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Heart rate complexity and cardiac sympathetic dysinnervation in patients with type 2 diabetes mellitus

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Abstract— **Cardiovascular autonomic neuropathy (CAN) is one of the most severe complications of type 2 diabetes mellitus (T2DM). The aim of this study was to investigate associations of cardiac sympathetic dysinnervation (CSD; by ^{123}I-MIBG scintigraphy) with short-term heart rate variability (HRV) measured by traditional vs. complexity markers. ECG was measured in 31 diabetic patients during rest over a period of 5 minutes and HRV quantified in different domains (time and frequency domain, scaling properties, symbolic dynamics). ^{123}I-MIBG scintigraphy identified 16 patients with CSD. Resting heart rate was increased and HRV reduced in these patients. In a subgroup of 16 patients ECG was also measured during standing. Changes in several HRV measures upon standing demonstrated cardiac responsiveness to orthostatic stress. Strong correlations between HRV, measured during standing, and CSD were observed with metrics based on symbolic dynamics. In conclusion, HRV assessment during standing may be useful for assessing cardiac sympathetic dysinnervation in patients with type 2 diabetes mellitus.**

I. Introduction

Cardiovascular autonomic neuropathy (CAN) is one of the most severe complications of type 2 diabetes mellitus (T2DM) and has been associated with increased cardiac mortality [1]. In the clinical setting, CAN is diagnosed using a battery of autonomic reflex tests (Ewing battery). There is substantial evidence, however, that the assessment of heart rate variability (HRV) provides a simple, reliable tool to detect early onset CAN [1]. Traditionally, HRV is quantified applying a set of time and frequency domain measures on time series of RR intervals obtain from body surface ECG as specified by the HRV Task Force guidelines [2]. Physiological fluctuations in heart rate are primarily mediated via activity of the vagus nerve with additional sympathetic inputs, the latter being augmented during periods of heightened physical, mental and emotional stress [2].

During the last 15 years, a variety of additional metrics has been used to quantify the complexity and temporal dynamics of RR time series [3]. Several studies argued for better discriminative power of those measures for cardiac risk stratification and diagnosis of CAN compared to time and frequency domain measures [3]. Physiological interpretation of heart rate complexity (metrics) is difficult as links to physiological rhythms (i.e. respiration and vasomotor activity), which cause the large part of physiological HRV, may not always be apparent. A great part of our understanding on physiological correlates of HR complexity has been gained through autonomic stress tests and pharmacological intervention (i.e. block or activation of vagal and sympathetic branches of the autonomous nervous system) [4, 5]. Evidence originating from direct measurement of autonomic nervous system activity is limited [6].

The aim of this study was to investigate short-term HRV using standard metrics and complexity measures in patients suffering from T2DM with varying degrees of cardiac sympathetic dysinnervation (CSD). We hypothesized that measures of HRV may be associated with CSD and thus provide clinically useful markers for sympathetic dysfunction in T2DM.

II. Methods

A. Patients

Patients with T2DM (n = 33) with no history of cardiovascular disease, cancer or psychiatric or other severe illness were recruited from the community. Some of these patients' data have been reported previously [7, 8]. Exercise echocardiography studies were performed in all patients to verify normal ejection fraction (> 50%) and the absence of coronary artery disease (i.e. no inducible wall motion abnormalities indicative of ischemia). Two patients with an abnormal resting ECG were subsequently excluded, including one patient with frequent ectopy (> 50%) and one patient with abnormal QRS morphology precluding valid measurement of HRV. Patients provided written informed consent and the study protocol was approved by hospital and university human research ethics committees.

B. ^{123}I-MIBG imaging

Protocols for recording and analysis of ^{123}I-MIBG images have been described in detail [7, 8] Patients were pre-medicated with 600 mg potassium perchlorate to block thyroid uptake of radioiodine. A standard camera with a low-energy, high-resolution collimator (Symbia, Siemens, Erlangen, Germany) was used in the acquisition of anterior planar and single photon emission computed tomography (32 projections for 50s each) images 15 minutes (early) and 4 hours (delayed) following injection of 150MBq of ^{123}I-MIBG. Global cardiac uptake of ^{123}I-MIBG was calculated from both early and delayed planar images by the ratio of tracer activity (mean count per pixel) in the heart and mediastinum. Due to non-neuronal uptake affecting early images, the delayed heart-to-mediastinum (H/M) ratio was primarily used in analyses and to define the presence of CSD (H/M ratio <1.8, as previously applied in diabetic patients [9].

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C. ECG recording

Studies were performed in accordance with standard conditions for clinical cardiac autonomic function testing, as previously described [7, 8]. ECG was recorded during spontaneous, rather than paced breathing, and patients were also instructed to relax, to avoid talking or moving, and to remain awake. The median time and interquartil range separating ^{123}I-MIBG imaging and ECG acquisition was 4 (0 – 82) days. Following at least 15 minutes supine rest in a quiet room, an ECG (lead II) was recorded continuously over 5-minutes at a sampling frequency of 1kHz using a Powerlab 8SP data acquisition system linked with commercially available software (LabChart Pro, AD Instruments, Sydney, Australia). In a subgroup of 16 patients, a second 5-min ECG recording was performed in the standing position. Standing ECG acquisition commenced following a stabilization period of at least 2 minutes. Patients were again instructed to maintain this position with minimal movement.

D. Heart rate variability and complexity analysis

For traditional time domain analysis of HRV [2], we computed *meanNN*–the mean beat-to-beat interval of normal heart beats; *sdNN*–standard deviation of NN intervals; and *RMSSD*–the root-mean-square of successive beat-to-beat differences.

For frequency domain analysis of HRV we generated equidistant time series, using a linear interpolation at 500 ms sampling intervals. The power spectrum was then estimated, using the Fast Fourier Transform according to HRV Task Force recommendations: *LF*–low frequency power (0.04-0.15 Hz), *HF*–high frequency power (0.15-0.4 Hz) and *LFn* – ratio of *LF* to the sum of *LF* and *HF*.

Nonlinear characteristics of HRV were quantified according to the symbolic dynamics approach described by Porta *et al.* [8]. Here, the series of RR intervals was transformed into an alphabet of six symbols {0, 1, 2, 3, 4, 5}. As a transform rule, non-uniform quantization was applied, keeping the number of points associated with each quantization level constant [10].

The patterns of symbolic sequences of length three were grouped into four families according to the number and types of variations from one symbol to the next one. The pattern families were: 1) patterns with no variation (*0V*–all three symbols are equal); 2) patterns with one variation (*1V*–two consecutive symbols are equal and the remaining one is different); 3) patterns with two like variations (*2LV*–the three symbols form an ascending or descending ramp), 4) patterns with two unlike variations (*2UV*–the three symbols form a peak or a valley). The rates of occurrence of these patterns are indicated as *0V%*, 1*V%, 2LV%* and *2UV%*.

In addition, we quantified scaling features of RR time series using Higuchi's fractal dimension (*HFD*) and the short-term scaling exponent α_1 obtain with detrended fluctuation analysis (DFA). For details, see [11, 12].

E. Statistical analysis

For statistical analysis we compared resting HRV measures in patients with and with no CSD using the student's t-test. Further, we compared HRV recorded during rest versus HRV during standing using the paired student's t-test. To examine association between HRV and CSD, we computed Pearson's linear correlation coefficients. All statistical analyses were carried out in GraphPad Prism 6 (GraphPad Software Inc.). Frequency domain measures were log-transformed prior to statistical analysis. Values of p less than 0.05 were considered to be statistically significant.

III. Results

The group mean and standard deviation of H/M ratio were 1.78 ± 0.19. Sixteen of the patients had an H/M ratio < 1.8, indicative of CSD. Comparing resting HRV and complexity between subgroups of patients with and without CSD, we found a significant reduction in all time and frequency domain measures, except for the normalized low frequency power. No significant difference in any of the complexity measures of HRV were observed between the two groups. Numerical values of all HRV measures are summarized in Table I.

Linear correlation analysis showed a significant relationship between H/M ratio and SDNN (r = 0.37, p = 0.04) and log*LF* (r = 0.41, p = 0.02), respectively.

TABLE I. Heart Rate Variability of Patients with T2DM with (CSD+) and with no (CSD-) Cardiac Sympathetic Dysinnervation Measured in the Supine Position

HRV	CSD+	CSD-	*p*
meanNN	818 ± 104	922 ± 142	0.03
sdNN	20 ± 12	39 ± 24	0.01
RMSSD	11 ± 7	22 ± 16	0.03
log*LF*	1.85 ± 0.53	2.37 ± 0.56	0.01
log*HF*	1.37 ± 0.52	1.93 ± 0.51	0.006
LFn	0.70 ± 0.22	0.71 ± 0.15	0.84
α_1	1.22 ± 0.25	1.20 ± 0.18	0.80
HFD	1.69 ± 0.15	1.69 ± 0.12	0.94
0V	33.4 ± 16.7	34.5 ± 16.3	0.85
1V	45.9 ± 7.5	45.0 ± 6.0	0.70
2LV	6.3 ± 4.5	6.8 ± 5.3	0.80
2ULV	14.4 ± 9.2	13.8 ± 8.7	0.85

Heart rate variability data of T2DM patients who had undergone measurements in both, supine and standing positions are summarized in Table II. Among them, six patients had CSD. Mean RR interval was shorter during standing and power in the LF and HF ranges significantly decreased. Normalized LF power, on the other hand, was increased during standing. The short-term scaling exponent was higher and Higuchi's fractal dimension lower decreased during standing. The relative frequency of words with changes in two symbols was significantly reduced.

Results of the correlation analysis between H/M ratio and HRV measures are shown in Table III. During standing the H/M ratio showed significant linear associations with symbolic dynamics parameters *0V* (r = -0.65, p = 0.006) and *1V* (r = 0.71, p = 0.002) (Figure 1).

TABLE II. HEART RATE VARIABILITY OF PATIENTS WITH T2DM MEASURED IN THE SUPINE POSITION AND DURING STANDING

HRV	supine	standing	*p*
meanNN	869 ± 141	776 ± 102	0.0006
sdNN	33 ± 24	27 ± 11	0.31
RMSSD	18 ± 15	13 ± 7	0.19
log*LF*	2.17 ± 0.58	2.12 ± 0.48	0.06
log*HF*	1.71 ± 0.57	1.40 ± 0.51	4.34E-
LFn	0.71 ± 0.18	0.82 ± 0.09	0.04
α_1	1.21 ± 0.20	1.42 ± 0.20	0.008
HFD	1.67 ± 0.13	1.59 ± 0.11	0.01
0V	38.5 ± 16.5	47.1 ± 11.4	0.13
1V	42.6 ± 6.5	40.2 ± 8.4	0.45
2LV	5.9 ± 4.4	2.9 ± 2.1	0.03
2ULV	13.1 ± 8.1	9.8 ± 4.3	0.07

TABLE III. CORRELATION BETWEEN H/M RATIO AND HRV MEASURED IN THE SUPINE POSITION AND DURIGN STANDING

HRV	supine		standing	
	r	*p*	*r*	*p*
meanNN	0.31	0.09	-0.18	0.51
sdNN	0.37	0.04	-0.31	0.24
RMSSD	0.31	0.09	-0.28	0.29
log*LF*	0.30	0.10	-0.19	0.48
log*HF*	0.41	0.02	-0.21	0.44
LFn	0.35	0.05	-0.14	0.61
α_1	0.17	0.37	-0.14	0.60
HFD	0.11	0.54	-0.28	0.30
0V	-0.12	0.53	-0.04	0.89
1V	0.08	0.67	-0.65	<0.01
2LV	-0.14	0.45	0.72	<0.01
2ULV	0.02	0.90	0.33	0.22

IV. DISCUSSION

In this study we investigated associations between CSD and a variety of HRV measures in patients with T2DM. Our main findings are the reduction in resting HRV in patients with CSD, and moderate linear correlations with the H/M ratio. Upon standing, besides the expected reduction in HRV, strong correlations with the H/M ratio were observed in symbolic measures of HRV.

Diabetic autonomic neuropathy affects parasympathetic and sympathetic branches of the autonomic nervous system, where vagus nerve damage is primarily responsible for reduced HRV in patients with T2DM observed during rest. Our data show that a reduction in the H/M ratio, which indicates cardiac sympathetic dysfunction at the level of nerve terminals, is paralleled by elevated resting heart rate and reduced heart rate variability in the time and frequency domains, demonstrating a reduction in magnitude of heart rate fluctuations. Measures of temporal structure and complexity with RR time series, which are not directly affected by the magnitude of variability, on the other hand, do not appear different in diabetic patients with or with no CSD. Given the results of the correlation analysis, which suggests only a moderate association between HRV and degree of CSD, one may speculate whether the relation between resting HRV and CSD may be primarily due to concomitant vagus nerve damage. Although pharmacological intervention demonstrated sympathetic influences on resting HRV, measurement of resting HRV may not be useful for assessing the level of sympathetic neural outflow to the heart [13].

Changes in magnitude and complexity of HRV upon standing indicate a prevailing level of autonomic responsiveness to orthostatic stress, where the increase in heart rate and reduction in power of HF oscillations, respiratory sinus arrhythmia, are the most prominent features. Of the complexity measures, both indices of scaling features, detrended fluctuation analysis and Higuchi's fractal dimension, as well as symbolic analyses point towards a change in the structure of RR time series rather than merely a reduction in the magnitude of HRV. The α scaling exponent as well as HFD – both mathematically interrelated – approach values towards those of Brownian motion ($\alpha = 1.5$; $HFD = 2$) during standing compared to values obtained during rest. Symbolic analysis of RR dynamics indicates a reduction in dynamics during standing as word types with changes in two symbols are relatively less frequent. Our results are comparable to those obtained during graded head-up tilt in healthy volunteers [13].

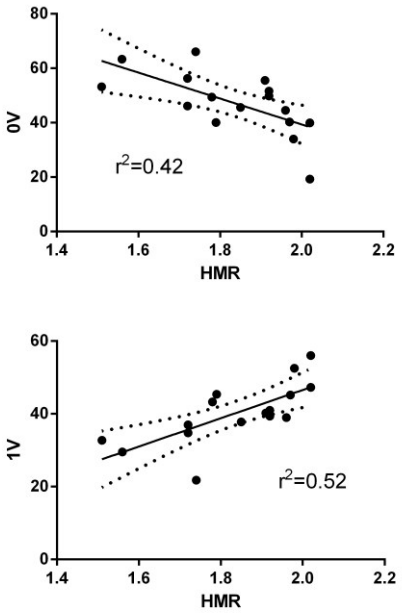

Figure 1 Scatter plots of symbolic measures of heart rate variability measured during standing and H/M ratio of patients with type 2 diabetes mellitus.

Correlation analysis of H/M ratio and magnitude and complexity measures of HRV suggests a linear association between CSD and symbolic dynamics of RR time series when measured during standing, i.e. in a state of sympathetic activation. The more severe the CSD in patients with T2DM, the less the dynamics of heart rate during standing as demonstrated by the inverse correlation between H/M ratio and the relative frequency of zero variability word types (*0V*) and the positive correlation with the percentage of word types with variability in one symbol (*1V*). These data suggest that the sympathetic tone modulates HR dynamics during

standing and that those patients with progressed sympathetic dysinnervation also have also the lowest RR dynamics. We previously investigated QT interval variability (QTV) in the same group of patients and observed a similar behavior, i.e. no association between QTV and H/M ratio during rest, but a negative correlation between both measures during standing, indicative of increased repolarization in CSD [14]. Elevated QT variability has been previously reported in patients with diabetic autonomic neuropathy [15] as well as changes in heart rate complexity [9, 16-18].

The main limitations of our study are the lack of a control group of healthy subjects and the small number of patients, who had ECG recorded during standing, which are a consequence of the retrospective nature of this study. The association between symbolic dynamics of RR time series measured during standing and sympathetic dysinnervation may be specific to CAN and not imply a general association between cardiac sympathetic function and symbolic dynamics of HRV during standing, although this requires further study.

V. Conclusion

Measurement of short-term heart rate complexity during standing using symbolic dynamics may be useful for assessing the degree of cardiac sympathetic dysinnervation in patients with type 2 diabetes mellitus in clinical routine.

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