Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

Novel heart rate variability metrics as a predictor of peripheral nerve damage in Type 2 diabetes mellitus

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Abstract:

Background: Diabetic peripheral neuropathy (DPN) often diagnosed at later stages when damage to the sensory receptors has already taken place in Type 2 Diabetes Mellitus (T2DM) patients. It is frequently accompanied by cardiac autonomic dysfunction. This study aimed to compare heart rate variability (HRV) between T2DM patients with and without neuropathy, find its association with DPN, and evaluate the predictive utility for early neuropathy detection.

Methods: In this cross-sectional study, 66 T2DM patients at AIIMS Kalyani and categorized into neuropathy (n=37) and no neuropathy (n=29) groups. Neuropathy was assessed using vibration perception threshold (VPT). Five-minute resting electrocardiograms were recorded and HRV was analyzed in Kubios software. Independent t-test followed by corrleation and binomial logistic regression were performed. Receiver operating characteristic analysis identified significant HRV predictors, while principal component analysis characterized two distinct HRV patterns between groups.

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

Results: Compared with the no-neuropathy group, neuropathy group had higher fasting and postprandial glucose, longer diabetes duration and higher VPT ($p<0.01$). Neuropathy group showed significantly lower RMSSD, SDNN, HF power, SD1, and sample entropy, and higher LF power and LF/HF ratio. RMSSD, LF power, and sample entropy significantly predicted neuropathy (AUC= 0.799). Principal component analysis identified two distinct components representing autonomic dysfunction and sympathovagal modulation, with PC1 differing significantly between groups ($p<0.01$).

Conclusion: The integrated assessment of HRV parameters using PCA revealed two distinct autonomic phenotypes, elucidating heterogeneous patterns of autonomic dysfunction in DPN. Our findings also suggest that autonomic impairment in DPN is not uniform, but it occurs through multiple underlying physiological pathways.

Keywords: Autonomic dysfunction, Diabetic peripheral neuropathy, RMSSD, LF power, Sample entropy, Principal component analysis,

Introduction

Diabetic peripheral neuropathy affects nearly 50% of the long-standing diabetes patients and heightens the probability of foot ulcers, amputations of the limbs and increased cardiovascular complications [1]. Type 2 diabetes mellitus (T2DM) becomes a chronic, progressive, metabolic disease characterized by altered blood glucose level and is associated with a wide spectrum of chronic complications. Despite somatic nerve involvement, T2DM also disrupts the autonomic nervous system (ANS), leading to cardiac autonomic neuropathy (CAN) associated with cardiac arrhythmias, ischemia, increased mortality [2]. However, small nerve fibers are responsible for cardiac autonomic neuropathy are affected earlier than large nerve fibers

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

Diabetic peripheral neuropathy is mostly caused by damage to the large peripheral nerve fibers [3]. Till date there is absence of reliable and accurate test for early detection of diabetic peripheral neuropathy which was often diagnosed at later stages when damage to the sensory receptors has already taken place [4]. If diabetic peripheral neuropathy (DPN) can be detected in early stages, the small nerve fibres in the foot can be protected from progressive damage. If diagnosed during the late stages, it is too difficult to improve the condition and provide healthy treatment. Thus, early detection and management would help reduce the incidence of foot ulcers and amputations which has been increasing by 25% every year among T2DM patients [5]. However, DPN is often associated with cardiac autonomic neuropathy, leading to cardiac autonomic dysfunction that can be evaluated non-invasively using heart rate variability (HRV) [6]. In our present study, neuropathy was assessed in T2DM patients using vibration perception threshold (VPT), a measure of large nerve fibre function, whereas, HRV evaluates small nerve fibers involved in cardiac autonomic regulation. Literature showed that the small C fibers in T2DM patients were affected earlier [3]. Therefore, cardiac autonomic neuropathy (CAN) may develop at an earlier stage, even before detectable changes appear in neurophysiological tests or peripheral nerve morphology in patients with DPN. HRV analysis elucidates the balance between sympathetic and parasympathetic activity in individuals by analyzing time domain, frequency domain, and nonlinear indices, which provide information about the adaptability of cardiac autonomic regulation. It has been reported that alteration in those parameters is associated with neuropathic changes in T2DM [6]. Literature suggests that prolonged hyperglycemia, oxidative stress, and microvascular complications are significant factors to promote peripheral neuropathy in T2DM [7,8]. Literature suggests that prolonged hyperglycemia, oxidative stress, and microvascular complications are significant factors to

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

promote peripheral neuropathy in T2DM [7]. Apart from this, it is plausible that cardiac autonomic dysfunction and peripheral neuropathy may coexist and progress in parallel.

Therefore, we aimed to elucidate the interaction between HRV indices and peripheral neuropathy in T2DM patients, which remains underexplored. We also aimed to find the difference in cardiac autonomic function among diabetic patients with and without neuropathy. We hypothesize that peripheral neuropathy will significantly affect cardiac autonomic functions and can be predicted from HRV indices. Identifying HRV indices as biomarkers could provide a valuable, non-invasive approach in routine clinical practice for early detection of peripheral neuropathy in diabetic patients. Furthermore, understanding the distinct HRV pattern among the neuropathy and non-neuropathy groups, this study focuses on the machine learning approach, which provides valuable insights into the regulatory patterns of the autonomic nervous system in diabetic peripheral neuropathy.

Methods

Participants

A total of 115 diabetic patients referred from the outpatient departments (OPDs) were screened in the Neurophysiology Laboratory of All India Institute of Medical Sciences (AIIMS), Kalyani, from August 2024 to September 2025. Individuals aged between 35 to 75 years and had more than 1 year of diabetes history (fasting blood glucose >126 mg/dL or HbA1c ≥ 6.5%) were included in this present study. Participants with a pacemaker, documented cardiac arrhythmia, recurrent syncope, uncontrolled hypertension, and chronic kidney disease were excluded. Individuals on medications that could interfere with autonomic or cardiovascular responses (e.g., beta-blockers, antidepressants, or anti-hypertensives with central action) were also excluded (Figure 1). The sample size was determined based on previous studies assessing nonlinear HRV indices in diabetic populations, with an expected moderate effect size (Cohen's

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

d = 0.7), a significance level of 0.05, and 80% power, resulting in a minimum of 25 participants per group. After excluding 47 participants, 68 subjects were selected for testing. Additionally, 2 participants were excluded due to being unable to complete the test. Finally, sixty-six diabetic patients were included for data analysis (Figure 1). The study was conducted according to the Declaration of Helsinki and ethically approved by the Institutional Ethics Committee (Certificate no. IEC/AIIMS/Kalyani/certificate/2024/418).

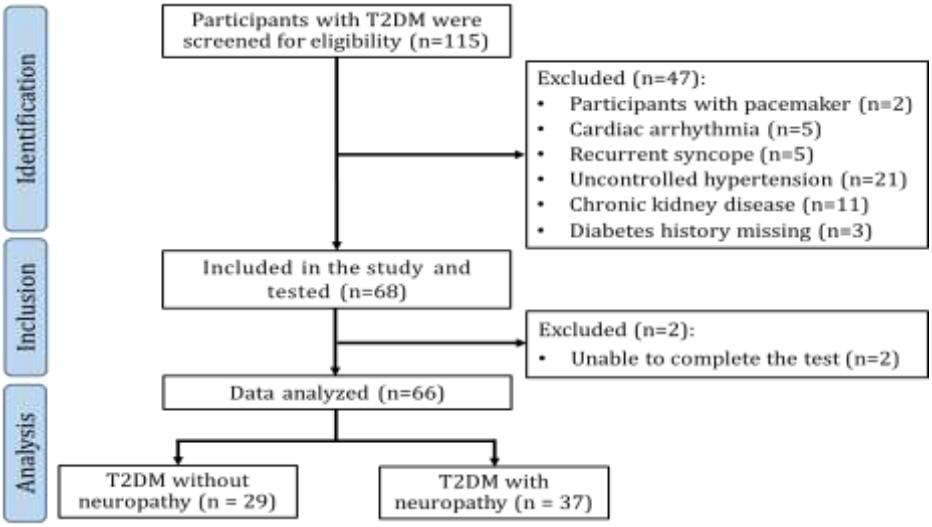

Fig. 1: Diagram showing participant flow.

Study design

In this analytical cross-sectional study, upon arriving at the lab, demographic details of the participants were collected, followed by primary health screening, including blood tests. Then, the participants underwent peripheral neuropathy assessment using the Vibration Perception Threshold (VPT) test. Based on the VPT result, the participants were stratified into two groups: T2DM with peripheral neuropathy (n = 37) and T2DM without neuropathy (n = 29) (Figure 1). Cardiac autonomic function was assessed in a temperature-controlled laboratory environment (ambient temperature 22–24 °C) in the morning between 9 am to 10 am after an overnight fast

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

and at least 12 hours of abstinence from caffeine and 36 hours for nicotine and alcohol. They also should have uninterrupted sleep for at least 8 hours.

Assessment of peripheral neuropathy

Peripheral neuropathy was evaluated using the Vibration Perception Threshold (VPT) test, a validated, non-invasive, and quantitative method. The VPT was measured bilaterally at the plantar surface of the great toe using a biothesiometer (Biothezi VPT from Kody Medical Electronics Private Ltd, India) in the lab with participants in a relaxed, supine position. After familiarization with the stimulus, the intensity of vibration was gradually increased from 0 V at a rate of approximately 1 V/s, and participants were instructed to indicate the first perception of vibration. The lowest voltage at which the vibration was consistently perceived in at least two out of three trials was recorded as the VPT value. The average of right and left foot readings was taken for analysis. A VPT value greater than 15 V was considered indicative of peripheral neuropathy, consistent with established clinical criteria [9].

Assessment of Heart Rate Variability

Patients were made to lie quietly in a bed in supine position for 10 minutes to acclimatize to the temperature-controlled (22–24°C) room. After that, a 5-minute resting electrocardiogram (ECG) was recorded in the supine position using Power Lab 16 channels data acquisition system (AD Instruments, Australia) with a sampling rate of 1000 Hz. The recordings were captured using Labchart Pro Version 8 (PowerLab AD Instruments) and analyzed using Kubios Software (version 2.1, Kubios Oy, Kuopio, Finland) to obtain time-domain, frequency-domain, and non-linear parameters. 10 Time domain parameters include standard deviation of RR intervals (SDNN), root mean square of the standard deviation of RR intervals (RMSSD), and percentage of number of successive NN intervals that vary by more than 50 ms (pNN50). Low frequency (LF; 0.04-0.15 Hz) and high frequency (HF; 0.15-0.4 Hz) power were assessed using

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

Fast Fourier Transformation (FFT) reflecting the balance between sympathetic and parasympathetic nervous system activity. The signals were further used to calculate LF/HF ratio. Nonlinear parameters include standard deviation (SD1 and SD2), sample entropy (SampEn), short-term (∝1) and long-term (∝2) scaling exponents. Representative plots of RR interval analysis in the neuropathy and no neuropathy groups have been shown in Figure 2.

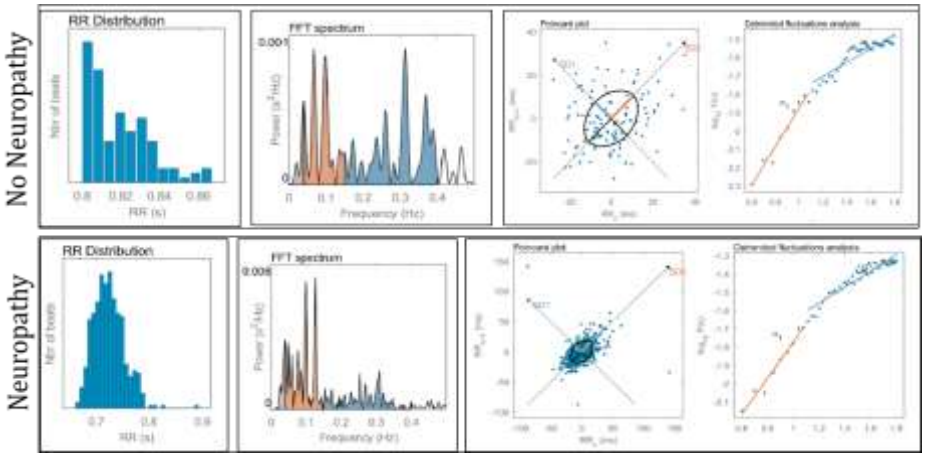

Fig. 2: Representative plots of RR Interval analysis in no neuropathy and neuropathy groups. FFT spectrum = Fast Fourier transform spectrum analysis

Assessment of blood glucose indices

Fasting blood glucose (FBG), postprandial blood glucose (PPBG), glycated hemoglobin (HbA1c), and duration of diabetes were recorded and compared between the neuropathy and non-neuropathy groups. Venous blood samples were collected in the morning after an overnight fast of at least 8 hours for estimation of FBG and HbA1c, while PPBG was measured 2 hours after a standardized meal on the same day. FBG and PPBG concentrations and HbA1C were determined using automated hemoautoanalyzer (ThermoFisher Scientific, Model: GENESYS 1XX).

Principal component analysis (PCA): Dataset were screened for missing values and errors, and invalid observations were excluded. Sampling adequacy and factorability were assessed using the Kaiser–Meyer–Olkin (KMO) measure and Bartlett's test of sphericity. Factor analysis was

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

performed on selected variables, including age, body mass index (BMI), and heart rate variability indices with factor loadings ≥ 0.30 were considered significant for interpretation. The number of factors was determined using the Kaiser criterion (eigenvalues> 1) and inspection of the scree plot. It was used to explore the underlying structure and to visualize the eigenvalues. The first PC captures the largest proportion of the total variance. The second PC explains the maximum remaining variance, with the constraint that it is uncorrelated with the first as previous literature revealed [11]. Although PCA can theoretically continue until all variance is accounted for, it is typically stopped after extracting a smaller number of PCs that collectively explain a significant proportion of the total variance [11,12]. The eigenvalue associated with each PC represents the amount of variance it explains. A higher eigenvalue indicates greater explanatory power [12].

Statistical analysis

All data were checked for normality using the Shapiro–Wilk test. Variables were expressed as mean ± standard deviation (SD). Between-group comparisons were performed using independent samples t-tests. Pearson's correlation was performed to find any correlation between HRV matrices with VPT followed by a binomial logistic regression analysis. False discovery rate (FDR) correction was also performed on HRV parameters to account for multiple comparisons-related corrections at $p_{FDR}<0.05$ [11]. All the analyses were performed using Statistical Package for Social Sciences (SPSS ver. 23) and Jamovi software (version 2.7.12). The graphs were made using R software (version 4.4.3). A $p<0.05$ was considered statistically significant.

Results

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

Clinical Differences between Neuropathy Phenotypes in T2DM

Among the 66 participants with T2DM, 37 individuals (56.1%) met the neuropathy criterion based on VPT (>15 V) and a significantly elevated threshold ($p<0.001$, $d = –1.98$) was observed in the neuropathy group, confirming substantial sensory impairment (Table 1). Significantly longer diabetes duration ($p = 0.001$, $d = –1.27$) and poor glycemic control, reflected in higher fasting blood glucose ($p = 0.003$, $d = –0.75$) and post-prandial blood glucose levels ($p = 0.008$, $d = –0.68$) were also seen in the neuropathy group compared to no neuropathy group. However, HbA1C did not differ significantly between the two groups.

Table 1: Comparison of glycemic parameters and vibration perception threshold between T2DM patients with and without neuropathy

Parameters	No neuropathy (n=29)	Neuropathy (n=37)	% difference	p-value	Cohen's d
History of diabetes (yrs)	5.89±3.25	10.75±4.21	82.51	0.001	-1.270
FBG (mg/dl)	130.51±40.51	164.10±47.52	25.74	0.003	-0.753
PPBG (mg/dl)	188.22±68.33	234.55±67.33	24.61	0.008	-0.684
HbA1c (%)	7.43±2.15	7.81±2.20	5.11	0.487	-0.173
VPT (V)	9.67±2.60	23.31±8.92	141.05	0.001	-1.984

Data is represented as Mean ± SD FBG = Fasting blood glucose; PPBG = Postprandial blood glucose; HbA1c = Glycated hemoglobin; VPT = Vibration perception threshold.

Differences in HRV metrics

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

In the neuropathy group, time-domain parameters SDNN (p=0.025) and RMSSD (p=0.005) were significantly lower, whereas SDNN/RMSSD ratio was significantly (p=0.011) higher compared to the no neuropathy group. The HRV triangular index (p=0.012) was also significantly less in the neuropathy group. Stress Index was significantly higher (p=0.030) in neuropathy group (Table 2). Among frequency domain parameters, LF power (p=0.007) and LF/HF ratio (p=0.04) were significantly higher, while HF power (p=0.02) was significantly lower in the neuropathy group, indicating a shift towards sympathetic predominance (Table 2). Non-linear parameters SD1 (p=0.004) and SD2 (p=0.050), and sample entropy (p=0.032) were significantly lower, whereas SD1/SD2 (p=0.011) was higher in the neuropathy group, suggesting altered autonomic imbalance (Table 2). DFAα1 (p=0.039) and DFAα2 (p=0.031) both were significantly higher in the neuropathy group as compared to no neuropathy (Table 2). Representative recordings and analysis of HRV of no neuropathy and neuropathy have been shown in Figure 2.

Table 2: Differences in HRV parameters in T2DM patients with and without neuropathy and their correlation with VPT

Parameters	T2DM		% difference	Cohen's d	p-value†	Correlation with VPT (r)
	No neuropathy (n=29)	Neuropathy (n=37)				
SDNN (ms)	20.02±8.74	15.52±7.93	-22.5	-0.54	0.025	-0.235
RMSSD (ms)	21.88±9.26	15.52±8.48	-29.1	-0.72	0.005	-0.274*
pNN50 (%)	4.30±7.51	1.42±3.35	-66.9	-0.52	0.041	-0.216
SDNN/RMSSD ratio	0.921±0.12	1.01±0.15	9.9	0.66	0.011	0.183

HRVTI	5.61±2.15	4.38±1.66	-21.9	-0.65	0.012	-0.292*
Stress index	18.46±7.26	23.80±11.46	-12.36	0.60	0.030	0.217
LF power (n.u.)	45.49±19.29	57.31±15.24	25.9	0.69	0.007	0.243*
HF power (n.u.)	52.33±17.43	42.70±15.20	-18.4	-0.59	0.020	-0.197
LF/HF ratio	1.16±0.88	1.63±0.93	40.5	0.52	0.040	0.234
SD1 (ms)	15.5±6.57	10.8±6.14	-30.3	-0.74	0.004	-0.272*
SD2 (ms)	23.6±10.8	18.5±9.67	-21.6	-0.50	0.050	-0.212
SD1/SD2 ratio	0.68±0.14	0.58±0.15	-13.2	-0.61	0.016	-0.171
Sample entropy	1.87±0.23	1.73±0.27	-7.5	-0.55	0.032	-0.246*
DFAα1	0.87±0.21	0.98±0.19	12.1	0.53	0.039	0.162
DFAα2	0.43±0.11	0.49±0.14	16.2	0.54	0.031	0.134

Data is presented as mean±SD. RMSSD = Root mean square of successive RR interval differences; SDNN = Standard deviation of NN interval; pNN50% = Percentage of successive RR intervals that differ by more than 50 ms; HRVTI = HRV triangular index; LF = Low frequency power; HF = High frequency power; SD1 = Poincaré plot standard deviation perpendicular the line of identity; SD2 = Poincaré plot standard deviation along the line of identity; DFAα1 = Detrended Fluctuation α1; DFAα2 = Detrended Fluctuation α2. †FDR corrected p-values ($p_{FDR}<0.05$). *$p<0.05$.

Correlation between HRV and neuropathy prognosis parameters

Association between neuropathy and cardiac autonomic function indices is shown in Table 2. VPT showed a significant negative correlation with RMSSD (r= -0.274; p=0.027), HRV triangular index (r= -0.292; p=0.019), LF power (r=0.24; $p<0.05$), SD1 (r= -0.272; $p<0.001$),

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

and sample entropy (r= -0.246; p=0.048). Whereas no significant correlation was found between VPT and SDNN, pNN50%, HF power, LF/HF ratio, DFAα1, and DFAα2 (Table 2).

HRV indices as a predictor of neuropathy

Binomial regression analysis has revealed RMSSD, LF power, and sample entropy as a significant predictor of VPT (Table 3). The overall model test was statistically significant (χ^2 =21.0; p<0.001), explaining 36.6% variance in the outcome (Nagelkerke R^2 = 0.366). ROC analysis also revealed that RMSSD, LF power and sample entropy, possess a significant discriminant factor in detecting neuropathy in T2DM patients with AUC 0.799 (Table 4). Collectively, these results demonstrate that diabetic neuropathy is associated with a reproducible pattern of reduced vagal tone, increased sympathetic modulation, and loss of HRV complexity.

Table 3: Summary of binomial logistic regression analysis showing the association of heart rate variability (HRV) parameters with the presence of diabetic peripheral neuropathy

Predictors	Estimate	SE	Z	p-value	Odds ratio	R^2_N	χ^2	p-value*
Intercept	6.814	2.98	2.29	0.022	910.88	0.366	21.0	<0.001
RMSSD	-0.095	0.04	-2.31	0.021	0.90			
LF power	0.043	0.02	2.21	0.027	1.04			
Sample entropy	-3.881	1.48	-2.63	0.009	0.02			

Dependent variable: Vibration perception threshold

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

Estimates represent regression coefficients (log-odds), with corresponding standard errors (SE), Z statistics, and p-values. Odds ratios (OR) indicate the change in odds of neuropathy per unit increase in each predictor. Model performance was evaluated using Nagelkerke's R^2 (R^2N) and the overall model chi-square (χ^2) test, indicating a statistically significant model fit.

Table 4: Predictive measures and Receiver operating Curve (ROC) analysis of predictors

Predictors	Sensitivity	Specificity	Accuracy	AUC
RMSSD	81.10%	58.60%	71.20%	0.799
LF Power				
Sample Entropy				

Combined Model estimated using sample size of N=66. AUC: Area under the Curve.

Principal Component Analysis (PCA)

As the large number of HRV matrices are intercorrelated, PCA was performed to reduce its dimensionality and address multicollinearity among the predictors (). The dataset demonstrated good suitability for PCA, with a Kaiser–Meyer–Olkin (KMO) value of 0.80. Bartlett's test of sphericity was highly significant (χ^2 = 935.5, p<; 0.001), confirming adequate correlations among variables. The components were retained based on eigenvalues greater than 1 showing in scree plot (Figure 3). The first two principal components explained the majority of variance: Dim1: 50.2% and Dim2: 26.2%. Together, PC1 and PC2 accounted for 76.4% of the total variance. Two statistically distinct and physiologically meaningful components were identified (Figure 4).

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

PC1 (Autonomic Dysfunction Axis): PC1 showed a strong negative loadings for RMSSD, SDNN, pNN50 and mean RR, along with a positive loading for stress index. This component represents overall reduction in HRV with increased sympathetic stress.

PC2 (Sympathovagal Balance Axis): PC2 was characterized by positive loadings for LF power, LF/HF ratio, and DFAα1, along with a negative loading for HF power. This component reflects the balance between sympathetic and parasympathetic activity.

PC1 was significantly higher in the neuropathy group (mean difference = 1.80, $p<0.01$), indicating greater autonomic dysfunction. In contrast, PC2 showed no significant trend (mean difference = 0.57, $p>0.05$). These findings suggest that overall HRV reduction is the primary distinguishing feature of peripheral neuropathy.

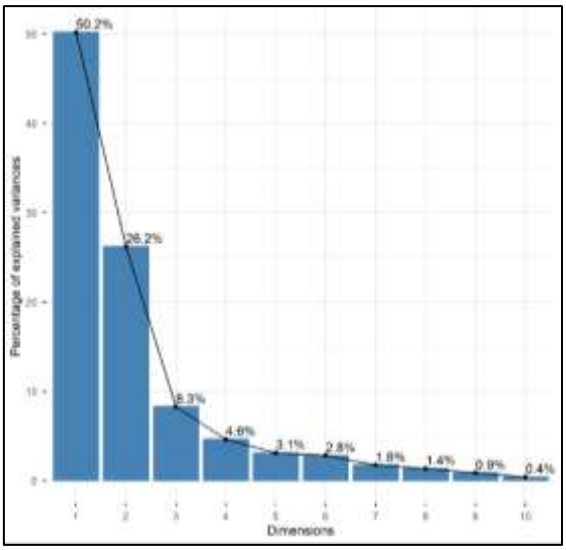

Fig. 3: Scree plot illustrating the percentage of variance explained by each principal component derived from heart rate variability (HRV) parameters. The first principal component (PC1) accounts for 50.2% of the total variance, followed by the second principal component (PC2) explaining 26.2%. Subsequent components contribute progressively smaller proportions of variance, with a clear elbow inflection observed after

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

the second component, indicating that the first two principal components capture the majority of the variability in the dataset.

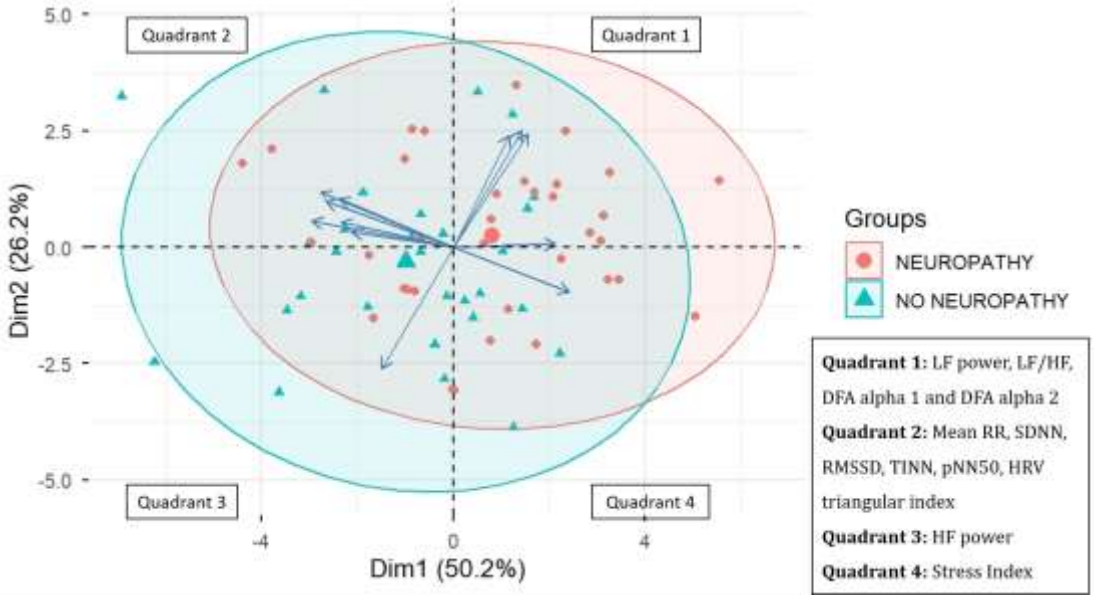

Fig. 4: Principal Component Analysis (PCA) biplot showing distribution of participants based on autonomic variables. The two principal components, Dim1 (50.2%) and Dim2 (26.2%), together explain 76.4% of the total variance. Red circles represent participants with neuropathy and blue triangles indicate participants without neuropathy. The ellipses indicate the 95% confidence intervals for each group, reflecting a distinct pattern of autonomic regulation. Arrows represent positive and negative loadings of each variable, indicating the direction and magnitude of contribution of each parameter to the principal components. The identified HRV matrices in each quadrant are mentioned inside the box (right side of the figure).

Discussion

We observed duration of diabetes as a significant variable between no neuropathy and neuropathy group. Literature suggests that duration of diabetic neuropathy was significantly

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

associated with risk of having diabetic neuropathy. They interpreted that duration of diabetes above 5 years can significantly develop neuropathy [13,14]. Our present study observed that the duration of diabetes was significantly longer in the neuropathy group compared to those without neuropathy. It indicates that longer exposure to hyperglycemia contributes to the development of diabetic peripheral neuropathy. This finding is consistent with previous studies demonstrating that prolonged disease duration is one of the strongest predictors of diabetic peripheral neuropathy (DPN) [15,16]. Longer diabetes duration leads to metabolic and microvascular damage which ultimately leads to peripheral neuropathy.

Interestingly, HbA1c levels did not differ significantly between the neuropathy and no neuropathy groups, despite significant differences in fasting and postprandial blood glucose levels. This also indicates that neuropathic changes are due to short term glycemic fluctuations or postprandial hyperglycemia. HbA1c is the measure of average blood glucose concentration over the past 2-3 months and indicates long-term glycemic fluctuations and may be due to this it did not show any significant differences between neuropathy and no neuropathy group [17]. Another study suggests that control of blood pressure, lipid levels or reduction of glucose fluctuations can also be monitored to prevent diabetic peripheral neuropathy in T2DM patients. Therefore, long-term glycemic variability and other non-glycemic risk factors play a critical role in neuropathy development, independent of the average HbA1c level at a single point of time.

The finding that both fasting blood glucose (FBS) and postprandial blood glucose (PPBS) were significantly higher in the neuropathy group further reinforces the role of poor short-term glycemic control and glucose variability in nerve damage. Elevated FBS and PPBS levels are associated with the generation of advanced glycation end-products, mitochondrial dysfunction,

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

and impaired nerve blood flow, all of which contribute to axonal degeneration and demyelination seen in DPN [18].

Neuropathy status assessed by Vibration Perception Threshold (VPT) showed a significantly higher threshold in the neuropathy group, confirming the presence of sensory loss which is a well-established, sensitive, and non-invasive method to quantify large-fiber dysfunction in diabetic patients [19].

In the present study, RMSSD, pNN50, and SDNN were significantly lower in patients with diabetic neuropathy, indicating impaired parasympathetic modulation and overall heart rate variability (HRV). These findings are consistent with previous studies demonstrating that vagal dysfunction represents one of the earliest manifestations of cardiac autonomic neuropathy in diabetes [6]. Both RMSSD and pNN50 primarily reflect short-term, high-frequency variations mediated by the parasympathetic nervous system, while SDNN represents overall HRV incorporating both sympathetic and parasympathetic influences [10]. Diabetic neuropathy affects multiple nerves involved in both autonomic neuropathy as well as sensorimotor polyneuropathy [6]. Among those, the vagus nerve, a major part involved in the parasympathetic nervous system, was affected in diabetic peripheral neuropathy as reported earlier [2,20,21]. Javorka et al., in 2008 who reported a decrease in SDNN, RMSSD, PNN50, LF and HF 17 patients diagnosed with Type 1 DM [22]. Therefore, the overall reduction of these HRV parameters in diabetic neuropathy patients underscores the loss of vagal tone and reduced cardiac adaptability, which are key indicators of early autonomic dysfunction in diabetes.

In our present study, LF power and LF/HF, which were significantly higher in peripheral neuropathy groups as compared to neuropathy suggesting autonomic imbalance that leads to sympathetic dominance. The higher normalized LF power reflects sympathetic overactivity,

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

while high LF/HF ratio shows a shift towards sympathovagal balance. Previous study done by Babu et al., 2016 investigated frequency domain parameters in diabetes mellitus patients and healthy population as well [23]. Our findings are in line with their findings. It is due to the reduction in vagal modulation of RR intervals occurring in the diabetic neuropathy group.

The nonlinear parameters SD1 & SD2 were significantly shown lower in neuropathy as compared to no neuropathy group. Reduction in HRV indicates early signs of cardiac autonomic neuropathy in diabetic population as previously reported [2]. All together, reduction in HRV indices discussed above, indicate autonomic, global and sympathetic modulation of the heart in T2DM patients.

The nonlinear parameters SD1 & SD2 were significantly shown lower in neuropathy as compared to no neuropathy group. Sample entropy, DFAα1 and DFAα2 were also significantly lower in the neuropathy group, indicating reduced responsiveness of cardiac control mechanisms to external or internal stimuli. Thus, it decreases the strength of cardiac feedback machinery in neuropathy patients [24]. All together, reduction in HRV indices as discussed above, indicate autonomic, global and sympathetic modulation of heart rate variability parameters in diabetic peripheral neuropathy patients.

Binomial logistic regression analysis revealed RMSSD, LF power and sample entropy as significant predictors to identify neuropathy in T2DM patients. Previous literature, as shown by Khandoker et al., in 2017 explored sample entropy as the only significant predictor in identifying cardiac autonomic neuropathy in T2DM [24]. Another study done by Wadhkor et al., in 2024 did prediction study on micro and macro complications in T2DM using HRV parameter [25]. They found HF & LF power, SDNN as significant predictors which were associated with complications in T2DM patients having an accuracy of 0.78 with 85%

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

sensitivity, 74% specificity with AUC of 0.83 was observed. No other study till date has been found where HRV parameters were used to identify peripheral neuropathy in diabetic patients. The integrated assessment of HRV parameters using PCA revealed two distinct autonomic phenotypes, elucidating heterogeneous patterns of autonomic dysfunction in diabetic peripheral neuropathy. Our findings also suggest that autonomic impairment in DPN is not uniform, but it occurs through multiple underlying physiological pathways. Further, it enhances our understanding about small fibre involvement at an early stage in diabetic peripheral neuropathy, highlighting the potential of autonomic phenotyping for targeted therapeutic interventions in patients with diabetic peripheral neuropathy.

From the above findings, we proposed a mechanism which may be involved in developing autonomic imbalance in peripheral neuropathy in Type 2 Diabetes Mellitus patients (Figure 5). The molecular pathway for progression of cardiac autonomic neuropathy in T2DM needs to be studied in future.

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

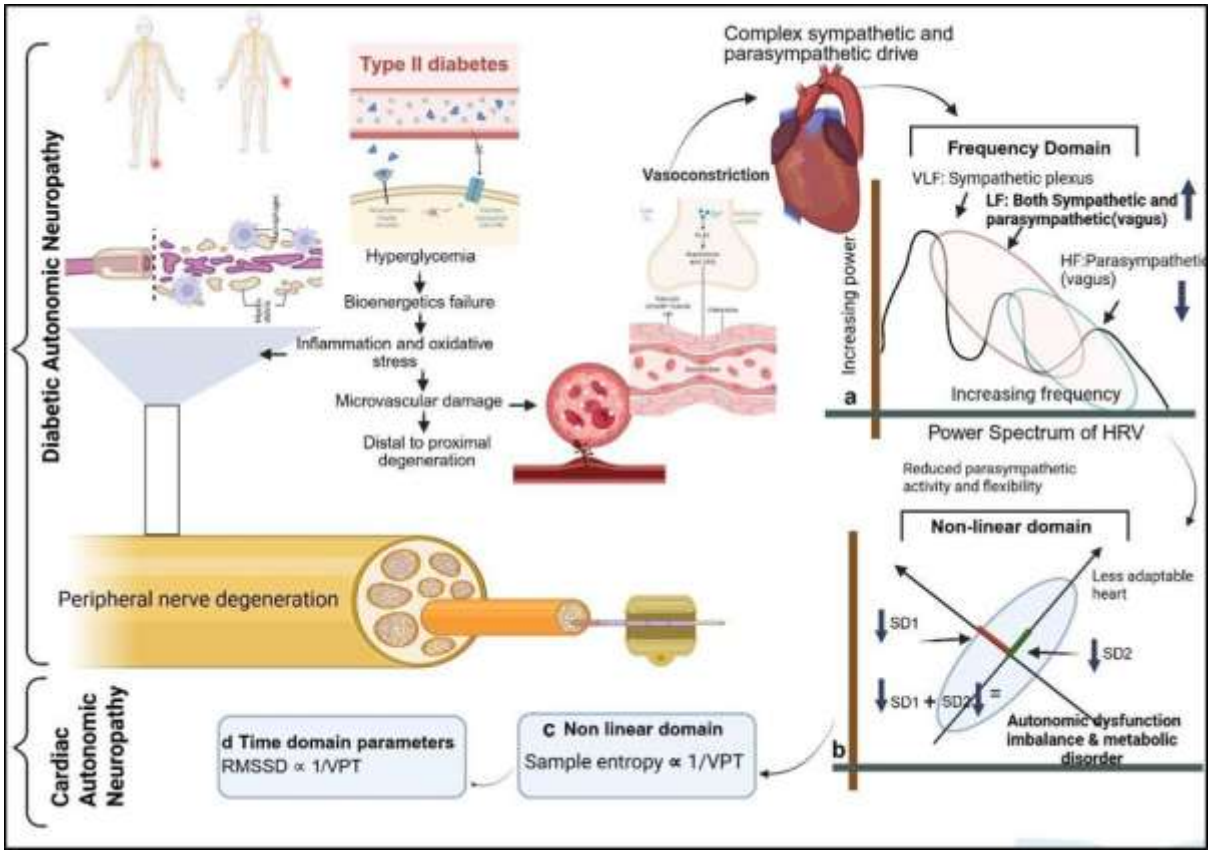

Fig. 5: Proposed mechanism involved in autonomic imbalance in diabetic peripheral neuropathy Unlike other studies, this study also has certain limitations that need to be addressed. This study has not considered male and female separately in with and without neuropathy groups due to relatively small sample size. The study was also carried out on the patients from a single-tertiary care hospital, which may restrict the generalizability of the findings. Therefore, future studies considering previous lifestyle habits and physical activity levels as covariates, large sample size, and multi-centric study should be carried out to strengthen the findings. Future studies should also include longitudinal designs to better understand the temporal relationship and pathways between autonomic dysfunction and neuropathy progression in type 2 diabetes mellitus. Incorporation of more sensitive and specific measures of small fiber neuropathy, alongside HRV, would provide a more comprehensive evaluation. Advanced analytical approaches, including machine learning models, may further

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

refine predictive accuracy for early neuropathy detection. Additionally, interventional studies exploring whether improving autonomic function can delay or reverse neuropathy progression would be valuable.

Since small fiber involvement occurs early, HRV assessment may help identify individuals at risk of developing diabetic peripheral neuropathy (DPN) before irreversible nerve damage occurs. Therefore, non-invasive and cost-effective HRV assessment along with Vibration Perception Threshold (VPT) test can be included into routine healthcare settings for screening and early detection of peripheral neuropathy, monitoring of disease progression, and response to therapeutic interventions in patients with type 2 diabetes mellitus. The use of advanced analytical techniques like principal component analysis (PCA) and clustering can also assist clinicians and healthcare professionals in identifying distinct autonomic phenotypes, enabling more personalized targeted interventions.

Conclusion:

The present study demonstrates that diabetic peripheral neuropathy, a chronic and progressive disease, is significantly associated with impaired cardiac autonomic function in patients with type 2 diabetes mellitus. Notably, this is the first study that showed heart rate variability (HRV) parameter RMSSD, LF power, and sample entropy significantly predicted neuropathy through regression and principal component analysis, which identified two distinct components representing autonomic dysfunction and altered sympathovagal modulation. These findings highlight the potential of HRV as a non-invasive, clinically feasible biomarker for the early detection of peripheral neuropathy, enabling diagnosis before severe sensory nerve damage occurs. Detecting DPN in early stages can protect T2DM patients from prolonged hyperglycemia, oxidative stress, and microvascular complications induced progressive nerve fiber damages. Overall, this approach will help doctors to take timely and targeted therapeutic

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

measures. Future studies should focus on integrating molecular biomarkers to further elucidate the underlying mechanisms of autonomic imbalance and disease progression in diabetic peripheral neuropathy.

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ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 6, 2026

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CRediT authorship contribution statement

AC: Writing – original draft, Validation, Methodology, Investigation, Formal analysis. RM: Writing – review & editing, ANSS: Review and editing; BDM: Writing, review & conceptualization: AD: Writing – Methodology, review & editing. SG: Visualization of data. TG: Writing –review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee AIIMS Kalyani (IEC/AIIMS/Kalyani/certificate/2024/418).

Funding

This work did not receive any specific grant from funding agencies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the Staff of the Department of Physiology and Department of General Medicine.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.