.70	0.578
Pulse (bpm)	83.12±9.86	87.72±16.26	93.82±12.38	0.007*
Respiratory Rate (cpm)	15.67±1.62	15.38±1.68	14.27±1.95	0.027*
Hemoglobin (g/dL)	12.27±1.19	12.08±1.00	11.81±1.13	0.349
FPG (mg/dL)	133.78±49.33	142.60±47.79	134.00±44.93	0.223
PPG (mg/dL)	205.28±74.24	214.32±72.45	209.00±58.32	0.425
HbA1c (%)	8.01±1.52	8.49±1.90	9.17±2.13	0.088
Vitamin D3 (ng/mL)	19.10±10.08	20.20±11.22	34.32±21.67	0.081
T3 (ng/dL)	1.11±0.63	2.35±10.53	1.02±0.17	0.903
T4 (µg/dL)	7.44±1.86	8.00±5.56	8.74±1.31	0.024*
TSH (µIU/mL)	9.30±47.33	3.94±7.38	3.39±1.17	0.425
HDL (mg/dL)	44.04±8.37	43.41±8.88	46.09±11.52	0.989
LDL (mg/dL)	79.00±28.82	81.37±30.39	77.44±27.31	0.654
TG (mg/dL)	142.36±71.35	150.56±57.41	129.00±64.29	0.145
Blood Urea (mg/dL)	31.69±9.73	33.73±11.38	33.55±11.47	0.468

Contd...

Table 2: Contd...

Parameters	CANS			*P*
	Normal (*n*=89)	Early (*n*=115)	Definite (*n*=11)	
Creatinine (mg/dL)	1.32±3.73	0.96±0.31	0.93±0.23	0.975
AST (IU/L)	24.71±11.09	27.12±13.35	21.70±10.10	0.092
ALT (IU/L)	29.11±16.39	31.25±23.92	20.30±9.16	0.049*
ALP (IU/L)	106.49±46.68	105.08±36.39	91.73±40.27	0.238
S. Bilirubin (mg/dL)	1.71±10.32	0.57±0.19	0.58±0.16	0.086
Urinary Protein (mg/24 hr)	382.03±609.22	751.89±1458.63	830.82±663.88	0.045*
Duration of diabetes (years)	11.24±7.44	11.37±7.57	16.45±7.97	0.076
eGFR (mL/min/1.73 m²)	93.46±37.91	91.23±32.93	74.39±11.73	0.264
Serum albumin (g/dL)	3.90±0.42	3.93±0.44	3.90±0.44	0.700
VPT (Right)	25.54±16.54	28.46±16.59	36.91±15.93	0.089
VPT (Left)	25.99±16.91	27.66±16.47	38.64±16.20	0.135
DTR: Knee				0.706
1+	2 (2.2%)	2 (1.7%)	0 (0.0%)	
2+	86 (96.6%)	113 (98.3%)	11 (100.0%)	
3+	1 (1.1%)	0 (0.0%)	0 (0.0%)	
DTR: Ankle				1.000
1+	2 (2.2%)	3 (2.6%)	0 (0.0%)	
2+	87 (97.8%)	112 (97.4%)	11 (100.0%)	
ABI (Right)	1.17±0.07	1.16±0.06	1.15±0.18	0.223
ABI (Left)	1.17±0.06	1.15±0.08	1.12±0.10	0.190

*P<0.05 considered significant. ABI: Ankle CAD: Coronary artery Disease; CHF: Congestive Heart Failure; CVA: Cerebral vascular accident; DTR: Deep tendon reflex; eGFR: estimated glomerular filtration rate; FPG: fasting plasma glucose; HDLc: High density lipoprotein cholesterol; LDLc: Low density lipoprotein cholesterol; PPG: Post-prandial plasma glucose TG: triglycerides; VPT: Vibration perception threshold

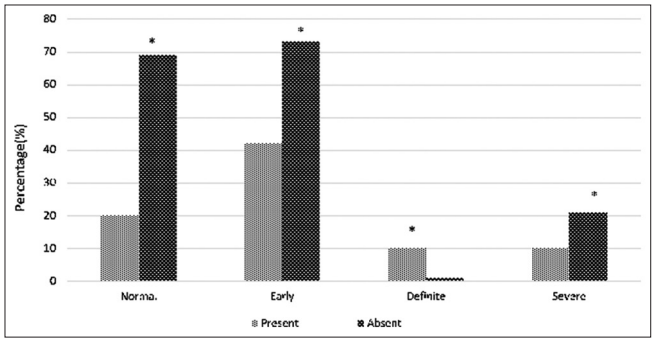

Figure 2: Prevalence of cardiac autonomic neuropathy in those with foot complications (present) compared to those without any foot complications (absent). * $P < 0.05$ (intergroup)

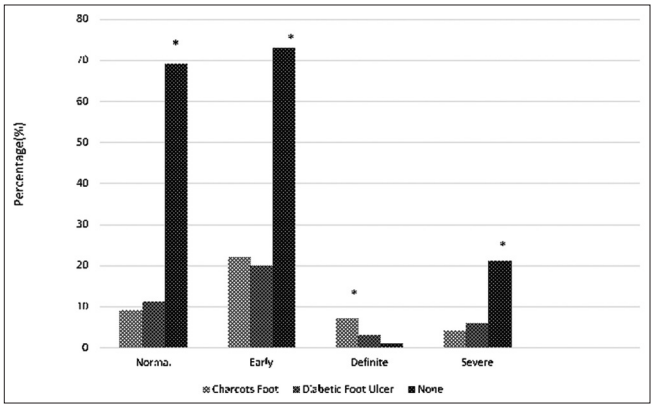

Figure 3: Prevalence of cardiac autonomic neuropathy in those with Charcot neuroarthropathy compared to those with diabetic foot ulcer or no foot complications. * $P < 0.05$ (intergroup)

pertaining to the involvement of autonomic nerve fibers but objective evaluation identifies many more patients of diabetes especially those with foot complications. In the present study, almost one-fourth of all participants with foot complications and CAN had gustatory sweating which was the most common symptom observed followed by rest palpitations and postural dizziness. The COMPASS-31 that is a validated autonomic symptom score was administered for the participants and we observed a significantly higher scores in CN and DFU group compared to those without foot complications in spite of similar duration of diabetes. Amongst patients with foot complications, CN patients were more likely to have gustatory sweating compared to other groups. Participants with DFU were more likely to provide history of rest palpitations or postural dizziness as compared to those with CN or those without foot complications. However, most diabetic patients with CAN were asymptomatic as symptoms of palpitations or gustatory sweating were observed in less than one-fifth of all patients that was not different from those without CAN. In a study from north-east India, symptoms of cardiovascular autonomic dysfunction were observed in only 17.1% of patients with CAN,14.2% were asymptomatic and others had symptoms pertaining to other autonomic system, similar to the present study.[18]

The prevalence of CAN has been reported to range from 25% to 75% depending upon the diagnostic criteria (definition of

Table 3: Regression analysis for the predictors of CAN depending upon the characteristics of the studied population

Dependent variable	CAN Absent	CAN Present	OR (univariable)	OR (multivariable)
Age (Years)				
Mean (SD)	52.1 (13.3)	55.1 (10.6)	1.02 (0.99-1.05, *P*=0.114)	1.02 (0.98-1.06, *P*=0.317)
Gender				
Male	56 (35.4)	102 (64.6)	-	-
Female	3 (33.3)	6 (66.7)	1.10 (0.28-5.36, *P*=0.898)	0.86 (0.18-4.71, *P*=0.848)
Duration of diabetes (years)				
Mean (SD)	11.3 (8.1)	11.5 (6.7)	1.00 (0.96-1.05, *P*=0.876)	0.97 (0.92-1.02, *P*=0.204)
History of CAD				
Absent	55 (35.3)	101 (64.7)	-	-
Present	4 (36.4)	7 (63.6)	0.95 (0.28-3.77, *P*=0.941)	0.50 (0.11-2.40, *P*=0.364)
Hypertension				
Absent	27 (40.3)	40 (59.7)	-	-
Present	32 (32.0)	68 (68.0)	1.43 (0.75-2.74, *P*=0.272)	1.51 (0.71-3.25, *P*=0.285)
Erectile dysfunction				
Absent	54 (33.8)	106 (66.2)	-	-
Present	5 (71.4)	2 (28.6)	0.20 (0.03-0.98, *P*=0.062)	0.25 (0.03-1.36, *P*=0.126)
Gustatory Sweating				
Absent	48 (35.3)	88 (64.7)	-	-
Present	11 (35.5)	20 (64.5)	0.99 (0.44-2.30, *P*=0.984)	0.95 (0.38-2.45, *P*=0.920)
Hemoglobin (g/dL)				
Mean (SD)	12.5 (1.3)	12.2 (1.1)	0.78 (0.58-1.02, *P*=0.073)	0.77 (0.54-1.07, *P*=0.126)
HbA1c (percent)				
Mean (SD)	8.1 (1.5)	8.6 (2.0)	1.19 (0.99-1.45, *P*=0.075)	1.35 (1.08-1.71, *P*=0.011)*
LDLc (mg/dL)				
Mean (SD)	79.0 (27.6)	77.4 (30.4)	1.00 (0.99-1.01, *P*=0.736)	1.00 (0.98-1.01, *P*=0.673)
eGFR (mL/min/1 73 m^2)				
Mean (SD)	100.6 (38.5)	91.5 (32.5)	0.99 (0.98-1.00, *P*=0.110)	1.00 (0.99-1.01, *P*=0.728)
VPT				
Mean (SD)	26.3 (17.1)	30.7 (16.4)	1.02 (1.00-1.04, *P*=0.102)	1.17 (1.02-1.39, *P*=0.043)*

MODEL FIT: $\chi^2(13) = 22$, *P*=0.055 Pseudo-R^2 = 0.1. Number in dataframe=167, Number in model=167, Missing=0; C-statistic=0.686. *P <0.05 considered significant. CAD: Coronary artery Disease; eGFR: estimated glomerular filtration rate; LDLc: Low density lipoprotein cholesterol; OR: Odds ratio; VPT: Vibration perception threshold

CAN) used. We observed that three-fourth of all patients with duration of diabetes more than 10 years had CAN. A study of 100 patients from India with mean diabetes duration of 13 years observed 70% prevalence of CAN using Ewing's criteria.[18] Similarly, a recent study from China found the prevalence of CAN be 62.6% amongst diabetic patients with mean age of 60 years and median diabetes duration of 10 years.[19] However, in other studies prevalence of CAN in type 2 diabetes individuals was found to be much lower as 20% and 15.3%, respectively.[6,19] The prevalence of CAN is shown to corelate with population characteristics specifically increasing age, duration of diabetes and glycemic control attributing to the differences in prevalence of CAN amongst various studies.[6,19,20-22]

However, it is not known that diabetic patients with foot complications matched for age and duration of diabetes would have differences in prevalence of CAN compared to the participants without foot complications. It is likely that CAN would be more prevalent in patients with foot complications of DFU or CN. We observed that patients with foot complications had higher prevalence of autonomic symptoms in the form of rest palpitations, postural dizziness, gustatory sweating, and urinary incontinence. Previously, it has been observed that patients with DFU have higher prevalence of CAN compared to those without CAN.[23-25] Patients with foot complications had twice the prevalence of definite and severe CAN than those without foot complications. A recent review identified the prevalence of CAN be 43%–66% amongst patients with DFU.[12] We observed that participants with Charcot foot were more likely to have CAN as compared with those with DFU or DSPN particularly severe CAN, suggesting significant involvement of the autonomic nerve fibers contributing to the pathophysiology of Charcot foot. A study from south India noticed that 70.8% of patients with chronic Charcot foot have CAN,[26] which is like our observation that 78.6% patients with CN having early, definite, or severe CAN. Other authors using different criteria for diagnosis of CAN have also highlighted an increased prevalence of CAN in patients with CN in small studies.[27-29]

We have previously observed that patients with CN had increased odds of sudden death as compared with patients without diabetic neuropathy over 5 years of follow up.[14]

We attributed an increased risk of sudden death to CAN as the included participants had no prior evidence of ASCVD. Prior large studies (EURO IDDM and ACCORD) have also identified an increased risk of all-cause or CV mortality in either type 1 or 2 diabetes individuals having CAN.[2,30] A meta-analysis of studies that defined CAN as the presence of two abnormal tests found a relative mortality risk of 3.45 (95% CI 2.66–4.47, $P < 0.001$) in diabetic patients with CAN.[4] However, these studies were not targeted to population with foot complications. Another meta-analysis that included studies with prospective follow up for CVE or mortality found pooled relative risk (RR) of 3.16 (95%CI 2.42 to 4.13; $P < 0.0001$) for mortality in people with CAN.[31] A heightened vigil for symptoms of autonomic neuropathy and periodic examination for CAN is the need of hour especially for patients with CAN.

The strengths include an appropriately powered, adequate sample size study with study population sub-grouped into those with foot complications (Charcot's foot and DFU) and with or without diabetic peripheral neuropathy matched for age and duration of diabetes to detect differences in prevalence of CAN. All possible confounding factors affecting heart rate and autonomic function assessment were properly excluded at the time of recruitment. Appropriate conditions were provided for CAN assessment including rest and quiet environment during testing maneuvers with strict protocol followed for every patient. A standard definition for CAN was used as per Toronto Consensus Panel on Diabetic Neuropathy using Ewing's criteria as well as extended criteria (includes resting heart rate). Certain limitations to mention include the diagnosis of diabetic peripheral neuropathy was considered on the basis of abnormal VPT and loss of monofilament perception than more sensitive measures including corneal confocal microscopy. Though matching was performed for baseline characteristics, however, certain differences in the prevalent co-morbidities cannot be ruled out. The CV of the analyzer could not be provided by the manufacturer. The CAN assessment was performed by the single investigator (inter-observer variances less likely), however, the tests were performed once so intra-observer variances could not be assessed. We also observed that during sympathetic function testing for dynamic hand grip tests, grip could be sustained for 120 s by most of the participants and not longer than recommended (>120 sec). We observed that one of the common reasons for exclusion was inability to perform Valsalva maneuver and hand grip test as required by testing protocol. Detailed ocular findings, objective autonomic function tests for other systems (eg: sudomotor dysfunction) or x-ray foot in patients with CN or DFU were not available. As both CAN and foot complications contribute to high mortality risk, a prospective follow up of the studied population for differences in mortality rates would be contemplated in future.

We conclude that CAN is present in more than two-third of patients with diabetes and foot complications. People with Charcot neuroarthropathy are especially at high risk for CAN than those with either DFU or diabetic peripheral neuropathy without foot complications. The symptoms related to autonomic dysfunction are non-specific and may not be elicitable requiring an objective evaluation for CAN in all patients of diabetes with foot complications especially those with CN.

Ethics statement

The study protocol involves human participants, and the protocol was reviewed and approved by the Institute Ethics Committee.

Informed consent

An informed and written consent was obtained from all the participants included in the study

Author responsibilities

SW conducted CAN tests, provided clinical care to the participants and wrote the initial draft of the manuscript. AR conceptualized and designed the study, provided clinical care to the participants, edited the manuscript and shall be the guarantor of the research work. AG and PD were involved in designing the protocol and edited the manuscript.

Highlights

What is already known on this topic – Patients with diabetes have higher risk of CAN. Do the patients with foot complications have higher prevalence of CAN independent of age and disease duration?

What this study adds – Symptoms related to CAN are nonspecific and present in less than one-fourth of all patients with CAN. Patients with foot complications have significantly higher prevalence of CAN particularly those with Charcot foot compared with those with or without diabetic peripheral neuropathy

How this study might affect research, practice or policy – An objective evaluation for CAN in all patients of diabetes with foot complications should be contemplated especially those with CN despite being asymptomatic.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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