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Original Article

Cardiac autonomic neuropathy in patients with type 2 diabetes mellitus having peripheral neuropathy: A cross-sectional study

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ABSTRACT

Aims: The aim was to see the frequency of CAN in type 2 diabetes mellitus patients with peripheral neuropathy, and its association with peripheral nerve conduction abnormalities.
Methods: A cross-sectional study at BIRDEM was conducted in 62 patients with type 2 diabetes mellitus having electrophysiologically diagnosed peripheral neuropathy. CAN was detected by four clinical tests - heart rate response to deep breathing and valsalva maneuver, blood pressure response to standing and sustained handgrip.
Result: The study showed that all patients had CAN – 14.52% had early, 26.67% had definitive and 59.68% had severe CAN. Patients with severe CAN had significantly reduced nerve conduction velocity and amplitude of peripheral nerves (sural 4.36 ± 12.77 vs 9.65 ± 17.77 m/s, $p = 0.009$; 2.23 ± 1.89 vs 3.01 ± 2.76 mV, $p = 0.001$; peroneal 7 ± 4.23 vs 8.53 ± 5.99 mV, $p = 0.047$; tibial 0.008 ± 0.03 vs 0.026 ± 0.05 mV, $p = 0.009$) and higher serum triglyceride levels (221.17 ± 120.61 vs 197.76 ± 68.43 mg/dl, $p = 0.033$).
Conclusion: Diabetic patients with peripheral neuropathy have CAN, the severity of which increases with worsening neuropathy.

1. Introduction

Neuropathy is an important complication of type 2 diabetes mellitus. Diabetic neuropathy does not only include somatic peripheral neuropathy, but also autonomic neuropathy. Autonomic neuropathy has a variety of manifestations. Autonomic neuropathy affects cardiovascular, gastrointestinal, genitourinary and integumentary systems among which cardiac autonomic neuropathy (CAN) is the most important [1]. The features of CAN include resting tachycardia, exercise intolerance, orthostatic hypotension and silent myocardial ischaemia. A study from Germany has reported that 25.3% of patients with type 1 diabetes mellitus and 34.3% of patients with type 2 diabetes mellitus suffer from CAN [2]. The prevalence of CAN in diabetes mellitus was high in the Asian population. A study in Vietnam demonstrated the prevalence of CAN in type 2 diabetes mellitus to be 67.7% and another study in South India showed a prevalence of 60% [3,4]. These values are higher than western figures. Therefore, the rate of CAN is influenced by geographical area and the type of population. Moreover, the prevalence of CAN varies depending on the number of tests and strictness of the criteria used to define CAN. When strict criteria were used to define CAN, the incidence was as low as 7.7% for newly diagnosed patients with type 1 diabetes to a high of 90% in potential recipients of a pancreas transplant [1].

A few studies were carried out to determine the frequency of CAN in type 2 diabetes mellitus patients with peripheral neuropathy. Ziegler et al. (1992) in Germany conducted a number of autonomic function tests (spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses) in 261 diabetic patients in different stages of peripheral neuropathy [5]. CAN, defined as the presence of ≥3 abnormal tests, was

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detected in 13% of those without peripheral neuropathy, 34.4% of those with subclinical neuropathy, 49.3% of those with symptomatic peripheral neuropathy, and in 100% of those with symptomatic peripheral neuropathy and autonomic symptoms. Earlier, Ewing et al. conducted a study in Edinburgh to see abnormalities in the conduction velocities of peripheral nerves in type 2 diabetes mellitus patients with autonomic neuropathy [6]. The studies showed that peripheral neuropathy paralleled changes in those with autonomic dysfunction.

However, it is one of the most overlooked of all serious complications of diabetes. In CAN, damage occurs to the autonomic nerve fibers that innervate the heart and blood vessels. This leads to abnormalities in the cardiovascular hemodynamics [1]. It manifests as resting tachycardia and orthostatic hypotension. Patients suffer from exercise intolerance, silent myocardial ischaemia, intraoperative and perioperative cardiovascular instability, increased risk of mortality and sudden death. Therefore, it is important to detect CAN at an early stage. CAN has a subclinical stage that can be detected by tests of baroreceptor function and heart rate variability. Heart rate response to deep breathing and to valsalva maneuver, blood pressure response to standing and diastolic blood pressure response to sustained handgrip are some of the clinical tests performed to determine CAN. These tests can identify CAN early in the asymptomatic stage. These clinical tests help to identify those patients at highest risk of developing clinically symptomatic CAN [7].

CAN is clinically important because of its association with increased mortality and modifiable risk factors [7–9]. In type 2 diabetes mellitus, CAN is associated with modifiable factors like smoking, central fat distribution, poor diabetes control, dyslipidemia, hypertension and other diabetic microvascular complications. Also, CAN is an independent risk factor for stroke. Therefore, its early detection and prevention is important for better management of type 2 diabetes mellitus patients.

As mentioned earlier, CAN is associated with increased mortality. Statistics show that 25%–50% of patients with symptomatic autonomic dysfunction die within 5–10 years of diagnosis [1]. Also the 5-year mortality rate among diabetic patients with autonomic neuropathy is three times higher than those without autonomic dysfunction. The most common cause of death is ischaemic heart disease. Cardiac ischaemia in patients with CAN is difficult to recognize. Hence, it is more dangerous and a higher degree of suspicion and vigilance is required. One study found a mortality rate of 47% after asymptomatic MI compared to 35% after MI with pain [1]. Ewing et al. reported a 2.5-year mortality rate of 27.5% that increased to 53% after 5 years in diabetic patients with abnormal autonomic function tests compared to a mortality rate of only 15% over the 5-year period among diabetic patients with normal autonomic function test results [6]. O'Brien et al. compared the relative importance of various factors associated with mortality by discriminate analysis of survivors and non-survivors using Rao's stepwise selection method and revealed that autonomic neuropathy was more of an independent predictive factor than systolic blood pressure, foot disease, BMI, sensory neuropathy, proteinuria and macrovascular diseases [10]. In addition, the investigators suggested that the cardiovascular autonomic dysfunction in individuals already at high risk (e.g., those with diabetes, high blood pressure, or a history of cardiovascular disease) may be particularly more hazardous. Burgos et al. found that perioperative vasopressor support was needed more often in diabetic individuals with autonomic dysfunction than in those without [11]. Sobotka et al. showed that some diabetic patients with autonomic neuropathy have a reduced hypoxic-induced ventilatory drive [12]. These data suggest that preoperative cardiovascular autonomic screening may provide useful information for planning the anesthetic management of diabetic patients and identify those at higher risk for intraoperative complications [13].

Therefore, it is important to detect CAN in patients with type 2 diabetes mellitus. Detection should be carried out early. Subclinical autonomic neuropathy can often be identified within 1 year of diagnosis of type 2 diabetes mellitus and within 2 years in those with type 1 diabetes mellitus [1]. The physician can then modify exercise programs and drug regimens accordingly. An increased monitoring for cardiac ischaemia should be instituted to reduce morbidities [14]. Autonomic impairment is an early event in type 2 diabetes mellitus which may go unnoticed long before symptoms appear. Early diagnosis of CAN in its subclinical phase by clinical tests would enable a closer metabolic control and prevent further deterioration of the condition [15].

In view of the above, it appears that CAN and peripheral neuropathy may be correlated in patients with type 2 diabetes mellitus. Although CAN is an important complication associated with significant morbidity, it is not adequately addressed. No study has yet been conducted to see the frequency of and factors associated with the severity of CAN in type 2 diabetes mellitus patients having peripheral neuropathy in Bangladesh. There are also limited studies elsewhere in the world. In the above context, the present study was designed to determine the presence of CAN in type 2 diabetes mellitus patients with peripheral neuropathy as well as the association of CAN with conduction abnormalities of different peripheral nerves, cardiovascular risk factors (overweight, hypertension, dyslipidaemia) and diabetic complications (retinopathy, diabetic kidney disease).

2. Subjects

This cross-sectional study was carried out from 1st September 2011 to 30th August 2012 on 62 adult type 2 diabetic patients with peripheral neuropathy admitted in BIRDEM general hospital. Those patients with abnormal red cell indices, history of alcohol consumption, connective tissue disease, Parkinsonism, ischaemic heart disease, taking drugs such as anti-ischaemic medication, vasodilators and anti-depressants, pregnancy and acute diabetic complications were excluded from the study.

3. Materials and methods

Diagnosed cases of type 2 diabetes mellitus patients were taken and nerve conduction study (NCS) was done to identify those with peripheral neuropathy. Tests of CAN were carried out in this group of patients. Their clinical (anthropometry, blood pressure, fundoscopy) and laboratory data (urine protein, HbA1c, lipid profile, serum creatinine) were also collected. The population was categorized into 3 grades of CAN and further divided into 2 groups (severe and non-severe CAN) based on the clinical tests. Non-severe CAN included early and definitive CAN.

Nerve conduction study: Peripheral neuropathy was diagnosed by nerve conduction studies. Nerve conduction velocity (NCV) and amplitude were measured by a standard electromyography (EMG) machine in a room with a temperature of 22 °C. All measurements were performed in the lower limbs with surface electrodes. Nerves were stimulated using 1 ms electrical pulses at a repetition rate of 1 per second with intensity sufficient to elicit maximum amplitude of compound muscle action potential and sensory nerve action potential. Motor nerve conduction velocity (NCV) was recorded for peroneal and tibial nerves. Sensory nerve conduction velocity was recorded for sural nerve. A reduced nerve conduction study (velocity or amplitude) of any one of three nerves (sural nerve, peroneal and tibial nerve) was taken as neuropathy [16,17].

Clinical tests of CAN: CAN was assessed clinically by heart rate response to valsalva maneuver, heart rate response to deep

breathing, blood pressure response to standing and sustained handgrip. However the first three tests were used to diagnose CAN.

a. Heart rate response to valsalva maneuver: Patients were asked to blow into mouthpiece which was connected to a modified manometer and to maintain a pressure of 40 mmHg for 15 s in sitting position. R-R intervals were recorded during the maneuver and for 15s following release with the help of an ECG. The ratio of the longest R-R interval following release to the shortest R-R interval during the maneuver (valsalva ratio) was calculated. A ratio of 1.10 or less was defined as an abnormal response, 1.11 to 1.20 as borderline, and 1.21 or more as a normal response [18,19].
b. Heart rate variation during deep breathing: The subject resting in a supine position was instructed to breathe deeply at a frequency of 6 cycles/min which produces maximal HRV in healthy persons. The inspiration and expiration intervals were 6 s and 4 s respectively. Continuous ECG recording was done during the whole procedure to record the difference between the maximum and minimum heart rates. The mean value was calculated from the six inspirations and expirations. The difference between heart rate during rest and after deep breathing was calculated. Heart rate normally falls by > 15 beats/min. A fall of <10 beats/min was abnormal [18,20].
c. Blood pressure response to standing: Blood pressure was determined 3 times over 1 min in a supine position and in the same way 2 min after standing up. Patients having either abnormal systolic or diastolic postural drop were considered to have CAN. For systolic BP normal response was a fall of <10 mmHg, borderline a fall of 10–29 mmHg, and abnormal a fall of >30 mmHg. For diastolic BP a fall of >15 mmHg was abnormal [18].
d. Blood pressure response to handgrip: Patients were asked to grip the sphygmomanometer with their right hands (using 30% of their maximum power for 5 min or 60 mmHg of pressure for 3 min). Systolic and diastolic blood pressures were recorded before and during each of the 3 consecutive minutes of the exercise in the other arm. Increase in diastolic pressure by 16 mmHg or more was considered as normal response, increase in pressure between 11 and 15 mmHg was accepted as borderline response and increase in pressure lower than 10 mmHg was regarded as abnormal response [18].

CAN was diagnosed by abnormal heart rate tests (heart rate response to valsalva maneuver and deep breathing) and blood pressure change on standing (postural drop). It was graded into three types:

a) Early CAN: any one of two heart rate tests abnormal or both heart rate tests borderline.
b) Definitive CAN: both heart rate tests abnormal.
c) Severe CAN: definitive CAN with postural drop [21,22].

Non severe CAN included early CAN and definitive CAN without postural drop.

Clinical and laboratory measures: Height was measured by stadiometer and weight with a scale. Blood pressure was measured three times using a mercury sphygmomanometer and the average measure taken. Ophthalmoscopy was carried out by the Department of Ophthalmology to determine presence or absence of retinopathy. Proteinuria was detected by dipstick method at the bed side. HbA1c was estimated on the principles of ion-exchange high-performance liquid chromatography (HPLC) by BIO-RAD Variant II Turbo analyzer [23]. Serum creatinine was measured by Siemens Dimension Clinical Chemistry system using a modification of the kinetic Jaffe reaction as described by Larson [24]. Serum total cholesterol was measured by Siemens Dimension Clinical Chemistry system using the method based on the principle described by Rautela et al. [25]. Cholesterol esterase and polychromatic technique were used. LDL (low density lipoprotein), HDL (high density lipoprotein), triglyceride were each measured by Siemens Dimension Clinical Chemistry system. The assays were performed as per the manufacturer's instruction manual.

Among the cardiovascular risk factors, BMI of 25 kg/m^2 was defined as overweight. Systolic and diastolic hypertension was defined as blood pressure level of more than 140 and 90 mmHg respectively. Dyslipidaemia was defined as either an LDL level greater than 100 mg/dl or HDL less than 40 mg/dl or triglyceride level of more than 150 mg/dl. Presence of non-proliferative retinopathy (NPDR), proliferative retinopathy (PDR) or maculopathy indicated diabetic retinopathy. Proteinuria on dipstick with or without raised creatinine (>1.2 mg/dl) was taken as evidence of diabetic kidney disease (DKD).

Data were analyzed by SPSS version 11.5. Results were expressed as mean ± SD and frequency with 95% confidence interval. Unpaired *t*-test was used to compare clinical variables between severe and non-severe CAN. Pearson's correlation coefficient was done to see the relation between tests of CAN and variables. P value of less than 0.05 was taken as significant.

4. Results

The study population consisted of 62 adults with diabetes mellitus and peripheral neuropathy. 50% were male. Mean age of the participants was 55.58 ± 10.73 years and mean duration of diabetes was 14.15 ± 7.99 years. The mean HbA1c level was 11.02 ± 2.86%, which reflected that the population had uncontrolled diabetes. Clinical and biochemical characteristics of the study population are shown in Table 1.

4.1. CAN of the study population

Table 2 shows the frequency of CAN in the study population. All patients with peripheral neuropathy had some grade of CAN. More than half had severe CAN.

Among the 4 tests of CAN performed, abnormal valsalva ratio was observed in the majority of participants - 98.34% cases. This was followed by an abnormal heart rate response to deep breathing (88.71%) (Table 3).

4.2. Cardiovascular risk factors and diabetic complications in patients with CAN

The rate of diabetic complications and risk factors in patients

Table 1
Clinical and laboratory parameters of the study population (n = 62).

Variable	Mean ± SD	Range
Age (years)	55.58 ± 10.73	18–72
Duration of diabetes (yrs)	14.15 ± 7.99	1–35
BMI (kg/m^2)	25.14 ± 4.71	12.82–41.28
Systolic Blood Pressure (mmHg)	133.09 ± 20.49	90–197
Diastolic Blood Pressure (mmHg)	76.12 ± 10.82	50–100
HbA1c (%)	11.02 ± 2.86	6.1–18.6
S. Cholesterol (mg/dl)	167.43 ± 48.82	255–326
S. LDL (mg/dl)	87.59 ± 35.37	22–190
S. HDL (mg/dl)	35.82 ± 8.84	21–57
S. Triglyceride (mg/dl)	211.57 ± 102.44	47–520
S. Creatinine (mg/dl)	1.17 ± 0.49	0.5–2.7

Table 2
Frequency of CAN and its grades in the study population (n = 62).

Frequency of CAN		Frequency of early CAN		Frequency of definite CAN		Frequency of severe CAN	
n	%	n	%95%CI	n	%95%CI	n	% 95%CI
62	100	9	14.52 (5.7–23.2)	16	25.80 (14.9–36.7)	37	59.68 (47.4–71.8)

Table 3
Frequency of individual abnormal clinical tests of CAN in the study population (n = 62).

Clinical test of CAN	Number of cases with abnormal test	Percentage
Heart rate response to valsalva maneuver (ratio ≤ 1.20)	61	98.34
Heart rate response to deep breathing (≤15 beats/min)	55	88.71
SBP response to standing (fall in SBP ≥ 10 mmHg)	43	69.35
DBP response to standing (fall in DBP ≥ 15 mmHg)	11	17.74
Heart rate response sustained handgrip (≤15 mmHg)	48	77.42

SBP = systolic blood pressure, DBP = diastolic blood pressure.

with CAN and severe CAN are shown in Table 4. One-third (33.87%) of the patients with CAN had hypertension, more than half (56.45%) were overweight and almost all (98.39%) had dyslipidaemia. 51.62% patients had retinopathy, of which NPDR was the most common. 66.13% had DKD. Compared to the study population, which included all grades of CAN, patients with severe CAN had a greater frequency of hypertension, but similar rates of other risk factors and complications.

Comparison of peripheral nerve conduction, cardiovascular risk factors and diabetic complications between severe and non-severe CAN.

The study variables of the two groups, namely severe and non-severe CAN were compared. Among the 25 variables, sural NCV, amplitude of sural, peroneal and tibial nerves were significantly lower in severe CAN group compared to non-severe group (4.36 ± 12.77 vs 9.65 ± 17.77 m/s, $p = 0.009$; 2.23 ± 1.89 vs 3.01 ± 2.76 mV, $p = 0.001$; 7 ± 4.23 vs 8.53 ± 5.99 mV, $p = 0.047$; 0.008 ± 0.03 vs 0.026 ± 0.05 mV, $p = 0.009$ respectively). Serum triglyceride level was significantly higher in those with severe CAN compared to non-severe CAN (221.17 ± 120.61 vs 197.76 ± 68.43 mg/dl, $p = 0.033$). All the other variables did not show any significant difference (Table 5).

Table 4
Frequencies of cardiovascular risk factors and diabetic complications.

Clinical condition	CAN (n = 62)	Severe CAN (n = 37)
Overweight (≥25 kg/m^2)	35 (56.45)	17 (45.95)
Hypertension	21 (33.87)	24 (64.86)
Systolic (>140 mmHg)	16 (25.80)	
Diastolic (>90 mmHg)	4 (6.45)	
Retinopathy	32 (51.62)	20 (54.05)
NPDR	16 (25.81)	
NPDR with maculopathy	14 (22.58)	
PDR	2 (3.22)	
PDR with maculopathy	0	
Uncontrolled diabetes (HbA1c > 7%)	57 (91.92)	35 (94.59)
Dyslipidaemia	61 (98.39)	37 (100)
Serum cholesterol (>200 mg/dl)	16 (25.81)	
Serum LDL (>100 mg/dl)	19 (30.65)	
Serum HDL (<40 mg/dl)	45 (72.58)	
Serum Triglyceride (>150 mg/dl)	45 (72.58)	
DKD	41 (66.13)	25 (67.56)
Serum creatinine (>1.2 mg/dl)	23 (37.10)	
Presence of proteinuria	31 (50)	

Dyslipidemia = abnormality in any one lipid component; within parenthesis are percentages.

4.3. Correlation of clinical tests of CAN with peripheral nerve conduction, cardiovascular risk factors and diabetic complications

Among the variables analyzed, systolic blood pressure was positively correlated with systolic blood pressure response to standing ($r = 0.32$; $p = 0.011$) and negatively with diastolic blood pressure increase to sustained handgrip ($r = -0.31$; $p = 0.014$). Serum creatinine was negatively correlated with diastolic blood pressure increase to sustained handgrip ($r = -0.26$; $p = 0.045$) and positively correlated with heart rate response to valsalva ($r = 0.34$, $p = 0.007$). Other variables did not show any significant correlation.

5. Discussion

The present study was undertaken to investigate CAN among type 2 diabetics with peripheral neuropathy. The study showed that all the cases with diabetic peripheral neuropathy had different grades of CAN. Among the type 2 diabetics with peripheral neuropathy and CAN, more than half had severe CAN. Abnormal valsalva ratio followed by an abnormal heart rate response to deep breathing were the most common positive tests of CAN. Dyslipidaemia was the most common cardiovascular risk factor in patients with CAN as well as severe CAN. A substantial proportion of patients with CAN had other microvascular complications. NCV and amplitude of peripheral nerves were lower and triglyceride level was higher in severe CAN group. Correlations of clinical tests of CAN with systolic blood pressure and serum creatinine were significant.

The rate of CAN in the present study was higher than the ones found by Spallone et al. in Europe, where the prevalence of CAN among type 2 diabetic patients was around 20% [22]. Another study by Ziegler et al. reported 25.3% of patients with type 1 diabetes and 34.3% of patients with type 2 diabetes had abnormal findings in more than two of six autonomic function tests [2]. In another study in Argentina, 29% and 54% of patients with type 2 diabetes had early and definitive CAN. The results in this study were higher as the study population was different. This study was carried out in type 2 diabetic patients with proven peripheral neuropathy of unknown duration, whereas the other studies were done among diabetic patients where the concomitant presence of peripheral neuropathy was not mentioned. It should be mentioned, that when looking at factors which increased the risk of CAN, presence of peripheral neuropathy had an odds ratio of 11.7 ($p < 0.001$) [26] and 25.94 (11.04–44.25) [27]. Another study demonstrated that peripheral neuropathy predicted development of CAN in type 2 diabetes [28]. If more strict criteria were used (i.e., abnormalities present in least

Table 5
Comparison of different variables between severe and non-severe CAN (n = 62).

Variable	Severe CAN (n = 37) mean ± SD	Non severe CAN (n = 25) mean ± SD	P value
Sural NCV (m/s)	4.36 ± 12.77	9.65 ± 17.77	**0.009**
Peroneal NCV (m/s)	30.28 ± 19.96	34.17 ± 21.14	0.948
Tibial NCV (m/s)	34.03 ± 12.35	36.36 ± 15.38	0.435
Sural amplitude (mV)	2.23 ± 1.89	3.01 ± 2.76	**0.001**
Tibial amplitude (mV)	7 ± 4.23	8.53 ± 5.99	**0.047**
Peroneal amplitude (mV)	0.008 ± 0.03	0.026 ± 0.05	**0.009**
BMI (kg/m2)	24.74 ± 3.88	25.74 ± 5.76	0.248
SBP (mmHg)	133.49 ± 21.06	132.51 ± 20.01	0.209
DBP (mmHg)	76.07 ± 10.83	75.93 ± 11.03	0.875
S. Cholesterol (mg/dl)	172.08 ± 54.75	160.48 ± 38.44	0.059
S. LDL (mg/dl)	89.74 ± 38.39	84.38 ± 30.78	0.220
S. HDL (mg/dl)	36.69 ± 9.21	34.50 ± 8.27	0.320
S.Triglyceride (mg/dl)	221.17 ± 120.61	197.76 ± 68.43	**0.033**
S. Creatinine (mg/dl)	1.18 ± 0.49	1.15 ± 0.50	0.799
HbA1c (%)	11.62 ± 2.89	10.13 ± 2.63	0.734

SBP = systolic blood pressure, DBP = diastolic blood pressure; p value was calculated using student's independent *t*-test.

three of six autonomic function tests), the prevalence of CAN was 16.8% for individuals with type 1 diabetes and 22.1% for individuals with type 2 diabetes [5]. In the present study CAN was diagnosed if there was only one abnormal heart rate test as described by Cardiovascular Autonomic Neuropathy Subcommittee of Toronto Consensus Panel on Diabetic Neuropathy, 2011 [21]. Therefore, the incidence of CAN varies depending on the strictness of the criteria used to define CAN. The stricter the diagnostic criteria used the lower the incidence of CAN. The prevalence of CAN also depends on the geographical area. The prevalence of CAN in diabetes mellitus was higher in the Asian population. A study in Vietnam demonstrated the prevalence of CAN to be 67.7% and another study in South India showed a prevalence of 60% [3,4]. The participants in this study were all Bangladeshi. This may explain why such a high rate of CAN was found. A similar study was done to see CAN in type 2 diabetic patients with nephropathy. This population was found to have an earlier onset and higher percentage of CAN (67%) as well as severe CAN. They also had higher prevalence of neuropathy (84%) [29]. Age, longer duration of diabetes mellitus and the presence of peripheral neuropathy are significant risk factors of CAN [4]. The population in this study had a mean age of 55 years and a mean duration of diabetes of 14 years. They all had peripheral neuropathy. All this may explain the high rate of CAN in this study.

This study showed that among the diabetic peripheral neuropathy patients with severe CAN, 94.59% had HbA1c > 7%, 100% had dyslipidaemia, 64.86% had hypertension, 54.05% had retinopathy and 67.56% had abnormal renal function. This is similar to the study by Zuern et al. where patients with severe autonomic neuropathy had longer duration of diabetes (25 years), high prevalence of diabetic neuropathy (70%), retinopathy (80%) and nephropathy (90%) and significantly higher levels of HbA1c [30]. A recent study also found that CAN was prevalent in those with poor glycaemic control, longer duration of diabetes and insulin therapy. Presence of concominant hypertension other microvascular complications was associated with the development of CAN [26]. The high prevalence of other microangiopathies in patients with CAN signify that diabetic complications occur concurrently. When CAN is detected, a search should be made for other complications or vice versa. This is important as diabetics with CAN have increased cardiac mortality [14]. All patients with long standing poorly controlled diabetes with retinopathy and nephropathy should undergo nerve conduction studies and clinical tests of CAN.

The study variables were compared between the two groups - severe and non-severe CAN. Significant difference was found among severe CAN group for triglyceride level, sural nerve conduction velocity and amplitude of sural, peroneal and tibial nerves ($p \leq 0.047$). So slower the sural nerve conduction velocity and lower the nerve amplitude on NCS, the greater the chance of having severe CAN. Ewing et al. conducted a study in Edinburgh to see abnormalities in the conduction velocities of peripheral nerves in type 2 diabetics with autonomic neuropathy [6]. Ziegler et al. conducted a number of autonomic function tests (spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses) in 261 diabetic patients in different stages of peripheral neuropathy. CAN, defined as the presence of $\geq$3 abnormal tests was detected 13% of those without peripheral neuropathy, 34.4% of those with subclinical neuropathy, 49.3% of those with symptomatic peripheral neuropathy, and in 100% of those with symptomatic peripheral neuropathy and autonomic symptoms [5]. The study showed that peripheral neuropathy paralleled changes in those with autonomic dysfunction. This means that any patient with diabetic peripheral neuropathy should be examined carefully for autonomic nerve involvement.

The clinical tests of CAN were correlated with some risk factors. There was significant correlation ($p \leq 0.007$). When systolic blood pressure increases, systolic postural drop increases and diastolic blood pressure response to sustained handgrip decreases. The diastolic blood pressure increase to sustained handgrip decreases with rising creatinine. Therefore blood pressure response to sustained handgrip has significant relation with other risk factors and should be included in the diagnostic criteria. This means that patients with systolic hypertension and nephropathy are more likely to develop CAN. In type 2 diabetes mellitus, CAN is associated with modifiable factors like smoking, central fat distribution, poor diabetes control, dyslipidemia, hypertension and other diabetic microvascular complications [7–9,27]. The significant correlation of CAN with systolic hypertension and elevated creatinine in the present study is in support of the above.

All diabetic patients with electrophysiologically diagnosed peripheral neuropathy had CAN and more than half of them had severe CAN. The presence of CAN should be explored in all patients with peripheral neuropathy. There was a high rate of cardiovascular risk factors and diabetic complications like hypertension, dyslipidaemia, retinopathy and nephropathy in patients with severe CAN. Therefore CAN should be screened in diabetic patients with other microangiopathies and vice versa. Reduced NCV and amplitude of peripheral nerves were found to be significantly associated with severe CAN. Correlations of clinical tests of CAN with systolic blood pressure and serum creatinine were significant. This means that patients with systolic hypertension and elevated creatinine are more likely to develop CAN. The study depicts the importance of determining the natural course of neuropathy in patients with type

diabetes mellitus. It is yet to be determined whether CAN precedes or occurs concurrently with peripheral neuropathy.

A high level of clinical vigilance should be exercised to detect CAN in this group of patients as CAN is associated with a high mortality and morbidity. To the best of our knowledge, this is the first study in Bangladesh to demonstrate the relationship between CAN and diabetic peripheral neuropathy in type 2 diabetic patients. However, further large-scale studies are required in type 2 diabetic patients with peripheral neuropathy to identify specifically the risk factors and predictors of different degrees of CAN. Future well controlled cohort studies are required to determine whether CAN precedes or occurs concurrently with the appearance of peripheral neuropathy. This will facilitate better management of diabetic patients with such complications.

Declaration

Ethics

The study was approved by the Research Ethics Board, BIRDEM hospital and informed written consent was obtained from each patient.

Conflict of interest

The authors declare that they have no competing interests.

Submission declaration

This article is not under consideration for publication elsewhere. If accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. Its publication has been approved by all authors and responsible authorities where the work was carried out.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

[1] Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. Circulation 2007;115:387–97.
[2] Ziegler D, Gries FA, Spuler M, Lessmann F. Diabetic cardiovascular autonomic neuropathy multicenter study group: the epidemiology of diabetic neuropathy. J Diabetes Complicat 1992;6:49–57.
[3] Thi NN, Paries J, Attali IR, Valensi P. High prevalence and severity of CAN in Vietnamese diabetic patients. Diabetes Med 2005;22:1072–8.
[4] Pappachan JM, Sebastian J, Bino BC, Jayaprakash K, Vijayakumar K, Sujathan P. Cardiac autonomic neuropathy in Diabetes mellitus: prevalence, risk factors and utility of corrected QT interval in the ECG for its diagnosis. Postgrad Med 2008;84:205–10.
[5] Ziegler D, Dannehl K, Mühlen H, Spüler M, Gries FA. Prevalence of cardiovascular autonomic dysfunction assessed by spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses at various stages of diabetic neuropathy. Diabet Med 1992;9:806–14.
[6] Ewing DJ, Burt AA, Williams R, Cambwell W, Clarke BF. Peripheral motor nerve functions in diabetic autonomic neuropathy. J Neurol Neurosurg Psychiatry 1976;39:453–60.
[7] Kuehl M, Stevens MJ. Cardiovascular autonomic neuropathies as complications of diabetes mellitus. Nat Rev Endocrinol 2012;10:1038.
[8] Voulgari C, Kokkinos A, Argiana V, Katsilambros N. The association between cardiac autonomic neuropathy with metabolic and other factors in subjects with type 1 and type 2 diabetes. J Diabetes Complicat 2011;25:159–67.
[9] Ayad F, Belhadj M, Pariés J, Attali JR, Valensi P. Association between cardiac autonomic neuropathy and hypertension and its potential influence on diabetic complications. Diabet Med 2010;27:804–11.
[10] O'Brien IA, O'Hare JP, Lewin IG, Corrall RJ. The prevalence of autonomic neuropathy in insulin-dependent diabetes: a controlled study based on heart rate variability. Q J Med 1986;61:957–67.
[11] Burgos LG, Ebert TJ, Asiddao C, Turner LA, Pattison CZ, Wang-Cheng R, et al. Increased intraoperative cardiovascular morbidity in diabetics with autonomic neuropathy. Anesthesiology 1989;70:591–7.
[12] Sobotka PA, Liss HP, Vinik AI. Impaired hypoxic ventilatory drive in diabetic patients with autonomic neuropathy. J Clin Endocrinol Metab 1986;62:658–63.
[13] Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. Diabetes Care 2003;26:1553–79.
[14] Maser RE, Lenhard JM, DeCherney SG. Cardiovascular autonomic neuropathy: the clinical significance of its determination. Endocrinololgist 2000;10:27–33.
[15] Alexandra DA. Subclinical diagnosis of cardiovascular autonomic neuropathy in type 2 diabetes: prevalence, severity, correlations with time, metabolic and vascular factors. J Neurol 2010;9:28–32.
[16] Preston DC, Shapiro BE. Electromyography and neuromuscular disorders: clinical-electrophysiologic correlations. second ed. Philadelphia: Elsevier; 2005.
[17] Electrophysiology and laboratory aids in the diagnosis of neuromuscular disorders. In: Ropper AH, Samuels MA, editors. Adams and victor's principles of Neurology. ninth ed. New York: McGraw Hill; 2009. p. 600.
[18] Ziegler D. Diabetic cardiovascular autonomic neuropathy: prognosis, diagnosis and treatment. Diabetes Metabolism Research and Reviews 1994;10:339–83.
[19] Rahman HZ. Autonomic neuropathy in relation to glycaemic status, C-peptide level and microtransferrinuria in MRDM. MD Dissertation. Bangabandhu Sheikh Mujib Medical University 1996.
[20] Swash M, editor. Hutchison's clinical methods. twenty-second ed. India: W.B. Saunders; 2007.
[21] Bernardi L, Spallone V, Stevens M. Toronto Consensus Panel on Diabetic Neuropathy. Methods of investigation for cardiac autonomic function in human research studies. Diabetes Metabol Res Rev 2011;27:654–64.
[22] Spallone V, Ziegler D, Freeman R, Bernard L, Frontoni S, Pop-Busui R, et al. Cardiac autonomic neuroparthy: clinical impact, assessment, diagnosis and management. Diabetes Metabol Res Rev 2011;27:639–53.
[23] Variant II Turbo haemoglobin A1c program instruction manual. BIO-RAD Laboratories Inc USA; 2010.
[24] Larson K. Creatinine assay by a reaction-kinetic approach. Clinical Chemistry Acta 1972;41:209–17.
[25] Rautela GS, Liedtke RJ. Automated enzymatic measurement of total cholesterol in serum. Clin Chem 1978;24.
[26] Romero SA, Ortin A, Mercado N, Caeiro G, Waitman J. Frequency and associated risk factors of cardiovascular autonomic neuropathy among patients with type 2 Diabetes. Rev Fac Cien Med Univ Nac Cordoba 2018;75(2):111–8.
[27] Moţăţăianu A, Maier S, Bajko Z, Voidazan S, Bălaşa R, Stoian A. Cardiac autonomic neuropathy in type 1 and type 2 diabetes patients. BMC Neurol 2018;18(1):126.
[28] Islam SKMA, Kim D, Lee YS, Moon SS. Association between diabetic peripheral neuropathy and heart rate variability in subjects with type 2 diabetes. Diabetes Res Clin Pract 2018;140:18–26.
[29] Viswanathan V, Prasad D, Chamukuttan S, Ramachandran A. High prevalence and early onset of cardiac autonomic neuropathy among South Indian Type 2 diabetic patients with nephropathy. Diabetes Res Clin Pract 2000;48:211–6.
[30] Zuern CS, Rizas K, Eick C, Sterz K, Gawaz M, Bauer A. Prevalence and predictors of severe autonomic failure in patients with insulin-dependent type 2 diabetes mellitus and coronary artery disease: pilot study. J Electrocardiol 2012;45(6):774–9.