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Original Article

Physiology Section

Short-term Heart Rate Variability to Evaluate Cardiovascular Autonomic Neuropathy in Newly Diagnosed Type 2 Diabetes Mellitus Patients: A Cross-sectional Study

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ABSTRACT

Introduction: Cardiovascular Autonomic Neuropathy (CAN) may be seen in patients of Type 2 Diabetes Mellitus (T2DM). In many previous studies it was seen that there is reduced heart rate variability in DM patients. Reduced heart rate variability is earliest indicator for the CAN. In different earlier studies, it was found that results of Short-term Heart Rate Variability (HRV) analysis of five minutes is comparable to standard 24 hours HRV analysis. In DM patients CAN is mostly found to be associated with a longer duration of disease, but according to some studies CAN may be present in newly diagnosed diabetes patients also, but the percentage is lower.

Aim: To assess the short-term HRV profile as cardiovascular risks among newly diagnosed T2DM patients and to find the correlation of HRV parameters with duration of disease and biochemical parameters; Fasting Plasma Glucose (FPG) and Postprandial Plasma Glucose (PPPG) for early detection and management of CAN.

Materials and Methods: The present study was a cross-sectional study conducted in the Department of Physiology in collaboration with the Department of Endocrinology at R.G. Kar Medical College and Hospital, Kolkata, West Bengal, India, from September 2020 to August 2021 on 56 newly diagnosed T2DM (age group 30-64 years). Study subjects were grouped according to the Standard deviation of NN intervals (SDNN) (millisecond) value as cardiovascular risk factor. Group 1 was the low risk group, group 2 was the moderate risk group, group 3 was the high-risk group. HRV testing was done with Physiograph Polyrite-D instrument with bio-amplifiers, 4 channels and accessories (RMS latest software-Version 3.0.16) in Autonomic Function Research Laboratory to measure HRV parameters. After collecting data, analysis was done using Statistical Package for Social Sciences (IBM, SPSS) version 23.0 Unpaired student's t-test, Chi-square test, Analysis of Variance (ANOVA) test, Pearson correlation test were performed and statistical significance of different parameters were evaluated. It was considered statistically significant with p-value <0.05.

Results: By assessing HRV parameters as cardiovascular risk factors among newly diagnosed T2DM patients the present study showed there was significantly (p-value=0.0065) high LF/HF ratio in males (1.70±1.19) than in females (0.99±0.69). The SDNN value was significantly highest in low cardiovascular risk group (137.29±25.49 ms) and lowest in high cardiovascular risk group (26.07±12.03 ms) (p-value=0.00001). Low Frequency and High Frequency Ratio (LF:HF ratio) was significantly more in age group of 51-60 years. Among all the patients, 34 (61%) patients had increased parasympathetic activity and 22 (39%) subjects had increased sympathetic activity. Female patients showed significantly more increased parasympathetic 24 (75%) activity than males 10 (41.7%). There was a significant association between SDNN values with the duration of disease (p-value=0.004).

Conclusion: The present study showed that cardiac autonomic neuropathy is present even at the time of diagnosis of newly diagnosed T2DM as there was sympathovagal imbalance. Female patients have more parasympathetic drive than males which indicates that females are more cardioprotective.

Keywords: High frequency, Low frequency, Parasympathetic activity, Standard deviation of NN intervals, Sympathovagal imbalance

INTRODUCTION

Diabetes Mellitus (DM) comprises of a group of metabolic disorders, resulting from defect in insulin action or insulin secretion. In the last two decades young adults of 30-39 years have become the fastest growing group for Type 2 Diabetes Mellitus (T2DM). Newly diagnosed diabetes was diagnosed according to the criteria of American Diabetes Association [1]. Chronically elevated blood glucose level may be attributed to autonomic neuropathy [2]. Diabetic neuropathy is the main cause of neuropathy in the world [3]. Cardiovascular Autonomic Neuropathy (CAN) is clinically considered as most important form of diabetic autonomic neuropathy. Reduced heart rate variability is earliest indicator for the CAN [4]. CAN can be presented as tachycardia at rest, exercise intolerance, orthostatic hypotension, asymptomatic ischaemia, myocardial infarction [5]. CAN in DM might be associated with abnormalities in control of heart rate, decreased baroreceptor sensitivity and late manifestations of impairment of vascular dynamics [6]. In DM patients CAN is mostly found to be associated with a longer duration of disease, but according to some studies CAN may be present in newly diagnosed diabetes patients also [7-9]. CAN may be reported in newly diagnosed T2DM patients without any prior history of ischaemic heart disease and it is associated with sudden death and increase in mortality rate in patients [10]. It is found in most of the times that Diabetic Autonomic Neuropathy (DAN) affects the nerves in length dependent manner, so in diabetic patients it is commonly seen that the longest parasympathetic autonomic nerve, the vagus nerve is first effected.

Vagal involvement decreases parasympathetic tone and can be manifested as resting tachycardia. CAN may be presented as sympathetic denervation in later stage [11]. The porges polyvagal

theory describes the pathway of Heart Rate Variability (HRV). This theory describes the influence of vagal tone on heart rate variability [12]. This suggests that standardised assessment of vagal tone is a very useful measure to assess cardiac health. Short-term HRV analysis was taken as five minutes recordings in earlier studies for early diagnosis of cardiac autonomic neuropathy [13,14]. Measurement of short-term HRV (5 minutes) is a clinical tool for screening and identifying patients at risk for cardiac mortality particularly in DM. Short-term HRV analysis (5 minutes) is very subject friendly (This technique was used in the present study). Study regarding short-term HRV to assess CAN in newly diagnosed T2DM is lacking, especially in eastern India. This study was conducted to find out whether sympathovagal imbalance is present in newly diagnosed T2DM patients as manifested by altered HRV which was measured by Short-term HRV analysis (5 minutes).

Study regarding short-term HRV to assess CAN in newly diagnosed T2DM is lacking, especially in eastern India. So, on this background the present study was conducted to find whether sympathovagal imbalance indicating CAN is present in newly diagnosed T2DM patients as manifested by altered HRV. The objectives of the present study were to assess the HRV parameters as cardiovascular risks among newly diagnosed T2DM and to find out any correlation of HRV parameters with duration of disease and biochemical parameters; Fasting Plasma Glucose (FPG) and Postprandial Plasma Glucose (PPPG) in newly diagnosed T2DM.

MATERIALS AND METHODS

This cross-sectional study was conducted on 56 newly diagnosed T2DM patients, with age group of 30 and 64 years (both genders) attending diabetic clinic run by the Department of Endocrinology and screened in the Autonomic Function Research Laboratory in the Department of Physiology, R.G. Kar Medical College and Hospital, Kolkata, West Bengal, India, from September 2020 to August 2021. Ethical clearance has been obtained from the Institutional Ethics Committee of the R.G. Kar Medical College, prior to study. Informed written consent was taken from each patient.

Sample size calculation: Sample size has been calculated by using the following formula [15]:

$$\text{Sample size (N)} = \frac{(Z1\text{-}a/2)^2 \times P \times Q}{D^2}$$

Where,

$(Z_{1-a/2})$=Standard normal variation (At 2SD of variation and at 95% confidence level i.e, $Z_{1-a/2}$=1.96

P=Prevalence of CAN in newly diagnosed T2DM 15.3% [16];

Q=100-P;

D=absolute error or precision (taken as 7%)

So sample size N=101.59. Taking into consideration of non responsive rate of 15% final sample size will be 117. But due the Coronavirus Disease 2019 (COVID-19) pandemic situation the present study was unable to include 117 study subjects and was able to include only 56 study subjects.

Inclusion criteria: Patients of both genders, newly diagnosed T2DM patients, age between to 30-64 years, patients willing to take part in the study. Newly diagnosed diabetes was diagnosed according to the criteria of American Diabetes Association [1] were included in the study.

Exclusion criteria: Patients who had already developed symptoms of autonomic neuropathy, patients with bowel and bladder disturbances, impotence (previously diagnosed, taken from the history sheet), postural hypotension, hypertension, hyperthyroid or hypothyroid patients, underlying cardiovascular illness like ischaemic heart disease, rheumatic heart disease, arrhythmia, cardiac failure, Chronic Obstructive Pulmonary Disease (COPD), asthma, sputum positive pulmonary tuberculosis, psychiatric diseases, recent surgery or trauma, patients on drugs known to affect autonomic functions like beta-blockers, diuretics, vasodilators, antiarrhythmics. Any hereditary history of T2DM was not taken in consideration.

Study subjects were grouped according to the Standard deviation of NN intervals (SDNN) (millisecond) value as cardiovascular risk factor.

- Group-1 was the low risk group
- Group-2 was the moderate risk group
- Group-3 was the high-risk group

Study Parameters

Time domain: Time domain parameters of HRV analysis were taken like Standard deviation of RR intervals (SDRR), Standard deviation of NN intervals (SDNN), Standard deviation of the average NN Intervals (SDANN).

Time domain analysis measures the heart rate changes overtime or the intervals between Successive normal cardiac cycles. A continuous Electrocardiogram (ECG) recording was obtained and each QRS complex (QRS complex is a group of waves seen on an electrocardiogram, representing ventricular depolarisation) was detected and the normal RR intervals due to instantaneous heart rate then determined. Normal sinus rhythm intervals or NN intervals obtained after eliminating artifacts like abnormal beats, ectopic beats, omitted heart beats and noise from machine. Standard Deviation (SD) of RR or NN intervals is generally considered to reflect the day-night changes of heart rate variability. SDNN is an index of heart rate variability and reflects circadian rhythms and long-term components which are responsible for variability of heart rate within the period of recording of the ECG.

Frequency domain: Frequency domain parameters of HRV analysis were Low Frequency (LF), High Frequency (HF), LF/HF ratio.

Frequency domain (power spectral density) describes periodic oscillations of the heart rate signal. The periodic oscillation of hearts sinus rhythm contains different frequencies with different amplitudes. One of the simple and rapid method to analyse the frequency domain or spectral density of heart rate variability is by Fast Fourier Transformation (FFT) method which is characterised by discrete peaks for the several frequency components of hearts sinus rhythm and individual R-R intervals stored in the computer are transformed into bands with different spectral frequencies [17]. The power spectrum consist of frequency band ranging from 0-0.5 Hz and can be classified into four bands [17]. Ultra Low Frequency (ULF), Very Low Frequency (VLF), Low Frequency (LF), High Frequency (HF).

Data Collection

Then each patient underwent history taking and physical examination. FBS and PPBS values were recorded from the history sheet of the study subjects which were available for each study subjects. The Waist Circumference (WC) was measured at the midpoint between the lower margin of the last palpable ribs and the top of the iliac crest, and Hip Circumference (HC) was measured around the widest portion of the buttocks, with the tape parallel to the floor using a non elastic tape [18]. The Waist Hip Ratio (WHR) was obtained by dividing the WC by the HC using the same units of measurements for both [18].

The conditions required for the HRV test were strictly maintained as per standard protocol; prior to the test for 12 hours atleast, the subject should not take tea, coffee, food, any sedative or any drug affecting central nervous system [17]. The subjects were advised to wear loose clothing, no metallic objects, have a sound sleep the night before the test, to evacuate bladder before doing the test and to avoid smoking in preceding 24 hours. The test procedures were performed in the morning as far as practicable and as standard guidelines. Autonomic function testing was done with Physiograph Polyrite-D instrument with bio-amplifiers, 4 channels and accessories (RMS latest software-Version 3.0.16) in Autonomic Function Research

Laboratory Department of Physiology R.G. Kar Medical College and Hospital. The room temperature of the laboratory was maintained between 18°C and 25°C. The lighting of room was maintained as minimum as possible. The subjects were asked to take rest in supine position for 15 minutes prior to the study.

Study Procedure

After proper counselling and adequate rest patients were asked to maintain following points during examination: not to sleep; no vigorous movements; no emotional over excitations; not to cough, sneeze or any other activities, to keep calm during examination. Both upper limbs were exposed up to elbows and the left lower limb was exposed up to knee. The exposed parts of limbs were properly cleaned with alcohol swab. All the clamp type limb electrodes (right hand, left hand and left foot) were attached using electoconductive jelly. Hand electrodes were placed around wrists and foot electrode was placed just above the ankle of left foot. All the electrodes were connected to bio-amplifier of the Physiograph polyrite-D via a transducer mechanism. The resting Heart Rate is obtained from the Polyrite-D. Frequency domain (spectral) and time domain analysis of Short-term (5 minutes) HRV was recorded. There was no single HRV number. To attain in depth reliability of HRV test relative and absolute reliability of the most common HRV parameters such as SDNN and LF/HF ratio are considered. European Society of Cardiology and North American Society of Pacing constituted task force to develop appropriate standards and proposals of HRV measurements [17]. But as a general rule the present study takes normal range of HRV values according to upper and lower 95% confidence limit. SDNN value over 100 ms can be considered normal, SDNN value less than 50 ms is considered high-risk, and a SDNN between 50-100 ms can indicate moderate risk, they are related to cardiovascular risks [19]. LF-HF ratio less than 1 indicates good cardiovascular health [20].

STATISTICAL ANALYSIS

After collection of data, statistical analysis was done by the Statistical Package for Social Sciences (SPSS) version 20.0. Unpaired student's t-test, Chi-square test, Analysis of Variance (ANOVA) test, Pearson correlation test were performed and statistical significance of different parameters were evaluated. It was considered statistically significant when p-value <0.05.

RESULTS

The baseline characteristics of the study subjects were considered, female patients had more waist circumference than males, but it was statistically insignificant (p-value=0.1659). In the present study, male patients had more waist hip ratio than females, and it was very significant (p-value=0.0001) [Table/Fig-1]. Mean hip circumference was significantly more in females (101.38±6.54 cm) than in males (95.71±7.31 cm) (p-value=0.0035). Mean height was more in males (162.88±4.64 cm) than females (153.88±5.89 cm) (p-value=0.0001). The mean of waist height ratio was significantly more in females than in males (p-value=0.0001).There were no significant difference of duration of diabetes mellitus FPG (p-value=0.099), PPPG (p-value=0.69) between males and females.

More SDNN value in male (45.21±28.94 ms) than female (43.42±46.47 ms), but that change was not significant (p-value=0.8692). There was significantly (p-value=0.0065) high LF/HF ratio in males (1.70±1.19) than in females (0.99±0.69) [Table/Fig-2].

Parameters	Male (Mean±SD)	Female (Mean±SD)	p-value (Unpaired student's t-test)
Hip circumference (cm)	95.71±7.31	101.38±6.54	0.0035*
Waist circumference (cm)	91.04±7.90	93.44±4.82	0.1659
Height (cm)	162.88±4.64	153.88±5.89	0.0001*
Waist hip ratio	0.95±0.03	0.92±0.02	0.0001*
Waist height ratio	0.56±0.05	0 .61±0.03	0.0001*
Duration of DM (month)	14.54±5.69	14.59±5.86	0.97
Fasting plasma glucose (mg/dL)	137.38±31.69	160.47±61.49	0.099
Postprandial plasma glucose (mg/dL)	228.63±78.26	238.38±100.24	0.69

[Table/Fig-1]: Baseline characteristics of study subjects.
*p-value <0.05 was considered statistically significant

Parameters	Male (Mean±SD)	Female (Mean±SD)	p-value (Unpaired student's t-test)
SDNN (ms)	45.21±28.94	43.42±46.47	0.8692
LF/HF	1.70±1.19	0.99±0.69	0.0065*

[Table/Fig-2]: Comparison of SDNN (ms) and LF/HF ratio among males and females.
SDNN: Standard deviation of NN intervals; LF: Low frequency; HF: High frequency
*p-value <0.05 was considered statistically significant

[Table/Fig-3] showed the distribution of study subjects according to the SDNN (ms) value as cardiovascular risk factor, there were significantly different SDNN values in three cardiovascular risk groups and the change was statistically (p-value=0.0001) significant. The mean of SDNN value 137.29±25.49 ms, was highest in group 1. Group 3 showed lowest mean SDNN value, 26.07±12.03. The result showed that 10.71% cases in group 1, 10.71% in group 2 and group 3 which was the high-risk group had 78.5% of the study subjects.

Groups	n, %	SDNN (Mean±SD)	p-value (Kruskal-Wallis test)
Group 1	6 (10.71%)	137.29±25.49	0.00001*
Group 2	6 (10.71%)	83.90±13.18	
Group 3	44 (78.5%)	26.07±12.03	

[Table/Fig-3]: Distribution of study subjects according to the SDNN value (ms) as cardiovascular risk factor.
*It was considered statistically significant when p-value <0.05; *p-value <0.05 was considered statistically significant

[Table/Fig-4] showed that mean of LF:HF ratio (1.97), was maximum in age group of 51-60 years. LF:HF ratio (0.99) was minimum in age group of 30-40 years (p-value=0.023). Mean of SDNN (ms) changes in different age groups were not statistically significant (p-value=0.610).

Age group (years)	LF/HF ratio (Mean±SD)	SDNN (ms) (Mean±SD)
30-40	0.99±0.72	37.63±29.84
41-50	1.10±.90	52.57±53.69
51-60	1.97±1.25	39.55±32.24
>60	1.31±0.05	56.35±21.89
p-value (ANOVA test)	0.023*	0.610

[Table/Fig-4]: Comparison of LF/HF ratio and SDNN value in different age groups.
SDNN: Standard deviation of NN intervals; LF: Low frequency; HF: High frequency
*p-value <0.05 was considered statistically significant

In the present study [Table/Fig-5] showed that 34 (61%) patients had parasympathetic overdrive and 22 (39%) subjects had sympathetic drive. In this study, the subjects significantly more male patients had sympathetic drive (n=14, 58.33%) than female patients (n=8, 25%). Female (n=24, 75%) patients showed significantly more parasympathetic drive than males (n=10, 41.7%). The results are statistically significant (p-value <0.05).

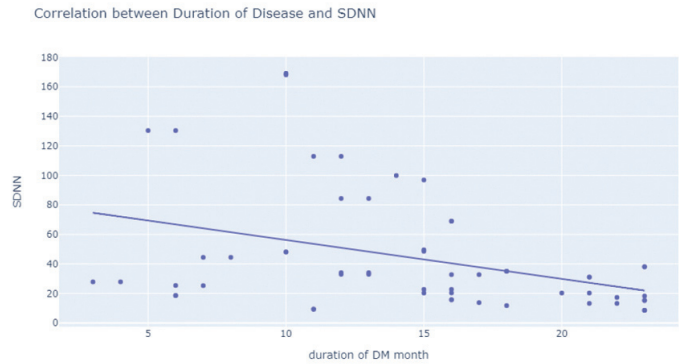
Parameters	Male	Female	Total
Sympathetic	58.33% (n=14)	25% (n=8)	39% (n=22)
Parasympathetic	41.7% (n=10)	75% (n=24)	61% (n=34)
p-value	<0.05*		

[Table/Fig-5]: Comparison of study subjects according to sympathovagal balance in males and females.
Test applied-Chi-square test; *It was considered statistically significant when p-value <0.05

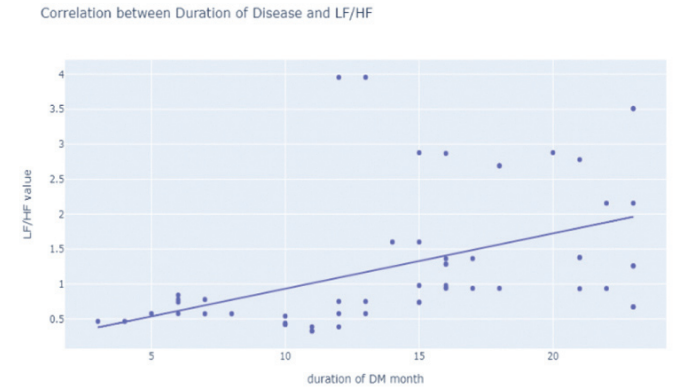
From [Table/Fig-6] we found that SDNN had a negative association with duration of diseases (r=-0.38) and the correlation was statistically significant (p=0.004). SDNN shows positive association with FPG (mg/dL) (r-value=+0.001) and PPPG (mg/dL) (r=+0.02), but their correlation was not significant (p-value=0.903). [Table/Fig-7] also shows strong negative correlation of SDNN with duration of diabetes. From [Table/Fig-6] we also found that LF/HF ratio had statistically significant positive association with duration of disease but no association with Fasting Plasma Glucose (FPG) and Postprandial Plasma Glucose (PPPG). [Table/Fig-8] also showed strong association of LF/HF ratio with duration of disease.

Parameters	SDNN (ms)		LF/HF ratio	
	r-value	p-value	r-value	p-value
Duration of T2DM (month)	-0.38	0.004*	+0.458	0.0001*
FPG (mg/dL)	+0.001	0.992	+0.146	0.282
PPPG (mg/dL)	+0.02	0.903	+0.186	0.169

[Table/Fig-6]: Pearson correlation of SDNN with age and anthropometric parameters of study subjects.
SDNN: Standard deviation of NN intervals; LF: Low frequency; HF: High frequency; Test applied-pearson correlation test; *p-value <0.05 was considered statistically significant



[Table/Fig-7]: Correlation of SDNN value with duration of disease (r-value=-0.38, p-value=0.004).
SDNN: Standard deviation of NN intervals



[Table/Fig-8]: Correlation between duration of disease and LF/HF (r-value=+0.458, p-value=0.0001).
LF: Low frequency; HF: High frequency

DISCUSSION

Recently, short-term HRV has been proposed as the most sensitive indicator of autonomic function, and CAN can be manifested as reduced HRV [21,22]. Malik M et al., found in a study that HRV indices mirror the sympathetic and parasympathetic activities in a subject [17]. In a previous study of Welborn TA et al., waist hip ratio was found to be a better indicator than waist circumference in evaluation of obesity and associated cardiovascular risk factors [23]. Waist hip ratio can indicate severity of CAN [24]. The present study showed that waist hip ratio is more in male that female which signified that male patients were at higher cardiovascular risk. The present study showed that waist-height ratio was more in females than males which indicates that female patients were more obese than males. Though studies suggested that waist-height ratio is a new parameter and more research is needed to confirm it is as a valid measure to evaluate cardiovascular risk prediction [25].

In the present study, the frequency domains for evaluation of CAN were considered, as they are most widely used parameters. Previous study of Kuehl M et al., discussed that early diagnosis of CAN, using spectral analysis of heart rate variability or scintigraphic imaging techniques, might enable identification of patients at highest risk for the development of clinical CAN [26]. In the present study mean of SDNN was slightly higher in males than females, but p-value was non significant (p-value=0.8692). Whereas, LF/HF ratio in males were significantly higher than females. In a short-term HRV of 5 minutes study LF/HF ratio is a better indicator of sympathovagal balance than SDNN value [17]. Pop-Busui R, in their study found that diabetes autonomic neuropathy affects the nerves mostly in length dependent manner and the longest parasympathetic autonomic nerve, the vagus nerve affects first, though CAN causes sympathetic denervation also [11]. The present study found sympathovagal imbalance in study subjects which was indicated by high LF:HF ratio in males where male patients showed more sympathetic overdrive which is an indication of cardiac autonomic neuropathy [17] and female patients showed more parasympathetic overdrive. The present study found male patients had more sympathetic overdrive than females but Zoppini G et al., found that prevalence of cardiac autonomic neuropathy in newly diagnosed type 2 diabetes cases were not different between males and females [27]. The present study showed that male subjects had more LF/HF ratio than female subjects of newly diagnosed T2DM. Hogarth A et al., also showed that male subjects had more LF/HF ratio than female among T2DM patients [28].

The present study has shown that out of 56 patients, 22 (39%) patients expressed sympathetic overactivity. Among all the male patients 58% (14 out of 24) males have shown significantly more sympathetic overactivity whereas 75% female patients (24 out of 32) among the all female patients showed significantly more parasympathetic overactivity. So, male patients clearly suppressed the general trend of parasympathic overactivity and showed gross deviation from general trend and inclined towards sympathetic overactivity and females were more cardioprotective than males. This finding of sympathetic overdrive more in males can be explained by different causative factors suggested by a study by Morrison SF, and another study by Grassi G, and these study showed that cardiovascular and metabolic functions are modulated by sympathetic mechanism and increased adrenergic drive is a basis of several pathologic states in metabolic syndrome [29,30]. In this socio-economic strata daily struggle of existence is more in males than females which causes more stress in males [31]. Stress causes dysregulation of adrenocortical and sexual steroids, but it was not considered during history taking in the present study. In previous study it was found that stress causes release of more cortisol which impairs insulin secretion and shows diabetogenic effect [32]. Stress is major risk factor for developing sympathovagal imbalance. Fenollar-Cortés J et al., in a study during COVID-19 pandemic found that women group coped up with negative emotions like stress due to confinement in pandemic situation, more than males over time [33]. In a previous study of Sloan RP et al., HRV variables changed with stress which showed low parasympathetic activity and an increase in LF/HF ratio [34]. So the findings of the present study which showed increase in LF/HF ratio indicating low parasympathetic activity in males than the females may be due to stress.

Android obesity or central obesity in males i.e., the distribution of human adipose tissue around the trunk and upper body, in areas of abdomen, chest, shoulder and nape of the neck are associated with increased proinflammatory state with more liberation of adipokines, TNF-alpha and IL-6 men are found to have greater visceral and hepatic adipose tissue, show more insulin resistance and do not

have cardioprotective hormone like oestrogen unlike women [35]. In a previous study by Varlamov O et al., it was found that there are sex differences in cardiometabolic profile and women is at lower risk, due to main difference in sex steroids [36]. The present study finding was similar with the previous study and showed vagal dominance in female patients. Endorphins act on the opioid receptors in the brains, they reduce stress and pain, also boost up pleasure and has calming effect [37]. Earlier studies suggest that oestrogen may have different cardioprotective roles in females. Oestrogen acts as a vasodilator, can protect from endothelial dysfunction and thus reduces vascular diseases [38]. Specific action of oestrogen on Corticotropin-Releasing Factor (CRF) gene is yet undetermined, It may so happen that oestrogen might reduce the synthesis and release of CRF [39]. In effect less stress will be perceived by females than males. Aldosterone is fibrotic and proinflammatory. Oestrogen reduces aldosterone synthesis, hence minimises the chance of cardiovascular morbidity and mortality by preventing unwanted cardiac remodelling [40]. Evidence suggests that oestrogen may enhance baroreceptor sensitivity and by this fine tuning of baroreceptors oestrogen may reduce sympathetic overdrive to a large extent [41].

The present study subjects were distributed to different cardiovascular risk groups according to SDNN value and showed most of the subjects were high-risk patients so far SDNN value was concerned. A previous study showed that in the HR analysis, statistically significant differences were found in the time domain, specifically on short-term values such as standard deviation of NN intervals (SDNN) [42].

The present study showed that there was reduced HRV with advancement of age which was statistically also significant (p-value=0.023). One previous study, showed that the variables SDNN (p-value=0.04), RMSSD (p-value=0.007), pNN50 (p-value=0.004) and triangular index (p-value=0.01) were significantly lower in the older age group indicating that this group had lower parasympathetic activity [43]. The findings can be explained as homeostenosis is a problem of aging [44]. Aging can lead to increased vulnerability to different diseases [45]. The oxidative stress with aging process can also associated with cardiovascular risks [27,43].

In the present study, FFG showed positive correlation and PPPG also showed positive correlation with LF/HF ratio, results were statistically non significant. Any statistically significant association between SDNN value with biochemical parameters like fasting plasma glucose and postprandial plasma glucose was not found. Dhumad MM et al., found poor glycaemic control associated with CAN [46]. This finding has made us thought that degree of glucotoxicity is less severe in new onset DM patients. In the present study Pearson correlation test showed that there was negative correlation between SDNN and duration of disease of diabetes mellitus, and it was found to be statistically significant [Table/Fig-6]. The previous study showed that duration of diabetes emerged as a significant independent factor for the development of CAN among T2DM patients [27].

However what is more important is that CAN is present even at the time of diagnosis of newly diagnosed T2DM. So CAN screening should be routinely done in all patients even if T2DM is newly diagnosed.

Limitation(s)

- Sample size was less, due to COVID-19 pandemic situation authors cannot attend the required sample size.
- It was a cross-sectional study which did not enable us to establish a causality relationship.
- The lack of a non diabetic control group for comparison was another limitation.
- The present study has not grouped the subjects based on BMI.
- In developing countries majority of people with T2DM between 45-64 years age, thus the females from 30 to 64 years were included.
- As the present study included both males and females in the age groups 30-64 years so menstruation and postmenopausal woman were confounding factor.
- All patients included in the study diagnosed with newly diagnosed T2DM were not on similar drug.

CONCLUSION(S)

The present study clearly points towards the major difference between male and female newly diagnosed type 2 diabetic patient so far as sympathovagal balance is concerned. Female patients have more parasympathetic overdrive than males and cardiac autonomic neuropathy is present even at the time of diagnosis of newly diagnosed type 2 diabetes mellitus. To prevent future complications from cardiac autonomic neuropathy authors suggest regular aerobic exercise which improves vagal tone, and reduces cardiovascular morbidity and mortality, HRV monitoring as a routine test from the time of diagnosis of type 2 diabetes mellitus. Further prospective studies with larger sample size are required to confirm the association between study outcomes and other variables. Evaluation of cardiac autonomic neuropathy by HRV test on newly diagnosed subjects on similar drug, with larger sample size, excluding postmenopausal women and evaluating the subjects considering HbA1c as a good indicator among different groups based on BMI should be further studied.

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