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Original Article

Cardiac autonomic dysfunctions in type 2 diabetes mellitus: an investigative study with heart rate variability measures

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Abstract: Cardiac autonomic neuropathy (CAN) is a common yet underdiagnosed complication of Type 2 diabetes mellitus (T2DM). Heart rate variability (HRV), a sensitive diagnostic marker of cardiovascular risk, could help detect CAN at its earliest stage. However, the progression of CAN based on age and disease duration in T2DM is lacking. In this study, we propose to explore the occurrence of CAN in patients with varying stages and duration of T2DM. This cross-sectional study involves participants with T2DM (n = 160) and healthy volunteers (n = 40) with an age range of 30-60 years of both genders. Patients in the T2DM group were further subdivided into four subgroups based on their disease duration [Prediabetes, disease duration <5 yrs (D1), 5-10 yrs (D2), and >10 yrs (D3)]. All participants underwent short-term HRV recording for 20 minutes and analyzed for both time and frequency domain measures. The study results showed a significant increase in Heart Rate (HR) in D1 (P = 0.031) and D3 (P = 0.001) groups compared to healthy controls. The time-domain measures of HRV were significantly reduced in the T2DM group compared to the healthy controls. Furthermore, this reduction is more intense in the D3 group than in D2 and D1. Correspondingly, in frequency domain parameters: total power, high-frequency power, and low-frequency power were significantly reduced in all the T2DM groups compared to healthy controls. The study concludes that the overall HRV (as determined by total power), sympathetic activity (low frequency power) and parasympathetic activity (time domain measures and high frequency power) were significantly reduced in all the diabetic subgroups except prediabetes as compared to the healthy controls, implying that both sympathetic and parasympathetic limbs are symmetrically affected in T2DM patients even in the earliest stages (<5 yrs) implying subclinical cardiac autonomic dysfunction in the earliest stages.

Keywords: Autonomic nervous system, type 2 diabetes mellitus, heart rate, heart rate variability, cardiac autonomic neuropathy, sympathetic and parasympathetic activity

Introduction

Diabetes Mellitus (DM) is a chronic, widely prevalent metabolic disorder marked by increased blood glucose levels, leading to severe complications of vital organs such as the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), heart (cardiopathy and other vascular complications) [1]. Further, diabetes can cause impairment of the autonomic nervous system functioning, leading to cardiac autonomic neuropathy (CAN). Cardiovascular autonomic neuropathy (CAN) is defined as abnormalities associated with heart-rate control and vascular dynamics. CAN is the result of complex interactions involving numerous mechanisms and pathways that contribute to neuronal ischemia and, eventually, neuronal death [2]. This Diabetic CAN, is a serious complication that affects one-third of patients with T2DM and is associated with five-fold increased risk of developing cardiovascular mortality [3]. The symp-

toms of CAN range from resting tachycardia and a fixed heart rate (HR) to the development of "silent" myocardial infarction. The common risk markers associated with CAN are age, duration of DM, glycemic control, hypertension, dyslipidemia, and microvascular complications. Multiple studies have shown the varying incidence and prevalence of CAN in diabetes ranging from as low 1.6% in well-controlled diabetic patients to as high as 90% in those with uncontrolled longstanding diabetics undergoing pancreas transplantation [4, 5]. CAN is often underdiagnosed, whereas the cardiovascular mortality, associated with CAN was found to be the primary cause of death in patients with T2DM [6, 7].

The under diagnosis of CAN is due to the lack of standardized diagnostics in the typical hospital context. According to published studies, the prevalence of CAN varies from 2% to 91 % in type 1 diabetic mellitus (T1DM) and 25% to 75% in type 2 diabetes (T2DM), this wide range in prevalence is due to this lack of standardized diagnostic criteria and under diagnosis at the hospitals. The early detection of CAN could improve prognosis and prevent cardiovascular complications [8].

CAN prevalence vary greatly depending on the diagnostic procedures utilized, the population analyzed, and the stage of the disease. Studies show that the duration of diabetes is an independent factor for developing CAN irrespective of diabetes type [9-11]. Despite the fact that CAN is associated with a longer duration of disease, certain studies indicate that it may also be present in newly diagnosed diabetic individuals, but at a much lower frequency [11]. CAN is discovered in around 7% of patients with type 1 or type 2 DM at the time of diagnosis, and the risk is projected to increase by approximately 2% to 6% annually [4, 11]. Other risk factors that influence CAN on DM are poor glycemic control, age, obesity, smoking, hypertension, hypercholesterolemia, distal polyneuropathy, nephropathy, and retinopathy [12].

Recent evidence suggests that multiple complex pathways are involved in CAN, whereas its entire pathogenesis is obscure. CAN occurs in a range of subclinical and clinical manifestations, from resting tachycardia to cardiomyopathy. According to the Toronto Consensus Panel on Diabetic Neuropathy, all diabetic patients, especially those with a history of poor glycemic control, macro/microvascular problems, and elevated cardiovascular risk, should be evaluated for CAN [10]. In clinical practice, CAN assessment methods include symptom and sign assessment, HR and BP-based cardiovascular reflex tests, short-term electrocardiography (ECG), QT interval prolongation, HR variability (24 h, classic 24 h Holter ECG), ambulatory BP monitoring, HR turbulence, baroreflex sensitivity, catecholamine assessment and cardiovascular sympathetic tests, heart sympathetic imaging [3]. Whereas, CAN is traditionally determined by cardiovascular reflex testing [13]. The initial preclinical sign of this issue is reduced Heart-Rate Variability (HRV), which refers to changes in the time intervals between R waves of the QRS complexes [14]. HRV is a quick, economical, and authenticated tool to help diagnose CAN at a subclinical stage, providing a significant association between cardiovascular morbidity and the autonomic nervous system [15, 16].

Further, Ewing et al. in 1970's proposed a five simple tests to assess autonomic functions [17]. Which include, (1) the R-R changes in paced deep breathing (expiration:inspiration ratio and E:I ratio); (2) HR response to standing-30:15 ratio-is the ratio of the greatest R-R interval (between the 20th and 40th beat) to the shortest interval (between the 5th and 25th beat) induced by a shift in position from horizontal to upright; (3) the HR response to the Valsalva maneuver; (4) the BP response to standing; and (5) the BP response to a continuous handgrip produced by muscle contraction via a handgrip dynamometer [15]. Further studies have also attested that HRV aids in diagnosing CAN at a subclinical stage, consequently providing the possibility for better prevention and treatment as low HRV is related to the progression of diabetes, predicting the early onset of metabolic syndrome and diabetic CAN [2].

Despite these measures available CAN is often under diagnosed due to the lack of standardized diagnostics in the typical hospital context. Moreover, no comprehensive studies have focused on the progression of CAN based on age and disease duration. Hence, the objective of the present study was to compare the HRV of T2DM patients with healthy controls based on their disease duration, (i) prediabetes, (ii) diabetes for less than five years, (iii) between five

to ten years, and (iv) more than ten years. This study would help understand the severity of CAN, which varies with age and disease duration. Also, this study would help detect CAN-associated severe complications early and aid in planning treatment strategies in the early stages of the disease.

Materials and methods

Study population

This cross-sectional study involves participants with T2DM (n = 160) and healthy volunteers (n = 40) with an age range of 30-60 years of both genders. Subjects in the T2DM group (n = 160) were further subdivided into four groups covering different disease duration: (i) prediabetes, (ii) diabetes with onset less than five years (D1), (iii) diabetes ranging from 5-10 years (D2), and (iv) more than ten years (D3). Patients were recruited form Karnataka Institute of endocrinology and research (KIER), Bangalore, India and were escorted to National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India for HRV analysis.

Exclusion criteria

Patients on medications known to affect autonomic functions (Beta-Blockers, anticholinergics, antidepressants and antipsychotics), patients with psychiatric illness like anxiety and depression and psychosis known to affect autonomic functions, patients with uncontrolled long term hypertension more than 5 years in spite of medication, patient with Hypothyroidism/Hyperthyroidism, patients with any recent history of fever, meningitis or encephalitis that may significantly influence CNS function or structure, patients with alcohol/substance abuse, patients with a cardiac pacemaker or other implanted or external electrical device, as well as pregnant or lactating female patients, were excluded from the study.

Inclusion criteria

Subjects ranging from 30 to 60 years of both genders were recruited for the study. Prediabetic patients with no co-morbidities and who were not on oral hypoglycemic agents/other medications are included in the study.

Patients with T2DM were recruited from the Karnataka Institute of Diabetology and healthy controls matching for age and gender matched were recruited from NIMHANS. The study was explained to all participants, and their written informed consent was obtained. Following recruitment, the patients underwent complete clinical examination with short-term HRV recording for 20 minutes. The temporal and frequency domains of HRV were measured in T2DM patients and compared to age- and gender-matched healthy controls (n = 40).

HRV analysis

For HRV recording patients should refine from smoking, caffeine intake for 2 hours and alcohol intake for 36 hours. They should have had adequate rest, at least 8 hours of uninterrupted sleep on the night before the assessment of HRV and regular breakfast on the day of assessment. They were made to lie quietly in a couch in supine position for 5 min to alleviate the anxiety in a sound attenuated room with dim lighting and the temperature ranging from 20 to 25°C. After explaining the procedure to the subject, HRV was analyzed as per the guidelines of the Taskforce report of 1996 [18]. Patients were attached with Lead II ECG and the signals were conveyed through an analogue digital converter (Power lab, 16 channels data acquisition system, AD Instruments, Australia) with a sampling rate of 1024 Hz. The data was stored in a personal computer and analyzed offline using an automatic programme that allowed visual checking of the raw ECG and breathing signal to obtain the HRV parameters in time-domain and frequency-domain, known as 'linear methods'. Twenty-minute basal recordings were stored and later analyzed to obtain both time and frequency domain parameters of HRV using HRV Analysis Software LabChart pro version 8 (Powerlab AD instruments). An artifact-free 5-minute segment was analyzed to obtain time and frequency domain parameters.

Time-domain analysis: In this method, a descriptive statistical tool will be applied to quantify the variations in RR intervals and the following parameters are computed; (a) Standard Deviation of RR intervals which is sensitive to all sources of variation (SDNN); (b) Root mean square of the standard deviation of RR intervals - (RMSSD); (c) Number of successive NN intervals that vary by more than 50 ms (NN50); and (d) Percentage of NN50 counts which are more sensitive to the highest fre-

Table 1. Demographic* details of the subjects included in this study

Variables	HC (n = 40)	Pred (n = 40)	D1 (n = 40)	D2 (n = 40)	D3 (n = 40)
Age (years)	49.53 ± 5.70	50.08 ± 6.46	49.43 ± 6.44	49.83 ± 5.18	51.65 ± 4.53
DM Duration	NA	0.45 ± 0.22	3.13 ± 0.91	7.33 ± 0.97	13.7 ± 1.65
Weight (Kg's)	69.70 ± 9.95	70.7 ± 10.47	71.67 ± 11.39	71.20 ± 8.52	71.02 ± 10.98
Height (cm's)	2.58 ± 0.31	2.60 ± 0.26	2.67 ± 0.26	2.71 ± 0.27	2.75 ± 0.24
BMI (Kg/m^2)	27.31 ± 4.39	27.50 ± 5.17	26.90 ± 3.78	26.41 ± 3.45	25.94 ± 3.72
Waist Hip Ratio	-	0.95 ± 0.06	0.96 ± 0.06	0.97 ± 0.04	0.97 ± 0.05
FPG (mg/dL)	-	109.37 ± 16.14	145.15 ± 37.31	164.37 ± 51.93	175.44 ± 56.51
PPPG (mg/dL)	-	150.26 ± 30.59	224.53 ± 69.95	232.20 ± 69.48	251.38 ± 94.73
HbA1c (%)	-	6.08 ± 0.24	7.93 ± 1.45	8.24 ± 1.74	8.58 ± 1.74

*All demographic data are presented as Mean ± Standard deviation (SD). None of these variables showed any difference between the various stages of diabetes: FPG = Fasting plasma glucose, PPPG = Post prandial plasma glucose, Pred = Pre-diabetes, D1 = Diabetes <5 years, D2 = Diabetes from 5-10 years, D3 = Diabetes >10 years (as per ADA-American diabetes association guidelines).

quency component and best predictors of parasympathetic activity (pnn50).

Frequency-domain analysis: For frequency-domain analysis the machine uses Fast Fourier Technique (FFT), which transforms R-R intervals into waves with three essential components: very low frequency ≤0.04 Hz (VLF), low frequency 0.04-0.15 Hz (LF), and high frequency 0.15-0.4 Hz (HF). HF describes the vagal activity, whereas LF blends the effect of sympathetic and parasympathetic influence. The signals were further used to calculate LF normalized unit (LF nu) and HF normalized unit (HF nu), LF normalized unit (LF nu) and HF normalized unit (HF nu) which represents the relative value of each component in proportion to the total power minus the very low frequency component and LF/HF Ratio reflects the sympathovagal balance and sympathetic modulation. Literature suggest a decrease in HF (a sign of parasympathetic dysfunction) with increased LF (sympathetic predominance) is observed in the early stages of autonomic dysfunction in diabetes, leading to an increase in LF/HF [18].

Furthermore, the fasting and post prandial blood sample was taken for estimation of plasma glucose by hexokinase method. HBA1C was measured by the high-performance liquid chromatography method using the Bio-Rad Variant 2 turbo analyzer. Prediabetes was diagnosed by FPG 100 to 125 mg/dl, 2-hour PPPG 140 to 199 mg/dl and HBA1c 5.7 to 6.4%. Diabetes was diagnosed by using American diabetes association (ADA) criteria that recommends Fasting plasma glucose ≥126 mg/dl and HBA1c ≥6.5% (**Table 1**). The American Diabetes Association is a United States-based non-profit organization that seeks to manage, cure and prevent diabetes.

Statistical analysis

Data were analyzed using IBM SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). All the parameters in this study were not normally distributed. Hence, the data is expressed using descriptive statistics such as median, quartiles for continuous variables, frequency and percentages for categorical variables. Continuous variables were tested for normality and the differences between the subgroups were analyzed using non-parametric tests with $P<0.05$ was considered statistically significant.

Results

A total of 200 volunteers of all the groups have undergone the HRV test Analysis. The values are exhibited in terms of Median (25th-75th percentile) = [M (Q1, Q3)]. Kruskal-Wallis test with Dunn's Correction was employed to compare the multiple groups of the study population. The statistical significance was set at a *p*-value <0.05. Analysis of Time domain and Frequency domain HRV parameters have presented in **Table 2**. HR of prediabetes and the diabetes groups showed higher values than healthy control group, but statistical significance was found with D1 and D3 groups compared to healthy controls. The values of SDNN in prediabetes and diabetes groups showed

Table 2. Time domain and Frequency domain parameters of heart rate variability (HRV) measures

Variables	Data represented as Median (Q1, Q3)					Kruskal-Wallis test		Multiple comparison with Dunn's correction
	HC (n = 40)	Pred (n = 40)	D1 (n = 40)	D2 (n = 40)	D3 (n = 40)	Chi-square	*P*-value	
HR (BPM)	71.39 (66.1, 82.1)	78 (72.2, 85.4)	80 (75.9, 90.0)	79.37 (68.0, 88.1)	82.06 (77, 89.7)	16.607	0.002**	HC vs. D1 = 0.031* HC vs. D3 = 0.001**
SDNN	42.34 (28.2, 57.25)	34.99 (22.13, 45.97)	29.29 (19.04, 42.37)	29.87 (20.72, 43.95)	26.57 (14.11, 34.07)	22.77	<0.001***	HC vs. D1 = 0.022* HC vs. D3≤0.001***
RMSSD	30.05 (22.5, 39.3)	23.05 (9.7, 34.0)	19.41 (11.9, 27.0)	19.49 (12.4, 26.8)	16.52 (8.6, 23.2)	23.411	<0.001***	HC vs. D1 = 0.016* HC vs. D2 = 0.003** HC vs. D3≤0.001***
NN50	28 (9, 60.5)	12 (0.75, 45)	5.5 (0, 24)	5 (0, 15)	2 (0, 15.7)	21.573	<0.001***	HC vs. D1 = 0.035* HC vs. D2 = 0.002** HC vs. D3≤0.001***
NN50%	8.08 (2.1, 19.3)	2.96 (0.1, 12.2)	1.49 (0, 6.2)	1.54 (0, 4.9)	0.45 (0, 3.9)	22.696	<0.001***	HC vs. D1 = 0.023* HC vs. D2 = 0.002** HC vs. D3≤0.001***
Total_Power (ms^2)	1661.3 (899.3, 2943.4)	1115.9 (464.5, 2188.3)	748.2 (349.1, 1732.9)	1061.6 (458.7, 1736.3)	741.6 (219.7, 998.3)	22.722	<0.001***	HC vs. D1 = 0.015* HC vs. D3≤0.001***
LF (ms^2)	312.7 (206.7, 563.92)	250.13 (67.23, 416.31)	228.76 (72.44, 397.24)	189.82 (102.7, 423.71)	153.94 (62.45, 267.39)	16.02	0.002**	HC vs. D3≤0.001***
HF (ms^2)	355.50 (209.7, 588.53)	197.91 (39.65, 413.80)	92.61 (46.25, 258.05)	119.49 (53.09, 283.86)	80.44 (33.60, 158)	25.239	<0.001***	HC vs. D1 = 0.003** HC vs. D2 = 0.003** HC vs. D3≤0.001***
HF_NU	44.32 (33.91, 54.96)	41.54 (27.08, 51.43)	39.59 (31.74, 49.44)	35.87 (24.26, 47.64)	30.25 (24.65, 39.12)	12.412	0.014*	HC vs. D3 = 0.008**
LF/HF Ratio	1.12 (0.69, 1.65)	1.2 (0.72, 2.15)	1.32 (0.78, 1.86)	1.4 (0.98, 2.6)	1.94 (1.24, 2.83)	10.701	0.030*	HC vs. D3 = 0.028*

Abbreviations: HC = Healthy Controls; Pred = Pre-diabetes; D1 = Diabetes <5 years; D2 = Diabetes from 5-10 years; D3 = Diabetes >10 years; HR = Heart Rate (BPM); SDNN = Standard Deviation of N-N intervals, in milliseconds; RMSSD = Root Mean square of successive differences between adjacent R-R intervals, in milliseconds; NN50% = Percentage of consecutive N-N intervals that differ by >50 milliseconds; TP = Total Power (The variations of NN intervals over the temporal segment, ms^2); LF = Low Frequency (ms^2); HF = High Frequency (ms^2); LF NU = Low Frequency (Normalized Units); HF NU = High Frequency (Normalized Units); LF/HF Ratio = Ratio of Low and High Frequency power; *P*-Value = Probability value; *Statistical Significance = *P*-value <0.05. **p<0.01; ***p<0.001.

lower values compared to healthy control group. However, statistical significance was found with D1 and D3 group in comparison with Healthy controls. The values of RMSSD, NN50 count, NN50% and HF values of prediabetes and diabetes groups showed lower values than the healthy controls and were also found to be statistically significant with all three diabetic groups compared to healthy controls. The values of Total power of Prediabetes and all the diabetes groups were lower in comparison with the healthy control, but the statistical significance was found with D1 and D3 groups in comparison with healthy controls. The values of LF of prediabetes and all the diabetes groups were lower in comparison with healthy control groups, yet found the statistical significant only with D3 group in comparison with healthy controls. The normalized units of HF of the D3 group were lower among all the groups and were found to be statistically significant compared to healthy controls. The LF/HF ratio of the D3 group showed greater values among all the groups and was statistically significant compared to Healthy controls.

Discussion

The autonomic nervous system is one of the major homeostatic regulatory systems of the body. HRV is making a valuable contribution to the diagnosis of cardiovascular autonomic dysfunction and CAN by exhibiting reduced HRV. Further, the measures of short-term resting HRV were found to be more accurate in diagnosis tests for detecting CAN [19]. The present study confirms the evidence of CAN in all subgroups of T2DM compared to healthy controls. HRV measures reveal that sympathetic and parasympathetic limbs of the ANS are symmetrically affected in all subgroups. However, it has been previously proposed that poor glycemic control in diabetes may contribute to the development of CAN. In addition, an overall reduction in HRV has been employed as a sensitive biomarker of subclinical CAN. In the present study, the subclinical assessment of CAN and its progression was studied upon T2DM based on disease duration using HRV measures [20, 21]. Numerous studies suggest that CAN result from complex interactions involving various processes and pathways, leading to neuronal ischemia and death [10, 11]. Hyperglycemia is the leading cause of this pathogenic process of CAN that leads to various complications [4]. Chronic hyperglycemia has been linked to mitochondrial dysfunction, membrane permeability, and endothelial dysfunction. In addition, these different pathways result in changes in gene expression, transcription factors, the disruption of several cellular activities, and communication between cells and the surrounding matrix-leading to neuronal malfunction and death [22]. Although there is substantial evidence that HRV abnormalities indicate an autonomic imbalance in Diabetes mellitus, no study has systematically addressed this issue by comparing groups with varying disease durations. Since the prospective comparison of HRV measures over 5-15 years with autonomic imbalance requires a longer duration (of few decades), our current study evades this temporal prolongation of the study period by comparing these disorders over patients with different disease duration: prediabetes, newly diagnosed T2DM with onset less than five years (D1), diabetes ranging from 5-10 years (D2), and more than ten years (D3). Age has a stronger positive correlation with the changes in CAN severity score and LF:HF ratio. Further, it is interesting to note that a shorter duration of diabetes is significantly associated with CAN [23].

The time and frequency domain characteristics of HRV and CAN development in T2DM patients were found to be associated independently [24]. The present study results were similar to the findings of Park et al., who reported subjects with diabetes had lower HRV values than normal subjects (SDNN; 36.2 ± 15.5 vs. 30.1 ± 14.4, *p*-value = .024) [25]. Furthermore, the standardized testing of HRV for autonomic function tests in the diabetes group with poor glycemic control as assessed by HbA1c levels of ≥8% showed lower (SDNN; 26.57 (14.11, 34.07) vs. 42.34 (28.2, 57.25), *p*-value = .001) compared with healthy controls [25]. The main findings of our study were that T2DM patients had significantly lower HRV in both sympathetic and parasympathetic activity than healthy controls, which can be explained by the adverse metabolic consequences of high blood glucose levels on HRV.

In this study, T2DM participants in the subgroups D1 (3.13 ± 0.91), D2 (7.33 ± 0.97), and D3 (13.7 ± 1.65) had a statistically significant

reduction in RMSSD, NN50% and HF (ms^2) compared to healthy controls. At the same time, a significant reduction in HR (BPM), SDNN, LF (ms^2) and Total Power (ms^2) occurred in D1 and D3 compared to HC. There was no significant change in the LF/HF ratio between DM subgroups and HC except for D3, which increased significantly. These findings match a previous population-based study that identified HF power to be lower in people with diabetes than in normal subjects [15].

In most clinical circumstances, parasympathetic activity decreases while sympathetic activity increases [26]. However, the present investigation indicated that both parasympathetic and sympathetic activities were decreased in T2DM patients. A possible explanation is that T2DM affects both sympathetic and parasympathetic fibres in the heart, causing cardiac autonomic neuropathy. The fact that the LF/HF ratio did not alter among the patients in this study could be attributed to changes in both the LF and HF components that have undergone similar alterations.

In an earlier study, Verma et al. [27] showed that patients with T2DM showed a reduction of HRV and increased LF nu and LF/HF ratio. These impairments were significantly higher for the group. However, there was no difference in these measures regarding disease duration (<5 vs. 5-10 vs. >10 years). On the other hand, duration of diabetes was strongly associated with a decrease in HRV similar to present study in another trial by Tarvainen et al., 2014 which has demonstrated that the most significant decrease in HRV was observed within the first 5-10 years of the disease and negative correlations of blood glucose level and HbA1c with most of the HRV parameters [28]. In T2DM with onset less than five years (D1), the early stage of CAN could cause damage to the vagus nerve, thus leading to sympathetic predominance. This increase in sympathetic tone could continue until advanced CAN when sympathetic denervation also occurs.

This is the first population-based study to examine multiple HRV variables (SDNN, LF and HF power, LF/HF ratio) across the spectrum of different subgroups of DM with varying disease durations. Our findings extend the previous observation made in selected studies with diabetic patients compared with healthy volunteers [29]. Thus, in this cross-sectional study of autonomic dysfunction assessed by HRV measures, correlation of disease duration and autonomic dysfunctions: RMSSD and HF power have a significant negative correlation with disease duration ($p<0.05$), indicating a decrease in both these measures with increased disease duration. Moreover, glycemic control assessed by HbA1c showed a negative correlation with RMSSD. Thus, our study confirms the earlier review by Helleputte et al., 2020 [30], which found a significant negative correlation between HRV measures and clinical measures such as glycemic variability (HbA1c) and disease duration. Thus, these HRV measures (RMSSD and HF power) could be used as predictors of glycemic control in patients with diabetes.

The present study finding of a reduction in HRV measures in pre-diabetes compared to HC was similar to a large study (Coopmans et al., 2000) involving more than 2000 subjects showed cardiac autonomic dysfunction even in the pre-diabetes stage of the disease as assessed by HRV measures [31]. Since the objective of our study is to detect autonomic abnormality through HRV measures and not to follow these patients with any changes in autonomic balance, as studied by Jun et al. 2019 [23], recovery from these dysfunctions was not assessed by subsequent follow-ups. Nevertheless, this prospective design might be the future direction of research and provide more details about the clinical significance of HRV measures in prognostication, especially the cardiovascular domain.

Our study has the strength that three disease groups were assessed with a varied duration of disease ranging from <5, 5-10 and >10 years, thus providing longitudinal changes with time. Although Cha et al. (2018) [24] have demonstrated longitudinal follow-up in same cohort of patients for around 8 years and evaluated cardiac autonomic and cardiovascular morbidity measures, our study showed similar cardiac autonomic dysfunctions in patients with different disease duration by examining their CAFT in a cross-sectional study design. Nevertheless, it would be imperative to conclude the importance of cardiac autonomic dysfunction analysis in patients with DM and look for cardiovascular morbidity in these patients. Further, patients with T2DM who have subclinical CAN,

which precedes clinically evident CAN, may have reduced HRV but go undiscovered by standard autonomic tests. Whereas short-term HRV appears to be a good approach for detecting pre-diabetic CAN [32].

Conclusion

We found substantial evidence of a significant drop in HRV among T2DM patients across all study groups. Assessing and monitoring the severity of T2DM using HRV could be a potential non-invasive, reliable, and pain-free measurement strategy to detect any diabetes-induced CAN and other complications. Both sympathetic and parasympathetic activity were reduced, possibly as a result of the negative effects of altered glucose metabolism on HRV. Further, the reduction in Time- and frequency-domain measures of HRV could also independently predict cardiovascular outcomes in patients with T2DM. Hence, ECG and HRV evaluation should be considered early in patients with diabetes.

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Disclosure of conflict of interest

None.

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References

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33 Suppl 1: S62-69.

[2] Balcıoğlu AS and Müderrisoğlu H. Diabetes and cardiac autonomic neuropathy: clinical manifestations, cardiovascular consequences, diagnosis and treatment. World J Diabetes 2015; 6: 80-91.

[3] Serhiyenko VA and Serhiyenko AA. Cardiac autonomic neuropathy: risk factors, diagnosis and treatment. World J Diabetes 2018; 9: 1-24.

[4] Pop-Busui R. Cardiac autonomic neuropathy in diabetes: a clinical perspective. Diabetes Care 2010; 33: 434-41.

[5] Kennedy WR, Navarro X and Sutherland DE. Neuropathy profile of diabetic patients in a pancreas transplantation program. Neurology 1995; 45: 773-80.

[6] Tancredi M, Rosengren A, Svensson AM, Kosiborod M, Pivodic A, Gudbjörnsdottir S, Wedel H, Clements M, Dahlqvist S and Lind M. Excess mortality among persons with type 2 diabetes. N Engl J Med 2015; 373: 1720-32.

[7] Shah AD, Langenberg C, Rapsomaniki E, Denaxas S, Pujades-Rodriguez M, Gale CP, Deanfield J, Smeeth L, Timmis A and Hemingway H. Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1·9 million people. Lancet Diabetes Endocrinol 2015; 3: 105-13.

[8] Bissinger A. Cardiac autonomic neuropathy: why should cardiologists care about that? J Diabetes Res 2017; 2017: 5374176.

[9] Dombrowski K and Laskowitz D. Cardiovascular manifestations of neurologic disease. In: Handbook of Clinical Neurology [Internet]. Elsevier; 2014 [cited 2022 May 13]. p. 3-17. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780702040863000011.

[10] Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, Stevens M, Kempler P, Hilsted J, Tesfaye S, Low P and Valensi P; Toronto Consensus Panel on Diabetic Neuropathy. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev 2011; 27: 639-53.

[11] Dimitropoulos G, Tahrani AA and Stevens MJ. Cardiac autonomic neuropathy in patients with diabetes mellitus. World J Diabetes 2014; 5: 17-39.

[12] Ziegler D. Diabetic cardiovascular autonomic neuropathy: prognosis, diagnosis and treatment. Diabetes Metab Rev 1994; 10: 339-83.

[13] Bengel FM, Sherif HM, Saraste A and Schwaiger M. Cardiac neurotransmission imaging. In: Clinical Nuclear Cardiology [Internet]. Elsevier; 2010 [cited 2022 May 12]. p. 674-88. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780323057967000746.

[14] Schumer MP, Joyner SA and Pfeifer MA. Cardiovascular autonomic neuropathy testing in patients with diabetes. Diabetes Spectr 1998; 11: 227.

[15] Benichou T, Pereira B, Mermillod M, Tauveron I, Pfabigan D, Maqdasy S and Dutheil F. Heart rate variability in type 2 diabetes mellitus: a systematic review and meta-analysis. PLoS One 2018; 13: e0195166.

[16] Thayer JF and Brosschot JF. Psychosomatics and psychopathology: looking up and down

from the brain. Psychoneuroendocrinology 2005; 30: 1050-8.
[17] Ewing DJ. Assessment of cardiovascular effects in diabetic autonomic neuropathy and prognostic implications. Ann Intern Med 1980; 92: 308.
[18] Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the European Society of cardiology and the North American Society of pacing and electrophysiology. Circulation 1996; 93: 1043-65.
[19] Min JW, Chang JY, Lee H, Park Y, Ko EJ, Cho JH, Yang CW and Chung BH. Clinical significance of heart rate variability for the monitoring of cardiac autonomic neuropathy in end-stage renal disease patients. Nutr Metab Cardiovasc Dis 2021; 31: 2089-98.
[20] Kempler P, Tesfaye S, Chaturvedi N, Stevens LK, Webb DJ, Eaton S, Kerényi Z, Tamás G, Ward JD and Fuller JH; EURODIAB IDDM Complications Study Group. Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM complications study. Diabet Med 2002; 19: 900-9.
[21] Stein PK, Barzilay JI, Domitrovich PP, Chaves PM, Gottdiener JS, Heckbert SR and Kronmal RA. The relationship of heart rate and heart rate variability to non-diabetic fasting glucose levels and the metabolic syndrome: the cardiovascular health study. Diabet Med 2007; 24: 855-63.
[22] Albers JW and Pop-Busui R. Diabetic neuropathy: mechanisms, emerging treatments, and subtypes. Curr Neurol Neurosci Rep 2014; 14: 473.
[23] Jun JE, Lee SE, Choi MS, Park SW, Hwang YC and Kim JH. Clinical factors associated with the recovery of cardiovascular autonomic neuropathy in patients with type 2 diabetes mellitus. Cardiovasc Diabetol 2019; 18: 29.
[24] Cha SA, Park YM, Yun JS, Lee SH, Ahn YB, Kim SR and Ko SH. Time- and frequency-domain measures of heart rate variability predict cardiovascular outcome in patients with type 2 diabetes. Diabetes Res Clin Pract 2018; 143: 159-69.
[25] Park SB, Lee BC and Jeong KS. Standardized tests of heart rate variability for autonomic function tests in healthy Koreans. Int J Neurosci 2007; 117: 1707-17.
[26] Liao D, Sloan RP, Cascio WE, Folsom AR, Liese AD, Evans GW, Cai J and Sharrett AR. Multiple metabolic syndrome is associated with lower heart rate variability. The atherosclerosis risk in communities study. Diabetes Care 1998; 21: 2116-22.
[27] Verma S, Alam R, Ahmad I, Singla D, Ali K and Hussain ME. Effect of glycemic control and disease duration on cardiac autonomic function and oxidative stress in type 2 diabetes mellitus. J Diabetes Metab Disord 2018; 17: 149-58.
[28] Tarvainen MP, Laitinen TP, Lipponen JA, Cornforth DJ and Jelinek HF. Cardiac autonomic dysfunction in type 2 diabetes - effect of hyperglycemia and disease duration. Front Endocrinol (Lausanne) 2014; 5: 130.
[29] Singh JP, Larson MG, O'Donnell CJ, Wilson PF, Tsuji H, Lloyd-Jones DM and Levy D. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). Am J Cardiol 2000; 86: 309-12.
[30] Helleputte S, De Backer T, Lapauw B, Shadid S, Celie B, Van Eetvelde B, Vanden Wyngaert K and Calders P. The relationship between glycaemic variability and cardiovascular autonomic dysfunction in patients with type 1 diabetes: a systematic review. Diabetes Metab Res Rev 2020; 36: e3301.
[31] Coopmans C, Zhou TL, Henry RMA, Heijman J, Schaper NC, Koster A, Schram MT, van der Kallen CJH, Wesselius A, den Engelsman RJA, Crijns HJGM and Stehouwer CDA. Both prediabetes and type 2 diabetes are associated with lower heart rate variability: the Maastricht Study. Diabetes Care 2020; 43: 1126-33.
[32] Phurpa M and Ferdousi S. Short-term heart rate variability: a technique to detect subclinical cardiac autonomic neuropathy in type 2 diabetes mellitus. Mymensingh Med J 2021; 30: 447-52.