Original Research Article

R-R variability during deep breathing as a diagnostic marker of cardiac autonomic neuropathy in Type 2 diabetes

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Abstract

Background: Studies have shown that simple Heart Rate Variability (HRV) measurement is useful in screening for and diagnosing Cardiac autonomic neuropathy (CAN). **Aim:** This study was aimed to assess the usefulness of R-R variability during deep breathing as the earliest predictor of CAN and to evaluate the effect of glycemic status and duration of diabetes on HRV. **Materials and Methods:** After obtaining ethical clearance and written informed consent, 72 Type 2 diabetic patients and 98 normal controls were administered an autonomic symptom questionnaire and subjected to recording of resting HRV and HRV during 1 minute deep and controlled breathing. Statistical analysis was done by appropriate tests using SPSS 20.0. **Results:** 44.4% of participants in the diabetic group had symptoms of autonomic dysfunction in contrast to 7.1 % of the control group ($p < 0.0001$). Resting HRV parameters and indicators of R-R variability during deep breathing like E/I ratio and Delta HR were significantly reduced ($p < 0.001$) in the diabetic group compared to controls. Subgroup analysis within the diabetic group showed significant decrease in E/I ratio and Delta HR in groups with poor glycemic control. Simple linear regression sought between resting heart rate and E/I ratio showed a highly significant inverse relationship ($p = 0.0001$). **Conclusion:** Our findings suggested that R-R variability during 1 minute deep breathing and resting heart rate are reliable and early predictors of CAN. Employing these simple tests in newly diagnosed patients and those in the pre-diabetic state could help control the emergence of CAN.

Keywords: cardiac autonomic neuropathy, deep breathing, heart rate variability, glycemic control

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Introduction

Cardiovascular Autonomic Neuropathy (CAN) is the most dreaded complication among both type 1 and type 2 diabetics. A meta-analysis of 15 different studies done in 2003 revealed that the prevalence of CAN is variable in different parts of the world, ranging from 1 to 90 %.[1] The outcome of CAN ranges from increased resting tachycardia to acute silent myocardial infarction and death. We are in the era of early identification and control of risk factors for

diabetes and its related complications. Risk stratification should begin from the pre-diabetic state itself as it is well documented that macrovascular complications start from the pre-diabetes state according to the "ticking clock hypothesis".[2]

Heart rate variability (HRV) is one of the strongest predictors of autonomic dysfunction. There are seven important observations from time domain analysis, spectral analysis of HRV and autonomic reflex tests. The possibility of CAN is confirmed when any of the following criteria is present: R-R variation during deep breathing <15 beats, R-R variation <20 plus, a Valsalva ratio <1.5, or a decrease of >10 mm Hg in diastolic blood pressure at any point during 10 min of standing after a period of 30 min of supine rest.[3]

We studied the resting HRV pattern and R-R variability during deep breathing in Type 2 diabetics and normal subjects in the context of detecting earlier abnormality which may be used as an alarming signal for making clinical decisions. The secondary objective was to study the effect of duration of diabetes and glycemic control on the R-R variability and HRV parameters.

Materials and Methods

This is a cross-sectional, analytical study done over a period of 6 months in the department of Physiology, Chennai Medical College Hospital and Research Centre, after getting clearance from the Institutional Ethics Committee. A total of 72 patients with Type 2 diabetes mellitus in the age group of 35 to 60 yrs of both genders and 98 age-matched normal controls were included in the study after obtaining informed written consent.

Patients having present and past history of diabetic foot ulcers, myocardial infarction and ischemia, other causes of autonomic neuropathy like chronic alcoholism, cancer chemotherapy, advanced renal failure and diabetic retinopathy were excluded from the study. All the subjects were administered a semi-structured questionnaire related to autonomic symptoms. HbA1C levels were assayed in all the diabetic patients as a part of their routine glycemic check up.

Measurement of Resting HRV: The subjects were rested in supine position for 15 minutes. ECG signals were acquired in all the patients in lead II using polygraph, the analogue signals were digitized using NI-DAQ Signal Acquisition device from National Instruments, India and the data stored in a PC.[4] The HRV was then analysed using HRV Soft, version 1.1, developed by AIIMS, New Delhi.[5] The statistical measures that were analysed as representative of time domain included Resting heart rate, Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording [SDANN], Root square of the mean of the sum of the squares of differences between adjacent RR intervals [RMSSD], percentage of number of R-R interval differences ≥ 50 ms [pNN50].

Measurement of R-R variability to deep breathing: The subjects were trained to breathe slowly at a rate of 6 breaths per minute (5 seconds inspiration and 5 seconds expiration as guided by the investigator showing hand signals). The Expiration: Inspiration ratio (E: I ratio) and the difference between the maximum heart rate and minimum heart rate (Delta HR) were calculated. E: I ratio >1.2 and Delta HR < 15 were considered abnormal.[4]

The results were analysed using SPSS software 20.0. The comparison between study and control groups was done using student's independent t test after checking for the normality of data. Subgroup analysis was done with duration of diabetes and glycemic status as variables within the study group using One-way ANOVA followed by Tukey's post-hoc test. A p value of <0.05 was considered to be statistically significant.

Results

The socio-demographic data of the study participants are shown in Table 1. Analysis of autonomic symptoms using Fisher's Exact Test showed statistical significance between diabetic group and control group.

Table 1: Socio-demographic details of the participants

Parameter	Diabetic group (n = 72)	Control group (n = 98)
Age (mean ± SD)	55.02 ± 5.09	54.02 ± 4.96
Male: Female (n)	36:36	69:29
BMI (mean ± SD)	23.2 ± 2.86	22.8 ± 1.98
Positive for autonomic symptoms,n (%)*	32 (44.4%)	7 (7.1%)

*p<0.0001 using Fisher's Exact Test.

Table 2 shows the comparison of resting HRV and R-R variability in deep breathing between diabetics and control subjects.

Table 2: Comparison of resting HRV and R-R variability between diabetics and controls

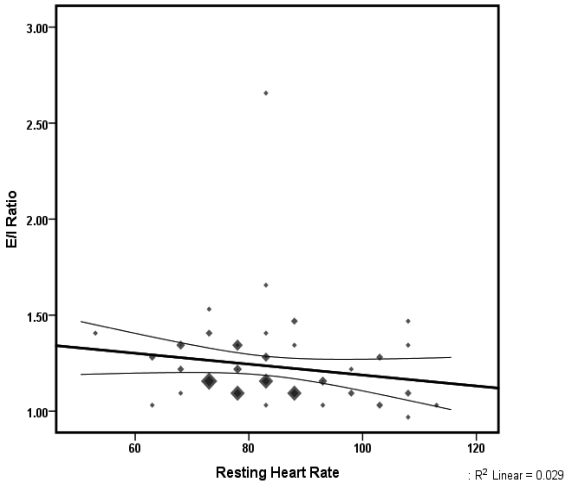
Parameter	Diabetic group (n = 72)	Control group (n = 98)
Resting heart rate*	83.22 ± 12.79	78.27 ± 12.98
RMSSD*	21.92 ± 18.11	29.02 ± 11.76
SDANN*	10.91 ± 8.06	52.29 ± 92.96
pNN50*	3.46 ± 9.06	19.46 ± 20.09
E: I ratio*	1.24 ± 0.213	1.53 ± 0.22
Delta HR*	15.09 ± 7.67	32.11 ± 10.2

* p < 0.001, extremely significant; all data were represented as mean ± SD. Analysis was done by Student's independent t test.

Resting HRV parameters included resting heart rate, RMSSD, SDANN and pNN50. R-R variability in deep breathing is assessed by E: I ratio and delta HR. All the above parameters were analysed using Student's independent t test and were found to be extremely significant (p < 0.001), as shown in Table 2.

Simple linear regression analysis was sought between resting heart rate and E: I ratio and was found to be highly significant (p <0.0001) with an inverse relationship. (Figure 1)

Figure 1. Linear regression between Resting heart rate and E/I Ratio



The figure explains the highly significant and linear decrease in the E: I ratio as the resting heart rate increases (p = 0.0001). The bold black fit line represents the regression line while the two faded black lines on either side represent the confidence intervals.

Subgroup analysis among diabetic patients was done with the duration of diabetes and glycemic control status as variables using One-way ANOVA with Tukey's Post-hoc test. It was found that there was no significant difference between both resting HRV and HRV during deep breathing as the duration of diabetes increased (Table 3).Subgroup analysis within the diabetic group showed significant decrease in E/I ratio and Delta HR in groups with poor glycemic control (Table 4).

Table 3: Subgroup analysis of diabetic patients based on duration of diabetes

Parameter	Subgroup 1 <5 years (n = 32)	Subgroup 2 5 to 10 years (n = 22)	Subgroup 3 >10 years (n = 18)
Hb A1C*	7.96 ± 1.60	9.21 ± 1.98	10.7 ± 2.31
Resting heart rate	83.22 ± 12.79	85.64 ± 11.97	81.72 ± 13.63
RMSSD	22.84 ± 21.38	20.77 ± 15.79	20.65 ± 14.97
SDANN	12.01 ± 6.43	12.12 ± 11.87	11.01 ± 4.43
pNN50	3.32 ± 8.08	3.77 ± 9.01	0.95 ± 1.47
E: I ratio	1.24 ± 0.14	1.18 ± 0.12	1.11 ± 0.36
Delta HR	17.75 ± 8.22	14.41 ± 8.73	14.78 ± 4.16

* p =0.0001, extremely significant; all data were represented as mean ± SD. Analysis was done by one-way ANOVA with Tukey's post-hoc analysis.

Table 4: Subgroup analysis of diabetic patients based on glycemic control

Parameter	Subgroup 1 HbA1C < 7 (n = 15)	Subgroup 2 HbA1C =7 to 9 (n = 27)	Subgroup 3 HbA1C > 9 (n = 30)
Hb A_1C**	6.65 ± 0.32	7.98 ± 0.55	11.16 ± 1.67
Resting heart rate	79.67 ± 11.46	83.41 ± 12.87	84.83 ± 13.39
RMSSD	27.16 ± 19.27	22.88 ± 20.26	18.43 ± 15.08
SDANN	14.72 ± 13.09	10.21 ± 6.85	9.03 ± 4.93
pNN50	4.74 ± 5.26	4.21 ± 11.66	3.15 ± 8.05
E: I ratio*	1.28 ± 0.11	1.21 ± 0.11	1.11 ± 0.29
Delta HR*	20.67 ± 11.8	15.15 ± 6.03	14.40 ± 5.44

** p =0.0001, extremely significant; *p = 0.02, significant between Subgroup 1 and 3; all data were represented as mean ± SD. Analysis was done by one-way ANOVA with Tukey's post-hoc analysis.

Discussion

This study has once again proved that the resting HRV of the 72 diabetic patients is significantly reduced when compared to the normal subjects. Previous studies done in the past 3 decades also have demonstrated the same results.[6] In this study, we limited ourselves to only one autonomic reflex test, i.e., R-R variability during deep breathing. In a meta-analysis of 15 studies done on diabetic patients by Vinik *et al.*, 2003,[1] 11 out of 12 studies[7-11] included HRV during deep breathing as a prime test and all of them have found a significant decrease in the R-R variability. Thus, HRV changes during deep breathing are the best predictor of autonomic neuropathy. HRV during deep breathing is a parasympathetic phenomenon. In the natural history of diabetic autonomic neuropathy parasympathetic involvement emerge earlier.[12]

But HRV in deep breathing does not seem to distinguish between minimal involvement and those with serious damage.[12] A similar feature is observed in our study as well and there was no significant difference between both resting HRV and HRV during deep breathing as the duration of diabetes increased. Another study by Hassan *et al.* also has demonstrated similar results.[13]

Contrary to the report given by Ewing *et al.*[12] poor glycemic status was found to affect the HRV changes deleteriously. Some important studies like the Diabetes Control and Complications Trial (DCCT) prove that the incidence of CAN is well controlled in the intensively treated group of diabetics.[14-16]

Resting tachycardia and a fixed heart rate with exercise intolerance is one of the earliest manifestations of parasympathetic involvement in CAN.[14] This study also showed that the resting heart rate was significantly higher in diabetics when compared to normal controls. As resting heart rate and R-R variability have both been found to be the early markers, we estimated the relationship between the two, using a simple regression equation which

proved a strong inverse relationship. Hence for the early diagnosis of cardiac autonomic neuropathy which is a life-endangering complication, physicians can use these two simple, non-invasive and relatively less expensive tests to alert the patients promptly on the need for strict glycemic control and risk factor modifications.

Conclusion

Resting Heart Rate Variability (HRV) especially resting heart rate and R-R variability during deep breathing are early and definitive predictors of cardiac autonomic neuropathy. Testing the diabetic patients on these two parameters routinely in the diabetic clinic will help to reduce the morbidity and mortality due to this dreaded complication.

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Conflicts of interest: Nil

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