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Autonomic nervous system function accessed by conventional and spectral analysis might be useful in terms of predicting retinal deterioration in persons with type 1 diabetes mellitus

L. Duvnjak[1,2], M. Tomić[1], K. Blaslov[1], S. Vučković Rebrina[1]

[1]Vuk Vrhovac Clinic for Diabetes, Endocrinology and Metabolic Diseases, University Hospital Merkur, Zagreb, Croatia

[2]School of Medicine Zagreb, Croatia

Correspondence to: Kristina Blaslov, MD; Vuk Vrhovac Clinic for Diabetes, Endocrinology and Metabolic Diseases, University Hospital Merkur, Dugi dol 4a, Zagreb, Croatia.

e-mail: kblaslov@gmail.com

Tel/Fax: 0038512353829 / 0038512331515

Abstract

Aims: To determine whether cardiac autonomic dysfunction represents a risk factor for diabetic retinopathy (DR) development and progression in persons with type 1 diabetes mellitus (T1DM).

Methods: The study comprised 154 normoalbuminuric persons with T1DM divided into two groups according to the DR presence: one with and another without DR. Cardiovascular autonomic functioning was accessed by conventional and spectral data analysis. Student's *t*-test and Fischer's exact test were used to access between group differences. Cox's proportional hazards regression analysis was used to examine the baseline variables predictive for development or progression of NPR 18 months after.

Results: The group with DR had longer disease duration compared to the group witout DR (20 *vrs* 11.5 years, $p<0.001$), heart rate coefficient of variation (HRV-CV) at rest and during deep breathing, as well as its vagal nerve mediated modulation, were lower in participants with DR ($p=0.001$; 0.004 and <0.001, respectively). No difference in glycaemic control, lipid status or hypertension rate was observed. Twenty-one (13.36%) participants developed non proliferative DR or progressed to proliferative DR. For each increase in the HRV-CV of 1%, the 18 months risk from retinal deterioration was reduced by 33.4% and by 12.7% for the same HRV-CV increase during deep breathing while the increase of parasympathetic nerve activity output of 1 ms^2 results in 8.6% DR risk reduction.

Conclusions: This study provides evidence that DR should not be considered merely a metabolic control manifestation and that HRV-CV might be the top predictor of retinal deterioration in normoabuminuric persons with T1DM.

Key words: type 1 diabetes mellitus, cardiac autonomic dysfunction, hearth rate variability

1. Introduction

Diabetic retinopathy (DR) is the most frequent cause of visual impairment and legal blindness among adults with type 1 diabetes mellitus (T1DM) [1]. The pathogenesis of its development and progression is not completely understood. To date, dysglycaemia, elevated blood pressure, dyslipidaemia and longer diabetes duration are the most pronounced risk factors for DR development and progression [2-4]. Diabetic kidney disease, often accompanied with urine albumin excretion above 30 mg/24 hours coincides with DR [5, 6]. Moreover, the structural changes in the kidney which include glomerular basement membrane thickening, micro aneurysm formation and mesangial nodule formation represent the similar small histological finding seen in DR [7, 8].

Cardiovascular autonomic neuropathy (CAN) is defined as impairment of autonomic control of the cardiovascular system in the setting of diabetes after exclusion of other causes with prevalence ranging from 16-20% among persons with diabetes [9]. There is a well established causative role of CAN in diabetic kidney disease development and progression in persons with T1DM [10, 11] while an association between CAN and DR has been reported in several cross-sectional studies [6, 12-14]. Clinical symptoms of CAN: resting tachycardia and a fixed heart rate represent the characteristic of structural vagal nerve impairment and may not appear until long after diabetes onset [9]. Subclinical CAN however represents functional abnormalities of the autonomic nervous system, i.e. the imbalance between the sympathetic and parasympathetic nervous system that can be discerned by using spectral analysis of heart rate variability (HRV) [15, 16, 17].

Spectral analysis involves decomposing the series of sequential R-R intervals into a sum of sinusoidal functions of different amplitudes and frequencies by several possible mathematical approaches, such as fast Fourier transformation [18]. The result (power spectrum) reflects the amplitude of the HRV present at different oscillation frequencies. The power spectrum of

HRV has been shown to consist of two major peaks: low-frequency (LF) component which represents the parasympathetic/vagal nerve contribution to HRV-CV at rest and high-frequency (HF) component which reflects parasympathetic contribution to HRV-CV during in relation to respiration.

It would be of a special interest to investigate whether early, subclinical CAN contributes to the risk of DR and whether it might represent an independent risk factor for its development and progression in normoabuminuric type 1 diabetic (T1DM). We aimed to determine whether CAN assessment accompanied by detailed spectral analysis might serve as a practical tool to identify a group of T1DM patients with a higher risk for retinal deterioration.

2. Materials and Methods

2.1 Study population

One hundred fifty four randomly selected normoalbuminuric persons with T1DM referred to tertiary care specialist between January 2011 and December 2014 were included in the study after informed consent was obtained. T1DM was diagnosed according to World Health Organization (WHO) criteria (< 35 years at the age of onset of diabetes, a previous episode of ketoacidosis or documented ketonuria, positive autoantibodies and permanent insulin treatment initiated within 1 year of diagnosis) [19]. Eligible participants were at least 18 years old, minimum duration of T1DM of 1 year, no medical history of cardiovascular diseases or electrocardiogram (ECG) evidence of ischemic heart disease, absence of any systemic disease and absence of any infections in the previous month. Urine albumin excretion (UAE) was measured from at least two 24-h urine samples and determined as the mean of 24-h urine collections in order to minimize variability. Normoalbuminuria was defined as a UAE<30 mg/24h [20]. Fasting venous blood samples were collected for the determination of a glycated

haemoglobin (HbA1c, %, reference interval 3.5–5.7), C-reactive protein (CRP), serum creatinine in order to exclude a wide range of disorders that might affect the study results. HbA1c was measured spectrophotometrically by turbidimetric immuno-inhibition (Olympus AU600, Beckman-Coulter, USA).

Blood pressure was measured in the sitting position with a mercury sphygmomanometer with a cuff appropriate to the length and circumference of the arm after a resting period of 10 minutes and expressed in mmHg. Patients taking blood pressure medications or with blood pressure >140/90 mmHg were considered to have hypertension.

Participants were re-examined after 18 months to update their glycaemic control, UAE and retinopathy status. The study protocol complied with the Declaration of Helsinki as well as local institutional guidelines.

2.2 Data acquisition

Complete eye examination included best corrected visual acuity (BCVA), Goldmann applanation tonometry, slit lamp biomicroscopy of the anterior eye segment, binocular indirect slit lamp fundoscopy and fundus photography after mydriasis with eye drops containing 0.5 % tropicamide and 5 % phenylephrine. Color fundus photographs of two fields (macular field, disc/nasal field) of both eyes were taken with a suitable 45° fundus camera (VISUCAM, Zeiss) according to the EURODIAB retinal photography methodology [21]. Macular field: positioned in such a way that the exact centre of the optic disc laid at the nasal end of the horizontal meridian of the field view. Disc/nasal field: such that the optic disc was positioned one disc-diameter in from the temporal edge of the field, on the horizontal meridian. EURODIAB classification scheme was used because it uses two-field 45° fundus photography and standard photographs to grade retinal lesions. In each person the "worse"

eye was graded for retinopathy using fundus photographs.

Autonomic nervous system testing was carried out on VAGUS 2100 (Sigma Medizin Technik, Thum, Germany) Test conditions was standardized as suggested by Spallone et al. (2011) [17] as follows: testing was conducted between 9-12 a.m., at least 2 hours after a light breakfast, a tested subject did not consume coffee, black tea or nicotine and blood glucose was checked prior to testing. Three disposable, self-adhesive ECG electrodes were applied to the chest with adhesive tape. Standard battery of cardiovascular tests to confirm diabetic autonomic dysfunction included: the coefficient of variation (CV) of RR intervals at rest indicating HRV at rest (HRV-CV), HRV-CV during deep breathing (dbHRV-CV), Valsalva manoeuvre in order to obtain Valsalva ratio: the ratio of the highest RR interval after the manoeuvre to the loveat during the manoeuvre,), active orthostatic test, blood pressure response to standing, deep breathing E / I ratio (dbE/I) and 30:15 ratio [22].. Although there are no unanimous criteria for CAN diagnosis, the presence of abnormalities in two or three abnormal results among mentioned autonomic tests is recommended for cardiac autonomic neuropathy diagnosis 17, 23].

Furthermore, as suggested by Barlund et al. (2009) [24], from the original data of RR interval series, using the fast Fourier transformation, the computer system obtained the power in the low frequency (LF) (0.04-0.15 Hz) and high-frequency (HF) (0.15-0.40 Hz). Normalised units of the LF and HF bands were calculated as follows: nLF=LF power/(LF+HF powers) and nHF=HF power/(LF+HF power) where n stands for normalised and HF for high and LF for low-frequency power.

2.3 Statistical analysis

The data distribution was assessed by Shapiro-Wilk test. All the continuous variables were log-transformed in order to reduce the skew, and reported as mean values and 95%CI of means or standard deviation (SD), whereas categorical variables were reported as numbers and percentages. The differences between two study groups baseline characteristics were tested by Student's *t*-test while the categorical variables were analysed by the Fischer's exact test.

Cox's proportional hazards multiple regression analyses was used to examine the baseline variables predictive for development or progression of non proliferative DR, taking in account of potential confounders and pre-existing retinopathy status. Results are described as relative risk (hazard ratio). A *p* value of less than 0.05 was considered significant.

3. **Results**

The mean age of study population was 37.5 years with 14 years of diabetes duration. Seventy four participants (48.05%) were male. CAN was established in 14 (9.09%) of them, however, non of the patients had pathological result of Valsalva manoeuvre nor orthostatic hypotension, as expected. The prevalence of non proliferative DR was 30.51% (N=47). There were no patients with the history of laser photocoagulation due to severe non proliferative, proliferative DR or macular oedema.

The mean UAE was 11.45 (95% CI 10.43-12.47 mg/24h) at the study beginning. Forty six participants (29.87%) were smokers, 58 (37.66%) were taking the nephroprotective dose of trandolaprilum (0.5 mg/day). The results of cardiovascular autonomic function assessment in 47 T1DM persons with non proliferative DR and 107 free of DR are given in Table 1. Persons with non proliferative DR showed significantly lower HRV-CV at rest and dbHRV-CV when compared to group of persons without DR. We also observed a significant attenuation for both

LF and HF bands in the group with non proliferative DR indicating that they have had lower parasympathetic or higher sympathetic nerve activity. calculate the LF/HF ratio that is considered an expression of the sympathovagal balance Although the in-between group duration of diabetes was significantly different, long-term glycaemic control assessed by HbA1c was similar.

Additionally, it did not show the significant change until the study end (7.0 (6.6-7.2) *vs* 6.9 (5.1-7.5) for all, p=0.566). Six (3.89%) participants developed albuminuria (UAE 29.87 (10.59-37.54) mg/24h for all) during 18 months follow up period while 21 (13.36%) developed non proliferative DR or progressed from non proliferative DR to proliferative DR. Retinal status deterioration was independent of the baseline retinal status (Figure 1.). The HRV-CV, dbHRV-CV and LF power showed inverse correlation with the disease duration (r=-0.633, p=0.043; r=-0441, p=0.012 and r=-0.391, p=0.040, respectively).

The Cox regression model (Table 2.) revealed that baseline HRV-CV and dbHRV-CV reduce the risk of DR incidence and progression. For each increase in the HRV-CV of 1%, the 18 months risk from retinal deterioration is reduced by 33.4% and by 12.7% for the same HRV-CV increase during deep breathing. In addition, increase in LF band of 1 ms^2 results in 8.6% DR risk reduction in the same period of time.

4. Discussion

In the present study a battery of non invasive cardiovascular autonomic function tests proposed by Ewing [23] and a power spectral analysis of HRV-CV in 154 strictly normotensive, normoalbuminuric, metabolically adequately regulated T1DM was performed in order to elucidate the possible pathogenic role of autonomic dysfunction in DR development and progression during 18 months of follow-up in. The possible predictive association between lower spectral indices of HRV that might derive from both

parasympathetic impairment and sympathetic activation with the DR development and progression was identified. This relation persisted after adjusting for age, gender, disease duration, HbA1c, ACE inhibitor use smoking status, UAE, total daily insulin dose, blood pressure and CAN presence which are already described as risk factors linked to DR pathogenesis [1, 5-8].

The manifest CAN with blunted parasympathetic activity was reported in persons with albuminuria [4, 7, 8], non proliferative DR [6] and proliferative DR [12]. However, there are only few studies conducted in order to establish the potential causative role of early, asymptomatic autonomic nervous system functional disturbances with changes in retinal microarchitecture [16, 26]. The small proportion of abnormalities obtained from the conventional autonomic nervous system tests compared to the differences in the spectral data indicates that we did access the group of patients with autonomic nervous system dysfunction [24] and that the presence of 14 (9.09%) with CAN diagnosis did not affect the final results.

It is well known that chronic hyperglycaemia promotes progressive dysfunction of autonomic nervous system in a manner that accompanies peripheral neuropathy development, i.e., beginning distally and progressing proximally. The vagus nerve, which is the longest autonomic nerve, mediates about 75% of all parasympathetic activity. Because neuropathy is seen first in the longest nerve fibres, the earliest manifestations of autonomic neuropathy in diabetes tend to be associated with parasympathetic denervation. Our data and those of others [27, 28] confirm that there is a compensatory increase in the cardiac sympathetic tone in the early phase of CAN. Since heart rate represents a balance between sympathetic and parasympathetic part of the autonomic nervous system activity, its alterations represent the earliest manifestation of disruption in autonomic nervous functioning, i.e. autonomic neuropathy. Moreover, CAN could be a manifestation of the disease duration and severity

since it is more frequent in the patients with longer diabetes duration and oftenly associated with other microvascular complications [27-29].

Although the correlation between CAN and DR in T1DM has been reported [6] and recent study by Huang (2016) [29] suggests that DR is the most significant risk factor predictive of the presence of CAN in 174 patients with type 2 diabetes., its implications in the patophysiological mechanism of retinal damage remains unresolved, especially because it has not been confirmed whether retinal vessels possess any sympathetic innervation [30]. The direct effect of autonomic nervous system on retinal blood flow is therefore dubious which brings to focus the proposed indirect mechanism through systemic hemodynamic changes. The pathophysiology of DR comprises a complex of hemodynamic disorders leading to endothelial dysfunction, intimal denudation, retinopathy induction and deterioration. The increase in symphatetic tone as observed in our study leads to systemic disbalance between vasoconstriction and vasodilatation [31] which consequently leads to blood pressure alteration and might mediate the association of CAN with DR as previously proposed [6]. More important, a possible increased blood pressure load during the night in patients with CAN might result in retinal ischemia [4, 6, 31, 32] because autonomic dysfunction may affect retinal vessels autoregulation, allowing even small blood pressure elevations to have a deterious impact on the retina caused by capillary hypertension, hyperperfusion and a consecutive damage.

Our study has several limitations that should be pointed out: we used the convenience sample method, which definitely contributes to the possible bias of the study for example: we do not have the complete data on the previous glycaemic control. As far the methodology, we did not take into account to precisely monitor breathing during deep breathing tests; it was estimated by a performing technician instead. At last, the number of patients with CAN must have had impact on the study results. However, we do believe that although we marked a significant

difference in the disease duration between T1DM persons with DR and those without DR, HRV-CV might one of the less recognised factors contributing to retinal deterioration in normoabuminuric T1DM patients. Early and detailed CAN assessment followed by spectral analysis of HRV might serve as a practical tool to identify a group of T1DM patients with a higher risk for retinal deterioration.

5. Conflict of interest

All authors disclosed no financial or personal conflict of interest.

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Table 1 – Clinical, laboratory data and cardiac autonomic function tests.

Variable		Patients without retinopathy ($N = 107$)	Patients with retinopathy ($N = 47$)	p value
Age (yrs)		37(34–39)	39.5 (36–42.5)	0.279
Gender (N, %)	Female	52 (48.6)	28 (59.6)	
	Male	55 (51.4)	19 (40.4)	0.211
Diabetes duration (yrs)		11.5 (10.5–13.0)	20.0(17.5–22.5)	<0.001
HbA1c (%)		7.2(6.8–7.5)	7.4(6.9–7.8)	0.489
eGFR (mL/min/1.72 m^2)		108.1(105.6–110.5)	105.6(101.2–110.1)	0.301
UAE (mg/24 h)		11.29(10.08–12.51)	11.82(9.86–13.77)	0.651
SBP (mmHg)		120 (110–140)	130 (115–140)	0.169
DBP (mmHg)		80 (70–85)	80 (70–85)	0.706
Total daily insulin dose (U/kg)		0.611 (0.032–0.943)	0.651 (0.349–0.851)	0.071
Autonomic neuropathy (N, %)		5 (4.7)	9 (19.15)	0.004
db E/I		1.35(1.29–1.41)	1.31(1.19–1.43)	0.008
Valsalva ratio		2.14(1.91–2.37)	2.23(1.79–2.68)	0.215
30:15 ratio		1.61(1.45–1.75)	1.44(1.29–1.58)	0.722
Spectral analysis of HRV				
HRV–CV (%)		4.64(4.32–4.96)	3.68(3.21–4.15)	0.001
dbHRV-CV(%)		8.21(7.48–8.91)	6.48(5.35–7.62)	0.004
RRI LF (ms^2)		444.6 (363.6–565.1)	431.6 (361.2–504.0)	0.003
RRI HF (ms^2)		252.0(229.2–280.2)	288.6(270.6–310.8)	0.022
RRI LF/HF		1.76 (1.63–1.93)	1.53 (1.35–1.63)	0.037
RRI nLF (%)		57.06 ± 1.89	49.19 ± 3.23	0.027
RRI nHF (%)		45.48 ± 1.97	39.04 ± 3.52	0.043

HRV-CV: hearth rate variability-coefficient of variation; dbHRV-CV: hearth rate variability coefficient of variation during deep breathing; LF-low frequency, HF-high frequency, RRI-the time interval between two consecutive R peaks of the ECG; nLF-normalised LF; nHF-normalised HF; dbE/I-the expiration/inspiration ratio during deep breathing, 30:15 ratio-the maximum:minimum 30/15 ratio of RR interval during active standing; SPB-systolic blood pressure; DBP-diastolic blood pressure.

Table 2 – Cox's multiple regression analysis model by means of baseline risk factors for development or progression of diabetic retinopathy.

Variable	Relative risk	95% CI
HRV–CV (%)	0.664	0.514–0.866
db HRV-CV(%)	0.873	0.784–0.972
RRI LF (ms^2)	0.914	0.554–0.973
RRI HF (ms^2)	0.936	0.901–1.225
RRI LF/HF ratio	0.610	0.481–0.844
CAN presence (yes)	1.432	0.916–6.498
Age (years)	1.017	0.970–1.066
Gender (male)	1.113	0.417–2.971
Disease duration (years)	1.632	1.190–2.239
HbA1c (%)	1.041	0.987–1.096
SBP (mmHg)	1.015	0.977–1.053
DBP (mmHg)	0.996	0.089–1.054
UAE (mg/24)	0.967	0.880–1.063
ACEI use (yes)	0.352	0.301–1.402
Smoking status (yes)	1.570	0.597–4.126
Total daily insulin dose (U/kg)	0.832	0.677–1.024

HRV-CV: hearth rate variability-coefficient of variation; db HRV-CV: hearth rate variability coefficient of variation during deep breathing; LF-low frequency; HF-high frequency; RRI the time consecutive R peaks of the ECG; CAN-cardiac autonomic neuropathy; HbA1c-glycated haemoglobin A1c; UAE-urin albumin excretion; ACEI-angiotensin converting enzyme inhibitors.