Original Article

Cardiovascular autonomic neuropathy in patients with type 2 diabetes mellitus and its association with serum omentin and leptin

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Abstract

Cardiovascular autonomic neuropathy (CAN) is a major cause of morbidity and mortality in patients with diabetes as it is associated with a high risk of cardiac arrhythmias. **Objectives:** This prospective observational cross-sectional study was done to estimate the prevalence of CAN in patients with type 2 diabetes and to study its association with serum omentin and leptin levels. **Methods:** This study included 100 patients with type 2 diabetes mellitus attending the outpatient department of JIPMER Hospital, Pondicherry, India, from January 2017 to December 2018. CAN was assessed in all subjects using four cardiovascular autonomic function tests. Blood samples were collected and stored at - 80˚C to estimate leptin and omentin levels. Comparison of leptin and omentin levels was done between diabetic patients with and without CAN. **Results:** CAN was present in 64% of the study subjects. Serum leptin levels were significantly higher in patients with CAN, whereas omentin levels, though elevated in those with CAN, were not statistically significant in diabetic patients without CAN. **Conclusion:** There is a high prevalence of CAN in patients with type 2 diabetes mellitus. Leptin levels were elevated in these patients, whereas omentin levels were not significantly different between diabetic patients with and without CAN.

Keywords: Diabetic autonomic neuropathy, leptin, omentin

Introduction

Diabetic autonomic neuropathy is a lesser-known complication of diabetes mellitus and it has a significant negative impact on the quality of life in affected patients. It can involve the cardiovascular, urogenital, and gastrointestinal systems. Poor glycemic control, long-standing diabetes, advanced age, female gender, and smoking are potential risk factors for diabetic autonomic neuropathy.[1] Specifically, cardiovascular autonomic neuropathy (CAN) contributes to morbidity and mortality in patients with diabetes as it carries a high risk of cardiac arrhythmias. Technological advances have made early detection of CAN in clinical practice a real possibility.[2]

The diagnosis of CAN is done by performing cardiovascular autonomic function tests (CAFTs), including heart rate variability, heart rate and blood pressure (BP) response to standing and deep breathing, and BP response to sustained handgrip. An improvement in the balance of the autonomic nervous system may help in reducing cardiovascular events and early mortality in patients with diabetes.[3] Early recognition of CAN by the primary

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care physician is possible with the aforementioned tests. The primary care physician can help in preventing the progression of CAN by tailoring an individualized treatment regimen for patients with diabetes encompassing lifestyle modification, physical activity, and careful use of oral hypoglycemic drugs.

Insulin resistance and adipocytokines are the probable underlying causes of vascular complications of diabetes. Inflammatory adipocytokines are being studied as potential markers for diabetes-related complications.

This study was performed to estimate the prevalence of CAN in a south Indian population with type 2 diabetes mellitus and to find out an association between CAN and inflammatory adipocytokines (i.e. leptin and omentin).

Aims and Objectives

The objectives of this study were (1) to estimate the prevalence of CAN in patients with type 2 diabetes mellitus and (2) to study the association of serum omentin and leptin levels with CAN in patients with type 2 diabetes mellitus.

Material and Methods

This cross-sectional study was conducted in the Medicine OPD and Diabetes Clinic of the JIPMER Hospital in Pondicherry, India, from January 2017 to December 2018 after obtaining clearance from the Institutional Ethics Committee (IEC Ref. No. JIP/IEC/2016/1139).

Considering a prevalence of 54% of CAN in patients with diabetes measured in a previous study with a precision of 10%, the sample size was calculated to be 100 (using the sample size formula for proportions).[1] The convenient sampling technique was used to include patients with diabetes attending the Medicine OPD and Diabetes Clinic of the hospital. Patients with known thyroid disease, cardiac arrhythmias, or on β-blockers or calcium channel blockers were excluded from the study.

Data regarding age and duration of diabetes were noted in the proforma of the study subjects. The clinical examination included an assessment of body mass index (BMI) and BP. CAN was assessed in the study subjects using four CAFTs completed in the autonomic function testing laboratory. CAN was diagnosed in a study subject when two or more tests were abnormal. CAFT data were also collected for study subjects with no or one abnormal test. This resulted in the formation of five groups among the study subjects (no abnormal CAFT, subjects with 1, 2, 3, or 4 abnormal CAFT).

The study subjects were asked to avoid heavy meals 2 h before undergoing the tests and to report to the CAFT laboratory after bowel evacuation and bladder emptying. The tests, for all patients, were performed between 2 and 4 PM to avoid diurnal variation. The room temperature was maintained between 23°C and 25°C.

CAFTs

Basal cardiovascular parameters and short-term heart rate variability (HRV)

The subjects were made to lie down on a couch comfortably and relax for 5 min. They were informed in detail about the procedure to reduce anxiety. Electrodes were connected to record the electrocardiogram (ECG) (lead II). The resting supine BP and heart rate were recorded using an automated BP monitor. Following this, the lead II ECG was recorded for 5 min in resting state for the short-term HRV analysis.

Heart rate (HR) and BP response to standing

After 10 min of rest in the supine position, the lead II ECG began recording and the subject was asked to stand after a mark was generated on the graph. BP and HR were recorded immediately, and after 3 min, using an automated BP monitor. The continuous ECG recording was saved for further analysis.

HR response to deep breathing

After standing, the subject rested for 5 min. Then, the subject was asked to perform deep breathing at a rate of 6 breaths per minute. With the help of a voice metronome and hand signals from the examiner, the subject was asked to inhale and exhale slowly, allowing 5 s for inspiration and expiration, for six cycles. A continuous ECG was recorded throughout the procedure and saved for further analysis.

BP response to sustained handgrip

The maximal voluntary contraction (MVC) during sustained handgrip was measured using a handgrip dynamometer and recorded. The basal HR and BP were recorded. The ECG began recording and, at the 15th s, the subject was instructed to sustain the handgrip at 30% of the MVC for 3 min. The maximal diastolic BP (DBP) attained during sustained handgrip was recorded.

Data retrieval from the CAFTs

Short-term HRV

The acquired 5-min lead II ECG was carefully analyzed for ectopics and artifacts, which were removed manually. The detection of R-waves was completed using a thresholding algorithm. The RR tachogram was then extracted in text format from the recording. The time and frequency domain measures were computed, using HRV analysis software Kubios version 2.0, from the RR tachogram. The values were compared to reference values as provided by a study by Nunan *et al.*[4]

HR and BP response to standing

The RR tachogram for the ECG recording was retrieved and copied to a Microsoft Excel worksheet. A line diagram of the RR tachogram was used to identify the minimum and maximum RR intervals after standing. The RR intervals at the 15th and 30th s after standing were noted and a 30:15 ratio was calculated. The difference between baseline BP and BP at 3 min after standing was also computed.

HR response to deep breathing

From the line diagram, the minimum and maximum RR intervals were averaged over six cycles of inspiration (I) and expiration (E); this was computed as an E: I ratio.

BP response to sustained handgrip

The difference between the maximum DBP attained during handgrip and the basal BP was calculated and recorded as the rise in DBP during isometric handgrip.

The values obtained from the CAFTs were compared with accepted normal values from a previous research.[5] Each abnormal test was given one point; CAN was considered present if two or more tests were abnormal.

Biochemical analysis

Thirty minutes before lunch, an amount of 6 mL of venous blood was collected from all study subjects. The samples were centrifuged; serum samples were collected and stored at –80°C for the estimation of leptin and omentin levels (by ELISA technique).

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 19.0. The data were subjected to normality testing using the Kolmogorov–Smirnov test. Categorical data were expressed as frequencies or percentages and continuous variables as the mean with standard deviation or median with range. The Mann–Whitney *U* test was used to compare the baseline parameters as well as the leptin and omentin levels among diabetic patients with and without CAN. The Kruskal–Wallis test was used to compare the levels of the biomarkers among the five groups with CAN. Logistic regression was used to study the ability of the parameters to predict CAN. A value of $P < 0.05$ was considered significant.

Results

One hundred patients were included in this study. The demographic characteristics among diabetic patients with and without CAN are given in Table 1.

Among the study subjects, 64% were found to have CAN diagnosed when two or more of their CAFTs were abnormal. Among the subjects with CAN, 36 had two abnormal tests, 23 had three abnormal tests, and 5 had four abnormal tests. The CAN points were calculated based on the number of abnormal CAFTs that the subjects produced.

An increase in age and the duration of diabetes were found to be significantly associated ($P = 0.005$ and < 0.001, respectively) with CAN. The data were represented as median as it was nonnormally distributed, along with the minimum and maximum values. The Mann–Whitney *U* test was used to compare the parameters [Table 1].

Comparison of the various CAFT parameters showed that the root mean square of standard deviation (RMSSD), low-frequency–high-frequency (LF/HF) ratio, low-frequency normalized units (LF nu), high-frequency normalized units (HF nu), and E: I ratio were significantly different among diabetic patients with and without CAN. The values are represented as median, along with the minimum and maximum values. The comparison among the different parameters was analyzed using the Mann–Whitney *U* test [Table 2].

When comparing the biomarkers, leptin and omentin, among the diabetic patients with and without CAN, leptin levels were found to be significantly higher in those with CAN. No difference in omentin levels was found between those with or without CAN ($P > 0.05$). The values are represented as median, with minimum and maximum values. The comparison was performed using the Mann–Whitney *U* test [Table 3].

Correlation between BMI and leptin and omentin values were also performed using the Mann–Whitney *U* test. When BMI and leptin values were compared in diabetic patients with CAN, the

Table 1: Comparison of the demographic characteristics among diabetic patients with and without CAN

Parameter	CAN absent	CAN present	*P*
Age (median years)	51 (40-63)	59 (40-71)	0.005
BMI (median kg/m^2)	25.8 (21-29)	26.8 (20.8-32)	0.14
Duration of diabetes (median years)	5.5 (1-30)	10 (1-30)	<0.001
HbA1c (median %)	6.8 (5-10.6)	7.4 (5.8-12.4)	0.205

Table 2: CAFT parameters associated with the presence and absence of CAN in diabetic patients

CAFT parameter	CAN absent	CAN present	*P*
Time-domain indices			
RMSSD	12.4 (2.7-33.6)	6.8 (2-31.7)	<0.001
SDNN	15.2 (5-40.6)	11.7 (4.5- 36.8)	0.715
Frequency-domain indices			
LF/HF	3.2 (0.7-9)	1.04 (0.5-1.1)	<0.001
LF nu	46.5 (23-60)	26.7 (9.8-770	<0.001
HF nu	59 (39-77.8)	73 (22.7-90)	<0.001
Cardiovascular autonomic reflex tests			
30:15 RR interval ratio	1.11 (1.0-1.2)	1.08 (1.01-1.25)	0.39
BP drop on standing	2 (-3-8)	7 (-6-21)	0.213
E:I ratio	1.09 (1.05-1.34)	1.05 (1.01-1.109)	<0.001
Diastolic BP rise with isometric handgrip	19 (16-24)	18 (4-26)	0.004

Table 3: Comparison of the levels of biomarkers among diabetic patients with and without CAN

Biomarker	CAN Absent	CAN Present	*P*
Leptin (median ng/mL)	14.9 (6.5-22.7)	27.8 (18-106)	<0.001
Omentin (median pg/mL)	3839 (678- 9987)	6078 (751-10879)	0.168

P value was found to be < 0.05, which is statistically significant. Similarly, BMI and omentin levels were compared in diabetic patients with CAN, the *P* value was found to be < 0.00001, which is also statistically significant.

Further *post hoc* analysis was performed to assess if leptin levels were significantly associated with the number of CAN points scored during the CAFTs. This revealed that leptin levels were significantly higher in subjects with two CAN points compared to that in subjects with three or four CAN points. However, no significant differences were found between leptin levels of patients with three or four CAN points.

Logistic regression analysis was performed to ascertain the effects of age, gender, BMI, duration of diabetes, and leptin/omentin levels on the likelihood that study participants had CAN. The logistic regression model was significant ($P < 0.005$); this model explained 94.3% of the variance in CAN and correctly classified 97% of the cases. Higher leptin levels were associated with a higher risk of developing CAN.

Summary

1. CAN was present in 64% of patients with diabetes
2. Age and duration of diabetes were significantly higher among subjects with CAN
3. Leptin levels were elevated in patients with CAN unlike that of omentin
4. Higher leptin levels were associated with a higher possibility of developing CAN

Discussion

CAN is defined as the impairment of autonomic control of the cardiovascular system and is a significant contributor to morbidity and mortality among patients with diabetes mellitus.[6] Though it remains undiagnosed in its early stage as it is asymptomatic, there is evidence to suggest that subclinical CAN occurs within a year of developing diabetes.[7] As the pathogenesis of CAN implicates adipose tissue involvement, several adipocytokines are being studied as potential markers for CAN in patients with diabetes.

The prevalence of CAN in this study (64%) is higher than that found in previous Indian studies. A study by Shukla *et al.* found the prevalence of CAN in patients with diabetes to be 53.2%. Another study by Mehta *et al.* found the prevalence of CAN to be 57%.[8] The prevalence of CAN was found to be higher in the South Asian population than in the Western population.[9] Indians have a higher percentage of visceral fat and insulin resistance compared to the population in western countries. Dysautonomia and adipose tissue inflammation have a strong pathogenetic link which could explain the high prevalence of CAN in this study that mainly included overweight subjects. Older age and a longer duration of diabetes were significantly associated with CAN in this study. This finding has also been observed in a study by Pop-Busui *et al.*[10]

The CAFT parameters in this study included time-domain indices and frequency-domain indices. Time-domain indices such as RMSSD and SDNN were lower in patients with CAN. They represent the HF variations in the short-term ECG recordings. A reduction of these parameters suggests decreased vagal tone in the cardiovascular system.

This is further substantiated by the findings of the frequency domain indices. Deviation of autonomic balance toward the adrenergic side is shown by an increased LF nu and reduced HF nu in patients with CAN. The high-frequency component (HF nu) represents the parasympathetic system; the low-frequency component (LF nu) represents the sympathetic system. An elevated LF/HF ratio in diabetes patients with CAN is another marker of the tilt of the sympathovagal scale toward the sympathetic side. This is consistent with prior studies that have shown that an increased sympathetic drive, a reduced parasympathetic tone, and reduced heart rate variability are markers of CAN in patients with diabetes.

In this study, the median level of leptin was significantly higher in diabetic patients with CAN compared to those without CAN. Leptin is a hormone produced by the adipose tissue that regulates appetite. Autonomic dysfunction leads to a dysregulated inflammatory process in the adipose tissue, leading to the release of leptin (an inflammatory adipocytokine).[11,12] Activation of the inflammatory process leads to leptin resistance and hyperleptinemia. Hyperleptinemia is linked to the presence of insulin resistance and vascular complications in patients with diabetes.[13] This study showed a significant association between markers of sympathovagal imbalance, such as LF/HF ratio, RMSSD, and leptin levels. This supports the fact that reduced vagal tone and increased adrenergic drive are associated with ongoing inflammation, particularly in the visceral adipose tissue. In a study done by Kurajoh M *et al.*, plasma leptin level was found to be associated with cardiac autonomic dysfunction in type 2 diabetic patients with visceral obesity.[14]

Omentin levels in diabetic patients with CAN were not found to be different from that of diabetic patients without CAN. Omentin is an adipocytokine that is expressed by visceral adipose tissue. It has been shown to have anti-inflammatory properties; an increase in omentin levels in patients with CAN could be a compensatory rise.[15] A study by Jung *et al.* showed that the incidence of CAN and the number of CAN points increased across the omentin tertiles. Another study showed that omentin was negatively associated with carotid atherosclerosis, but it was positively associated with CAN.[16] As omentin was previously shown to have anti-inflammatory and cardioprotective properties, the study findings appear to be contradictory. However, an increase in omentin levels is a compensatory mechanism to counter the autonomic dysfunction caused by insulin resistance and ongoing inflammation. A study by Niersmann C *et al.* showed that higher omentin level in blood is associated with an increased risk for cardiovascular disease in patients with diabetes. This may be due to an inadequate upregulation of omentin level in

response to stimuli that raise the cardiovascular risk in patients with diabetes.[17]

Cardiac autonomic neuropathy is an underdiagnosed complication in type 2 diabetic patients. A high index of suspicion is needed for the diagnosis of CAN in older patients with long-standing diabetes. Serum adipocytokines can help as a supplementary tool in the confirmation of CAN in patients with diabetes.

The limitations of this study are listed below:

1. The prevalence of CAN in patients whose duration of diabetes is less than 5 years has not been estimated in this study.
2. This study does not reveal if leptin levels are significantly associated with CAN in patients with diabetes who have BMI below 24.9 kg/m^2.
3. Among the anthropometric measurements, only BMI was taken into account as the waist circumference of patients were not measured.
4. Ankle-brachial index was not measured that could have helped in the correct stratification of the cardiovascular risk of patients with diabetes.

Conclusion

In this study, CAN was recorded in 64% of patients with type 2 diabetes mellitus. Older age and having a longer duration of diabetes were associated with the development of CAN as a complication of diabetes. Leptin levels were higher in diabetic patients with CAN, whereas omentin levels were not different between those with or without CAN.

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Conflicts of interest

There are no conflicts of interest.

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