Original Article

Nonlinear Methods to Assess Changes in Heart Rate Variability in Type 2 Diabetic Patients

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Abstract

Background: Heart rate variability (HRV) is an important indicator of autonomic modulation of cardiovascular function. Diabetes can alter cardiac autonomic modulation by damaging afferent inputs, thereby increasing the risk of cardiovascular disease. We applied nonlinear analytical methods to identify parameters associated with HRV that are indicative of changes in autonomic modulation of heart function in diabetic patients.

Objective: We analyzed differences in HRV patterns between diabetic and age-matched healthy control subjects using nonlinear methods.

Methods: Lagged Poincaré plot, autocorrelation, and detrended fluctuation analysis were applied to analyze HRV in electrocardiography (ECG) recordings.

Results: Lagged Poincare plot analysis revealed significant changes in some parameters, suggestive of decreased parasympathetic modulation. The detrended fluctuation exponent derived from long-term fitting was higher than the short-term one in the diabetic population, which was also consistent with decreased parasympathetic input. The autocorrelation function of the deviation of inter-beat intervals exhibited a highly correlated pattern in the diabetic group compared with the control group.

Conclusion: The HRV pattern significantly differs between diabetic patients and healthy subjects. All three statistical methods employed in the study may prove useful to detect the onset and extent of autonomic neuropathy in diabetic patients. (Arq Bras Cardiol. 2013;101(4):317-327)

Keywords: Heart Failure; Diabetes Mellitus, Type 1; Systole; Measurements, Methods and Theories; Statistics as Topic.

Introduction

Heart rate is dynamically regulated by intrinsic and extrinsic control systems, maintaining homeostasis. The major extrinsic control is provided by the autonomic nervous system. Heart rate variability (HRV) is a measure of the fluctuation in the interval between sequential sinus heartbeats, and reflects cardiac autonomic regulation [1-3]. Diabetes leads to autonomic neuropathy[4], thereby disrupting a major component of cardiovascular regulation and contributing to an increased incidence of cardiovascular diseases in diabetic patients, such as heart attack, sudden cardiac death, and silent ischemia [5-8]. Early diagnosis of autonomic diabetic neuropathy is difficult and the detection methods available, e.g., the Ewing Test Battery, are cumbersome and have poor sensitivity and reproducibility. In contrast, HRV analysis is noninvasive and the input data are easily obtained by conventional electrocardiography (ECG)[9-12]. However, because of the nonlinear heart dynamics, conventional time and frequency domain parameters of HRV may not always represent the nonstationary characteristics of ECG. Nonlinear methods such as the Poincaré plot, detrended fluctuation analysis (DFA), tone/entropy analysis and HR complexity analysis are newly developed tools used for identifying nonlinear patterns within ECG data [13-18].

In this study, we used nonlinear analytical methods to study the differences in HRV patterns between diabetic and healthy individuals. The purpose of this study was to identify new parameters useful for detecting autonomic dysregulation in diabetes.

Methods

The patient group consisted of 23 type 2 diabetes mellitus patients with no history of cardiac, neurological, psychiatric, or sleep disorders. Patients on heart rate-altering medications were excluded from the study. The study was approved by the ethical committee of the Indian Institution of Technology, Kharagpur, India. A total of 23 healthy subjects were selected as a control group using the same exclusion criteria. All participants provided

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Manuscript received July 17, 2012, revised manuscript September 09 2012; accepted April 26, 2013.

DOI: 10.5935/abc.20130181

written informed consent prior to inclusion in the study. Subjects were instructed to avoid caffeine, alcohol, and physical exertion the day before the study was performed. A 10-min ECG recording was acquired from the patients while on supine position following a 15-min relaxation period. All ECGs were recorded at a fixed time of day to avoid the effects of diurnal variations on HRV.

Matlab and SPSS software packages were used for statistical analysis. For comparative analysis between the groups, unpaired t-tests were applied as appropriate. Other statistical methods are individually described in details.

Poincaré Plot

The Poincaré plot is a scatter plot of RR_n vs. RR_{n+1} where RR_n is the time between two successive R peaks and RR_{n+1} is the time between the next two successive R peaks. When the plot is adjusted by the ellipse-fitting technique, the analysis provides three indices: the standard deviation of instantaneous beat-to-beat interval variability (SD1), the continuous long-term R/R interval variability (SD2), and the SD1/SD2 ratio (SD12)[15]. On the Poincaré plot, SD1 it is the width and SD2 the length of the ellipse. In addition to this conventional plot (RR_{n+1} vs. RR_n), we also used the generalized Poincaré plot with different intervals, including the m-lagged Poincaré plot (the plot of RR_{n+m} versus RR_n). The values of SD1 and SD2 were calculated for lag = m from the relations

$SD1 = (\Phi(m) - \Phi(0))^{1/2}$ and $SD2 = (\Phi(m) + \Phi(0))^{1/2}$, where the autocovariance function $\Phi(m)$ is given by

$$\Phi(m) = E[(RR_n - \overline{RR})\ (RR_{n+m} - \overline{RR})]$$

and $\overline{RR}$ is the mean RR_n[14]. For the purpose of our study, we set m at 1, 5, and 9. We then extended our analysis to reveal the association between these standard deviation (SD) values and m by using the Padé approximation[19]. We assumed a simple form of the Padé approximation for SD values as the ratio of polynomial in M of degree one.

$$Y = \frac{a + bM}{c + dM} = \chi \frac{1 + \beta M}{1 + \gamma M} \qquad (1)$$

Here Y = SD1, SD2, or SD12 and $\chi = a/c$. The terms $\beta = b/a$ and $\gamma = d/c$ are the new unknown parameters. In order to determine if these parameters are of value for assessing cardiovascular health, we considered eq. (1) for the case of small m. In this limit, equation (1) can be approximated as $Y = C + LM + QM^2$, where the slope is $L = \chi(\beta - \gamma)$ and the curvature is $Q = \gamma L$. The slope and curvature of the plot of SD vs. m were determined by the fitted parameters χ, β, and γ.

Detrended Fluctuation Analysis

Another analytic method to assess long-term correlation in the R–R-time sequence is based on DFA [20]. The measure of correlation was given by a scaling exponent (α) of the fluctuation function $F(\tau) \approx \tau^{\alpha}$. The fluctuation function $F(\tau)$ was computed as follows. For a given time sequence $R(t_i)$, $t_i = i\delta t$, where δt is the characteristic time interval for the sequence and i = 1, N is an integrated time series, $r(t_i)$ was defined as $r(t_i) = \sum_j^i [R(t_j) - \langle R \rangle]$, i = 1,N, where $\langle R \rangle$ is the mean of $R(t_i)$. The integrated series was divided into segments of equal duration, $\tau = n\ \delta t$ and a linear function used to fit the data within each segment. The fluctuation function $F(\tau)$ was calculated as the root mean square fluctuation relative to the linear trend and alpha was obtained by fitting the data to a power law function. It has been observed that an acceptable estimate of the scaling exponent alpha (from DFA) can be obtained from analysis of data sets with 256 samples or longer (equivalent to approximately 3.5 min of RR data at a heart rate of 70 beats/min). The analysis of RR data from an ECG recording period of 10 min was therefore expected to provide an adequate measure of the scaling exponent [21]. However, the alpha value obtained from this calculation may be under the mixed influence of both short-term scaling, reflecting parasympathetic control, and long-term scaling, reflecting sympathetic control, and thus may fail to fully distinguish parasympathetic and sympathetic influences. A separate analysis of both short- and long-term scaling is supposed to nullify the mutual effect and reveal the exact scaling variation [22]. Thus, we analyzed separate alpha values, short-term α_s and long-term α_l. For α_s, data from 25 beats were included, whereas for α_l, data from 30 to N/4 beats were included.

Correlation between successive differences in RRn interval

The coherence of the RR_n interval can be assessed from the map of interval variation:

$$rr_{n+1} = \frac{RR_{n+2} + RR_{n+1}}{(RR_n)} \quad \text{VS.} \quad rr_n = \frac{RR_{n+1} - RR_n}{(RR_n)}$$

where $\langle RR_n \rangle$ is the mean interval. This plot is expected to show the correlation between the variability of three consecutive R–R intervals.

Autocorrelation of fluctuation of RRn

We explored the autocorrelation of the deviation of RR_n from the mean $\langle RR_n \rangle$. The autocorrelation function C(m) of a particular subject was calculated from

$$C(m) = \sum_{n=1}^{N} \Delta RR_{n+m} \Delta RR_n / \sum \Delta RR_n^2$$

where the deviation is $\Delta RR_n = RR_n - (RR_n)$ and N is the total number of RR_n intervals.

Results

The mean heart rate was 74.7 ± 6.1 beats/min in the diabetic group and 72.4 ± 6.7 beats/min in the healthy control group. Mean age in the diabetic group was 46.3 years (range, 36−56 years) and 47.4 years (range, 39−57 years) in the control group. All study subjects were normotensive.

In the Poincaré plot analysis, plot scatter increased with lag number, yielding higher width (SD1) and length (SD2)

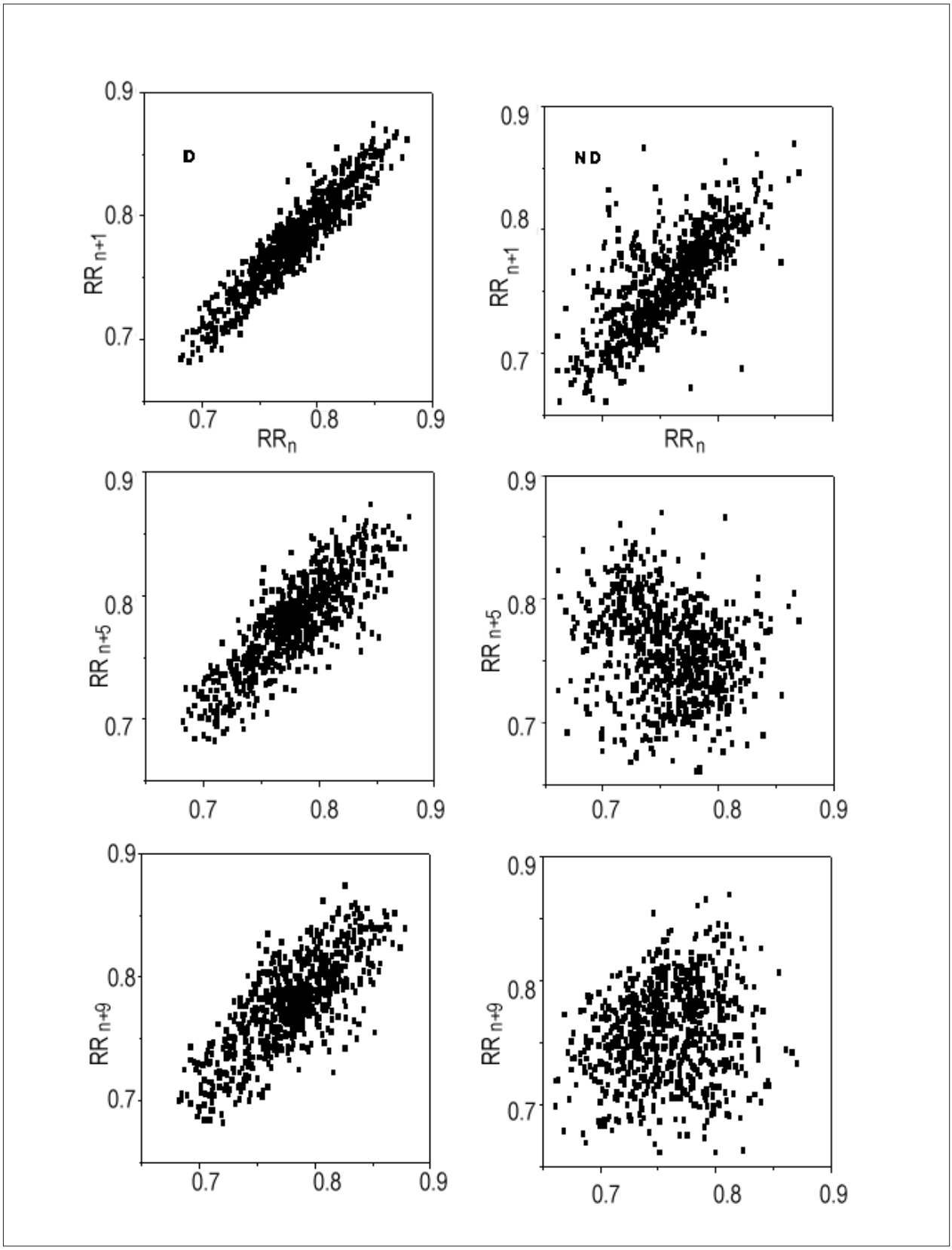

Figure 1 – *Poincaré plot of RRn+m vs. RRn from HRV analyses of one diabetic (D, left panels) and one nondiabetic subject (ND, right panels). In the upper panel, the lag factor m = 1, in the middle panel, m = 5, and in the bottom panel, m = 9. Note the greater scatter in the ND subjects, particularly as the lag factor is increased.*

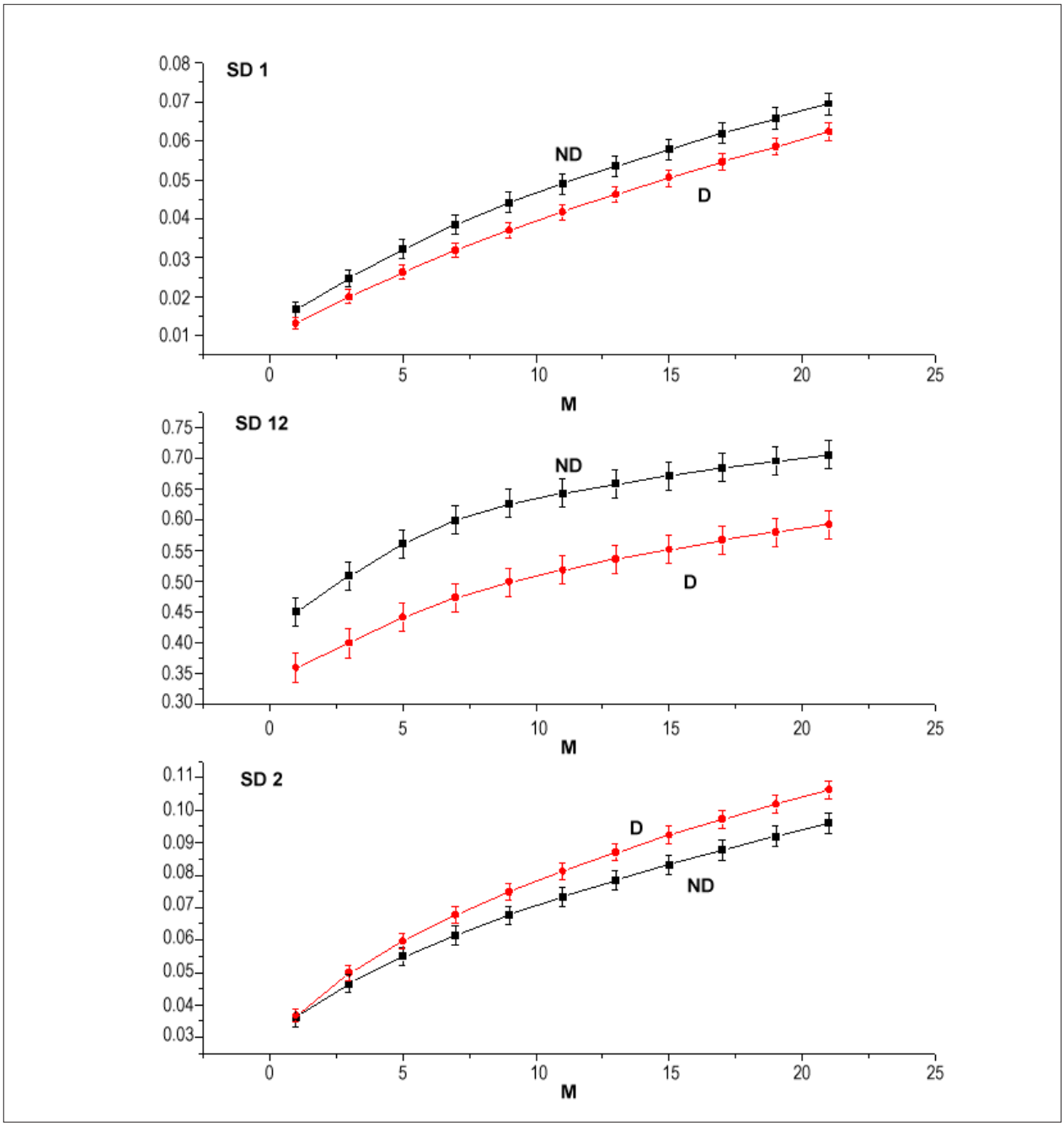

Figure 2 – *Variation of mean SD1 (upper panel), mean SD2 (middle), and mean SD12 (lower) with lag number m for diabetic (D) and nondiabetic (ND) groups (n = 23 subjects each).*

values. The incremental increase in width of the plot RR_{n+m} vs. RR_n as m increased was smaller in the diabetes group (Figure 1, D) than in the control group (Figure 1, ND). Differences in the values of SD1, SD2, and SD12 between the diabetes group and the control group were statistically significant ($p < 0.001$ for all). The values of SD1 and SD12 were higher in the control group, whereas SD2 was higher in the diabetic group. The difference in SD12 increased with lag number (Figure 2).

An excellent fit of the data with equation (1) (solid line on the curve, $R^2 = 0.999$) was found with the χ, β, γ value sets listed in Table 1. The values for L and Q as obtained by fitting of the data to eq. (1) are also presented in Table 1. The general features were that the slope (L) was positive but curvature (Q) was negative for all parameters and curvature was nearly one order of magnitude smaller than the slope.

From DFA, the mean value of alpha in the control group was smaller than that in the diabetic group (0.88 ± 0.17 vs. 1.02 ± 0.13; $p < 0.001$) (Figure 3). In control subjects, α_s was slightly larger than α_l (1.01 ± 0.14 vs. 0.80 ± 0.19), whereas α_l was larger than α_s for the diabetic group (α_s = 1.09 ± 0.17;

Table 1 - The values of parameters χ, β, γ obtained by fitting the data to eq. (1), as well as respective R2 values. The L and Q parameters are the coefficients of the linear and quadratic terms in expansion of Y in terms of m. Values of χ, L and Q for SD1 and SD2 are expressed in seconds

		$\chi \times 10^{-2}$	$\beta \times 10^{-2}$	$\gamma \times 10^{-2}$	$R^2 \times 10^{-2}$	$L \times 10^{-3}$	$-Q \times 10^{-4}$
SD1	ND	1.3 ± 0.03	39.1 ± 2.0	3.2 ± 0.2	99.9	4.7 ± 0.4	1.5 ± 0.2
	D	1.0 ± 0.02	38.2 ± 1.4	2.0 ± 0.1	99.9	3.6 ± 0.08	0.7 ± 0.02
SD2	ND	3.2 ± 0.06	20.3 ± 1.1	3.5 ± 0.2	99.9	5.4 ± 0.4	1.9 ± 0.2
	D	3.1 ± 0.07	26.4 ± 1.6	4.4 ± 0.3	99.9	6.8 ± 0.6	3.0 ± 0.5
SD12	ND	40.2 ± 0.5	25.0 ± 1.8	12.2 ± 0.9	99.9	51.3 ± 6.4	62.7 ± 2.4
	D	33.0 ± 0.3	15.3 ± 0.8	6.5 ± 0.4	99.9	29.0 ± 2.6	18.9 ± 2.8

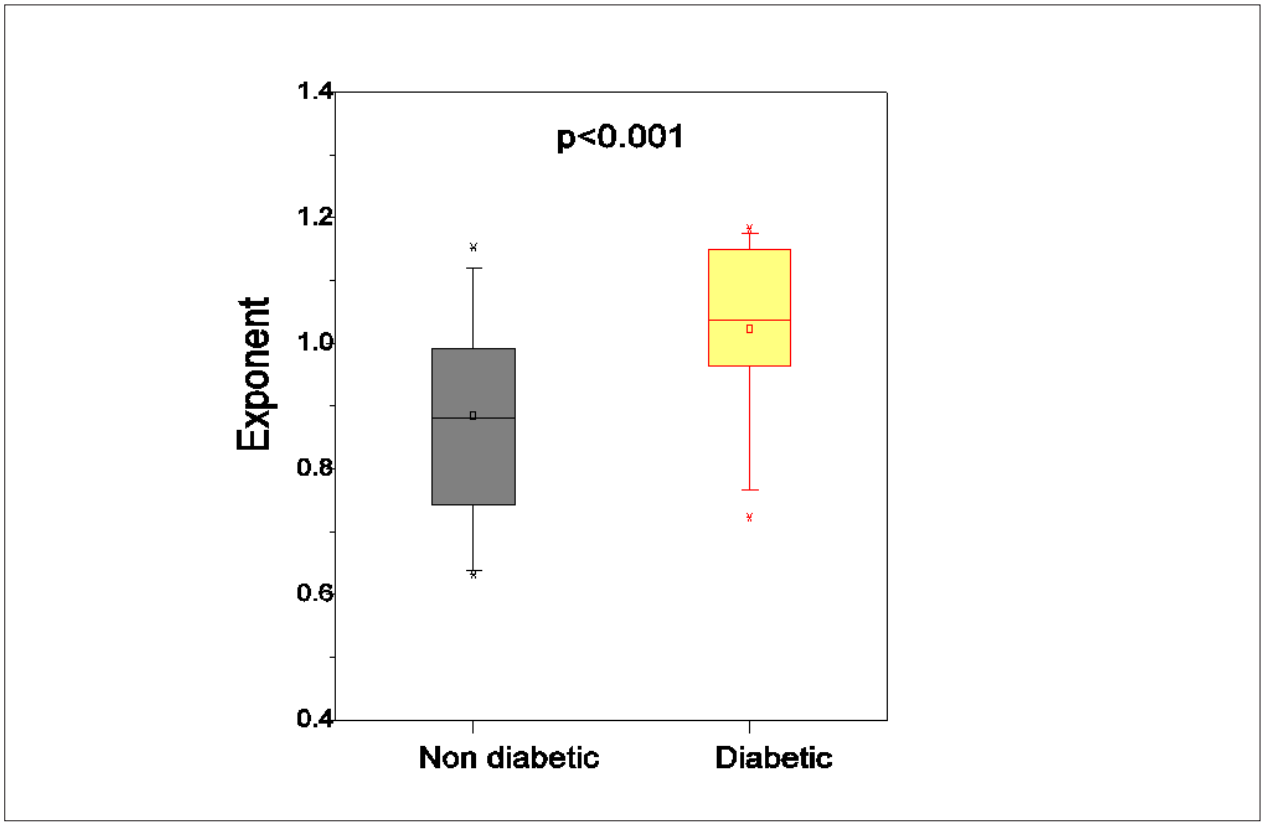

Figure 3 - *The DFA exponent α for healthy (nondiabetic) and diabetic subjects.*

α_l = 1.18 ± 0.19). When α_s was plotted against α_l (Figure 4), the diabetic and nondiabetic populations tended to form two separate clusters.

In the correlation plot, points were crowded around the origin for diabetic patients. In contrast, there was greater scattering about the origin and more asymmetry in the plot of control subjects (Figure 5, ND1, ND2). The strength of heart rhythm correlation was estimated by considering the autocorrelation of fluctuation in RR_n. Representative results from one control and one diabetic patient are plotted in Figure 6. The autocorrelation functions for diabetic and control patients were distinct. For diabetic subjects, the correlation function C(m) decreased slowly (black and green curve in the upper figure) with lag time. The time dependence was close to the sum of the two exponentials with superimposed small amplitude oscillation of low frequency. On the other hand, C(m) from the healthy subjects demonstrated a more rapid (exponential) fall as correlation time decreased compared with the diabetic cases. To confirm this difference in correlation pattern between control and diabetic subjects, we shuffled the actual time series of R–R interval using Matlab software and the autocorrelation functions of the shuffled data (red and blue for subjects 1 and 2 respectively) were plotted in Figure 6. The autocorrelation functions of the shuffled data from all subjects (2 diabetics and 2 healthy controls) were nearly identical.

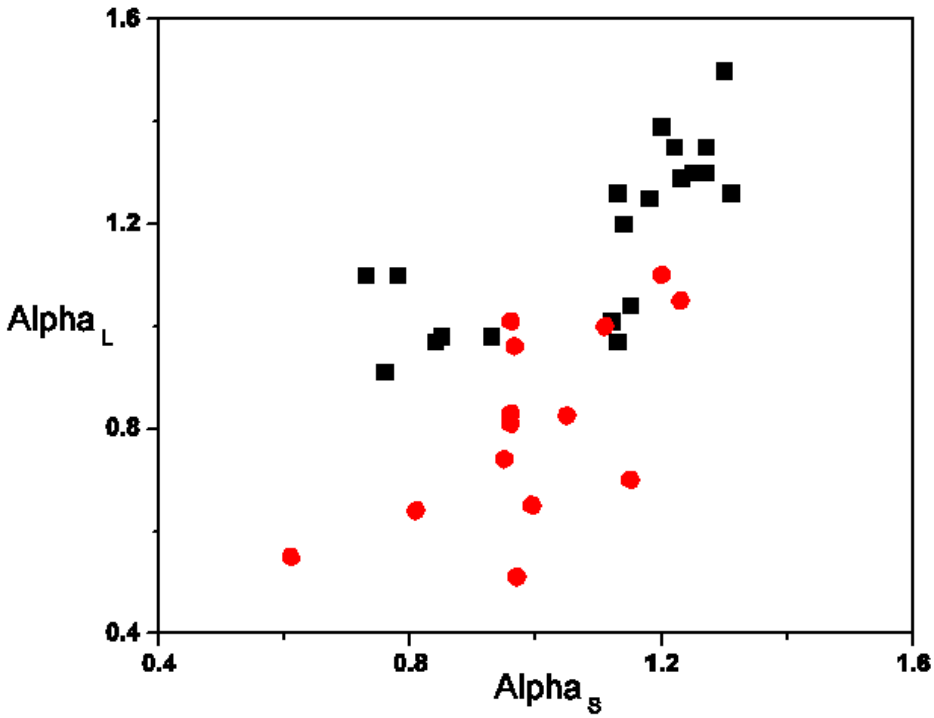

Figure 4 - *Scatter plot of DFA exponent long-term alpha (AlphaL) vs. short-term alpha (AlphaS) for nondiabetic subjects (red circles) and diabetic subjects (black squares).*

We also characterized properties of ΔRRn by the probability distribution function P(ΔRRn) (Figure 7). For diabetic patients, the probability distribution was almost symmetrical and could be fit by a Gaussian function ($R^2 = 0.93$) with width = 0.023. For healthy subjects, the probability distribution P was asymmetrical with positive mean and higher width = 0.036 as obtained by the Gaussian fit ($R^2 = 0.93$).

Discussion

We found marked differences in HRV pattern between diabetic and healthy control subjects using nonlinear analyses. Subjects were matched for both mean age and resting heart rate, the two major determinants of HRV [23], so that the difference in distribution would reflect changes in cardiovascular regulation resulting from the diabetic condition only.

Several modifications of the simple Poincaré plot have been proposed to more effectively reveal changes in HRV patterns, including the lagged plot. The concept of this m lagged plot emerged from the recognition that any given R–R interval can influence up to eight subsequent R–R intervals [24,25]. It has been shown that SD1 correlates with the short-term variability of heart rate and is mainly influenced by parasympathetic modulation, whereas SD2 is a measure of long-term variability [14,26] and reflects sympathetic activation. The lower SD1 in diabetic subjects indicates that parasympathetic regulation is weakened by the disease, presumably by peripheral neuropathy, whereas higher SD2 in diabetic patients indicates increased long-term variability because of compensatory sympathetic input.

The results from Poincaré plot analysis are further revealed by the slope (L) and curvature (-Q) of the plot. In the diabetic group, L and -Q for SD1 and SD12 were smaller, whereas L and -Q values for SD2 were higher than in the control group. The difference in Q was larger than the difference in L. In particular, the Q value for SD12 in the control group was >3 times greater than that for diabetic group. Low values of curvature are found in patients with cardiovascular disease [24]. These data strongly suggest decreased parasympathetic activity and excessive influence of sympathetic activity in the diabetic heart. In addition, this result provides indirect support for the notion that higher sympathetic influence over cardiovascular function is correlated with cardiac morbidity [27,28]. An increased SD12 is considered a good indicator of healthy heart dynamics, and the lower value in diabetic patients again supports altered sympathovagal balance in diabetes.

Previous reports using DFA showed that $\alpha_s > \alpha_l$ in healthy subjects, whereas the reverse was the case for subjects with cardiovascular disease [20]. We found a similar trend in this study, again confirming the adverse effect of diabetes on the heart.

In the absence of external modulation, the correlation plot is expected to scatter close to the point of origin, whereas random input will produce a uniform distribution. We observed a high density of points around the origin with greater symmetry in diabetic patients when compared with controls. Plots from

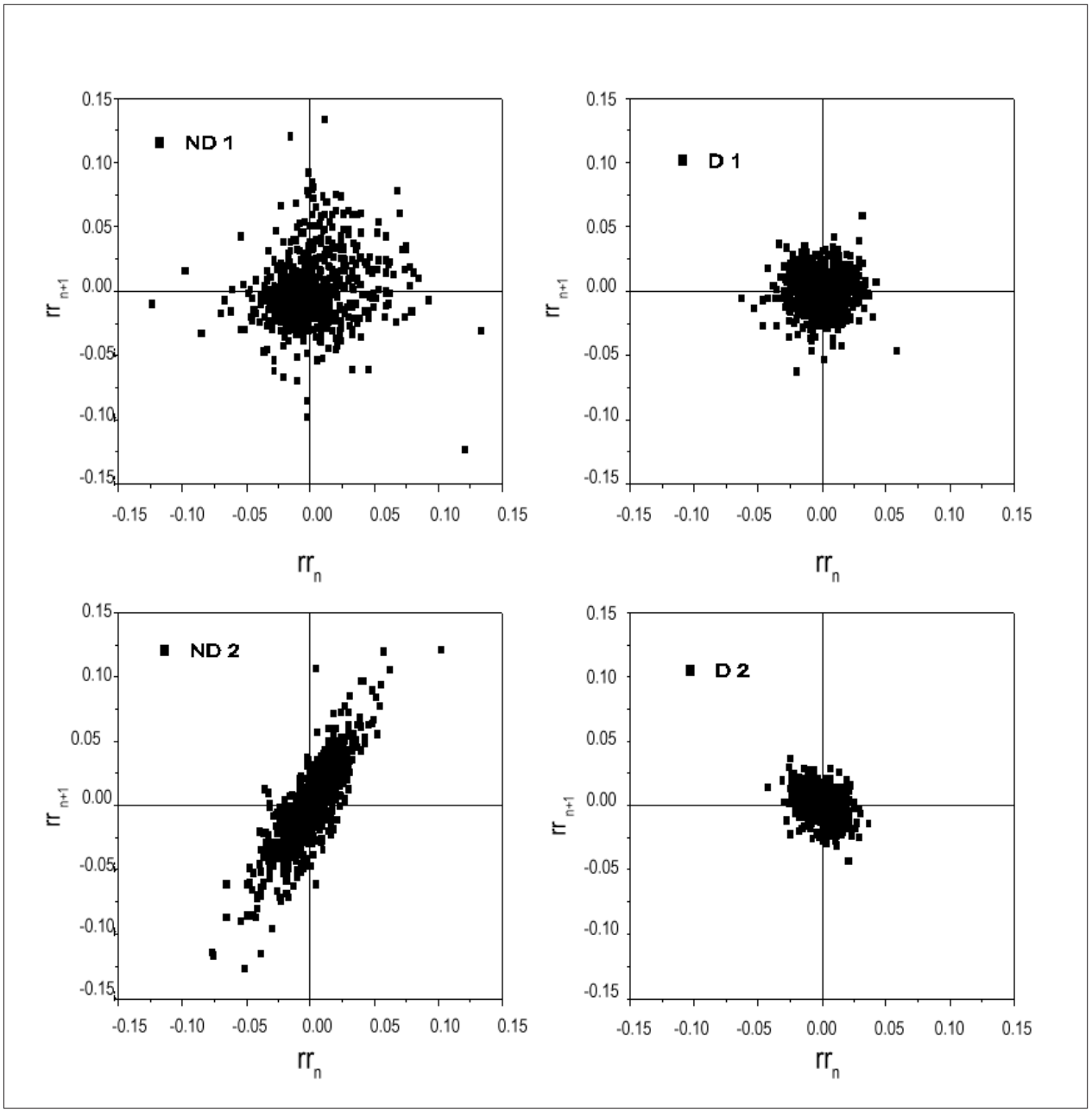

Figure 5 - *Plot of rrn+1 and rrn for two subjects from each group. Subjects were age matched (1 from each group in their mid-fifties, one from each group in their late thirties). The quantity rrn is the relative difference between RRn+1 and RRn normalized to the mean RRn of all intervals.*

healthy controls were generally asymmetrically scattered with large RR_n values. These results suggest that mechanisms for decelerating and accelerating HR over different time frames are substantially impaired in diabetic patients.

Application of autocorrelation to HRV analysis is a recent idea that regards HRV as the outcome of the interaction between coupled oscillators of various frequencies [29]. The degree of autocorrelation can also reflect on the embedded time scales within the HRV pattern. It is thought that each of these time scales in the coupled oscillator is represented by a separate self-oscillator, interacting with other oscillators with different physiological functions [18]. The lack of exponential fall in C(m) indicates the presence of a long-term memory effect in the diabetic condition and strongly suggests that mechanisms for short-term variation in heart rate are weakened or lacking in diabetic patients.

Heart rate variability analysis based on nonlinear dynamics has been shown to be superior to conventional methods for identifying hidden changes in cardiac autonomic modulation in various disease conditions. Previous reports have demonstrated

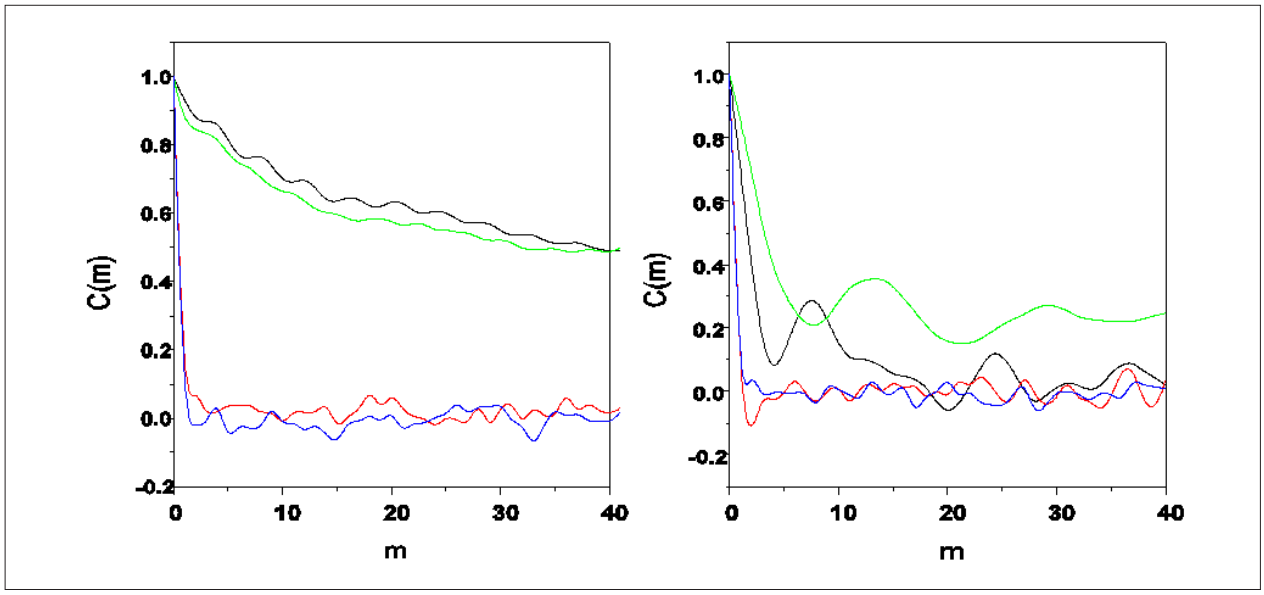

Figure 6 - *Plot of the correlation function C(m) with m for two diabetic (D) (left) and two control (ND) subjects. The lower curves were obtained from shuffled RRn intervals.*

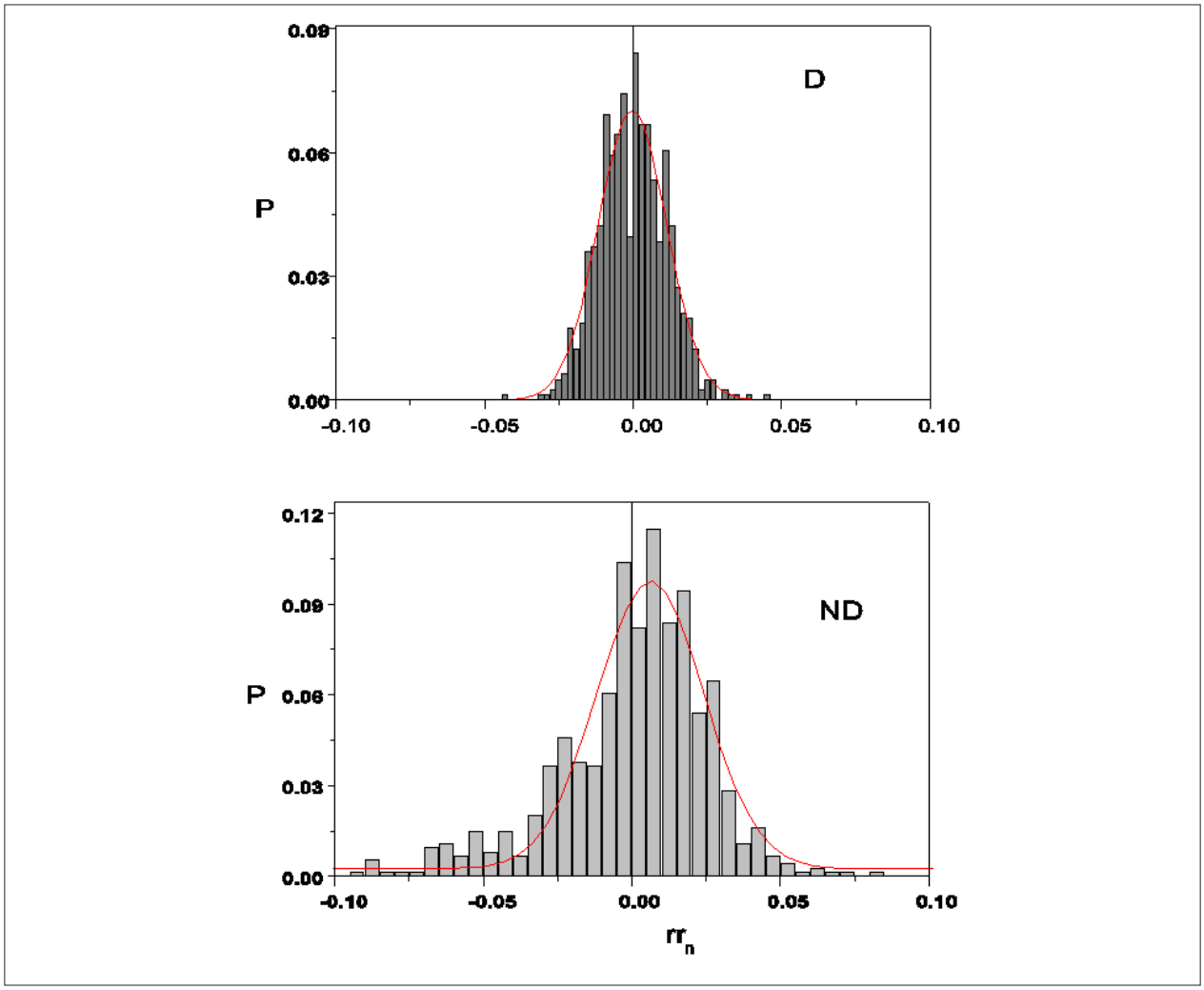

Figure 7 - *The plot of probability distribution P as a function of rrn for the two groups (upper panel is the diabetic group and the lower is the nondiabetic healthy group). Continuous Guassian curves are fitted to the distributions.*

differences in Poincaré plots, DFA, and sample entropy analysis between the hearts of diabetic and nondiabetic patients [17,30], but these differences often did not reach statistical significance because of the small sample sizes [17]. Our study not only enrolled larger numbers of patients and controls but also used multiple nonlinear analytic tools, including Poincaré plot analysis, DFA, and autocorrelation analysis to reveal changes in HRV due to diabetic neuropathy.

The major limitation of this study is the heterogeneous patient population. The duration of illness in the patient group was variable and many were on different antidiabetic medications. Moreover, a population of 23 patients may be sufficient to identify differences in HRV pattern between diabetic and healthy nondiabetic subjects, but a much larger group of patients is required to confirm the true diagnostic and prognostic values of the parameters derived from the analytic methods. Intra-group analysis in a larger group of diabetic patients of variable disease duration to assess progressive changes in HRV pattern is the next logical step. Our study establishes the potential of nonlinear methods of heart rate variability analysis to assess changes in HRV pattern indicative of cardiovascular disease, including effects associated with diabetes mellitus.

Conclusions

In summary, we have shown the effectiveness of nonlinear analytical methods to study differences in HRV patterns between diabetic patients and healthy-matched controls. We also emphasized the novelty of autocorrelation analysis to assess changes in the autonomic regulation of the diabetic heart. To our knowledge, this is the first attempt to distinguish normal from diabetic heart function using autocorrelation analysis. We believe these methods have the potential to identify diagnostic and prognostic markers for cardiac autonomic neuropathy in diabetes.

Author contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Statistical analysis, Writing of the manuscript, Critical revision of the manuscript for intellectual content: Roy, B, Ghatak S.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any post-graduation program.

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