European Review for Medical and Pharmacological Sciences 2016; 20: 2099-2105

# Computerized dynamic pupillometry indices mirrors the heart rate variability parameters

S. OKUTUCU[1], M. CIVELEKLER[2], M. APARCI[3], C. SABANOGLU[4], O. DIKMETAS[5], H. AKSOY[1], B. YETIS SAYIN[1], A. OTO[1]

[1]Department of Cardiology, Memorial Ankara Hospital, Ankara, Turkey
[2]Department of Ophthalmology, Etimesgut Military Hospital, Ankara, Turkey
[3]Department of Cardiology, Etimesgut Military Hospital, Ankara, Turkey
[4]Department of Cardiology, Kirikkale High Speciality Hospital, Kirikkale, Turkey
[5]Department of Ophthalmology, Hacettepe University School of Medicine, Ankara, Turkey

**Abstract. – OBJECTIVE: Dynamic pupillometry (DP) is a simple, non-invasive computerized assessment of pupillary light response which provides data concerning both branches of the autonomous nervous system (ANS). Heart rate variability (HRV) analysis assess cardiac health and the ANS modulation on the heart. In this study, we aimed to evaluate the utility of DP as a predictor of cardiac autonomic activity assessed by HRV.**

**PATIENTS AND METHODS: A total of 44 consecutive healthy subjects (mean age = 35.9 ± 7.4 years, 24 males) were enrolled. Pupil diameters (R0, R1, R2 and R%): latency (Lc), amplitude (Ac), velocity (Vc) and duration of pupil contraction (Tc): latency (Ld), velocity (Vd) and duration of pupil dilatation (Td) were measured in DP. Time and frequency domain indices of HRV were obtained from 24-h ambulatory electrocardiographic monitoring.**

**RESULTS: There were strong significant correlations of Vc with LF/HF (r = -0.672, $p$ = 0.001) and a measure of HRV: RMSDD (r = 0.654, $p$ = 0.001). R% significantly correlated with PNN50 (r = -0.432, $p$ = 0.003) and RMSDD (r = -0.422, $p$ = 0.004) and LF/HF (r = 0.340, $p$ = 0.024). Vc (β = 0.647, $p$ = 0.011) and Ac (β = 0.320, $p$ = 0.013) were found as independent predictors of RMSSD. Vc (β = 0.578, $p$ = 0.036) was found to be only significant predictor of PNN50. Vc (β = -0.617, $p$ = 0.008) and R% (β = 0.309, $p$ = 0.038) were found to be significant predictors of LF/HF.**

**CONCLUSIONS: Pupillary autonomic functions assessed by DP correlates with cardiac autonomic functions evaluated by HRV. Among the DP parameters analyzed, Vc was a predictor of parasympathetic indices, and R% was a predictor of sympathetic indicators of cardiac autonomic functions.**

*Key Words:*
Autonomous nervous system, Dynamic pupillometry, Heart rate variability, Parasympathetic, Sympathetic.

## Introduction

In recent years, a marked interest has emerged about the physiological or pathophysiologic features and clinical evaluation of the autonomic nervous system (ANS) regulation of the heart rate (HR)[1,2]. Cardiac autonomic dysfunction (CAUD) has been associated with increased all-cause mortality, death from cardiovascular disease and sudden cardiac death[3-5].

Heart rate variability (HRV) is the physiological phenomenon of variation in the time interval between heartbeats[6-8]. HRV is regulated by the ANS. Parasympathetic activity decreases HR and increases HRV, whereas sympathetic activity increases HR and decreases HRV[9-12]. HRV analysis has a considerable role to assess overall cardiac health and the ANS fluctuations in normal healthy individuals and in patients with various cardiovascular and non-cardiovascular disorders[6-8].

Dynamic pupillometry (DP) is a novel, standardized, fully automated computerized system for evaluating pupillary autonomic nerve activity[13,14]. The pupil is a convenient and accessible terminal station for studying autonomic function because the parasympathetic and sympathetic nervous systems innervate constrictor and dilator muscles of the iris[13-15]. Therefore, the infrared DP which uses pupillary light reflex allows independent evaluation of both branches of pupillary autonomic activity[15-17]. DP has been evaluated in patients with hypertension[14], heart failure[15], obstructive sleep apnea[16], diabetes mellitus[18] and neurological diseases[19,20]. However, correlation and association of DP indices with cardiac autonomic functions has not been assessed before. In this study, we aimed to investigate the clinical utility of DP indices as a predictor of cardiac autonomic functions via HRV.

*Corresponding Author:* Sercan Okutucu, MD; e-mail: sercanokutucu@yahoo.com

## Patients and Methods

### Study Population and Data Collection

A total of 44 (mean age = 35.9 ± 7.4 years, 24 males and 20 females) consecutive healthy subjects (volunteers such as healthy participants from our outpatient clinics, physicians and nurses) were enrolled in our study. During participation and data collection 4 (8.3%) cases were excluded. Exclusion criteria were arterial hypertension in three cases and diabetes mellitus in one case. All subjects had no systemic and neurological conditions with known ocular involvement, use of medication and topical eye treatment. All participants underwent a complete ocular examination consisting of slit-lamp bio-microscopy, fundus examination and intraocular pressure evaluation. All participants' best-corrected visual acuity (BCVA) was 20/20 with Snellen and ophthalmologic examination was normal.

Data collection was carried out by face-to-face interviews of subjects by two trained researchers. Demographic features, smoking habits, and use of medications were interviewed using a questionnaire. All subjects underwent a complete physical examination, and their height and weight were recorded. BMI was calculated as weight divided by square of height ($kg/m^2$).

After initial data collection, all participants underwent 24-h ambulatory electrocardiographic monitoring and DP. These two studies were performed by blinded researchers to results of one another. The current study complies with the Declaration of Helsinki. Informed consent was obtained from all participants and the study was approved by the Research Ethics Committee.

### Analysis of Heart Rate Variability Parameters

A 24-h ambulatory electrocardiographic monitoring was performed for all participants. Recordings were obtained using three-channel analog recorders and analyzed using the DMS300-4AL ambulatory electrocardiographic recording system [DM Systems (Beijing) Co. Ltd, Beijing, China]. All 24-hour periods were used to investigate HRV parameters. The standard parameters from the time domain analysis of HRV were SDNN (standard deviation of all NN intervals for a selected time period), SDANN (SD of the 5-minute mean R-R intervals tabulated over an entire day), RMSSD (square root of the mean of the sum of the squares of differences between adjacent RR intervals) and PNN50 (the proportion of differences in successive NN intervals greater than 50 ms)[6,21,22].

Spectral analysis of HRV included: total power which represents the variability of the entire signal and is obtained by summing powers of each frequency band; high-frequency (HF) component (0.15-0.40 Hz); low-frequency (LF) component (0.04-0.15 Hz) and very-low-frequency (VLF) component (0-0.04 Hz). The normalized high-frequency power (HFnu = 100 × high-frequency power/total power), normalized low-frequency power (LFnu = 100 × low-frequency power/total power), and low-/high-frequency power ratio (LF/HF ratio = low-frequency power/high-frequency power) were calculated to give the relative changes in HRV in the frequency domain[6,21,22].

### Dynamic Pupillometry

Monocular DP analysis was performed for each eye (darkness adaptation 300 seconds, duration of 90 seconds, sampling frequency = 30 Hz) with Metrovision MonPack one. Software which is provided in the DP device automatically outlines pupillary contour on the images, ensuring the accuracy of the measurements (accuracy of measurements of pupil diameter = 0.1 mm) under controlled illumination conditions. The DP device stimulator had near-infrared illumination (950 nm) and a high-resolution near-infrared image sensor which allows measurement of pupil diameter even in absolute darkness. From the DP analysis of response to visual stimulus: pupil diameters (initial, R0; maximum, R1; minimum, R2; R2/R0 expressed as (R%): latency (Lc), amplitude (Ac), velocity (Vc) and duration of pupil contraction (Tc): latency (Ld), velocity (Vd) and duration of pupil dilatation (Td) were measured.

### Statistical Analysis

Distribution of data was assessed by using a one-sample Kolmogorov-Smirnov test. Data are demonstrated as mean ± SD for normally distributed continuous variables, median (minimum-maximum) for skew-distributed continuous variables, and frequencies for categorical variables. Univariate linear regression analysis was performed to evaluate the effects of DP variables on HRV indices. Predictors obtained from univariate analysis were further checked with multivariate linear regression analysis. Pearson's correlation analysis was used in order to assess the relationship between the DP variables and HRV indices. Spearman's correlation analysis was used for skew-distributed continuous DP variables in order to evaluate the relationship with HRV indices. Statistical analysis of the data was conducted using SPSS 15 (SPSS Inc., Chicago, IL, USA) and two-tailed $p < 0.05$ was considered statistically significant.

## Results

Of the 44 participants (mean age = 35.9 ± 7.4 years, 24 males and 20 females), 10 (22.7%) were currently smokers. The mean BMI of the study group was 22.8 ± 1.7 kg/m$^2$, SBP was 110.1 ± 11.7 mmHg and DBP was 66.2 ± 8.6 mmHg. Heart rate variability indices of participants are represented in Table I.

Pupil sizes were determined 4.40 ± 0.86 mm for R0, 4.90 ± 0.80 mm for R1, 3.36 ± 0.76 mm for R2, respectively. Detailed DP indices and correlation with HRV parameters are shown in Table II. Pearson's correlation analysis revealed strong significant correlation of Vc with LF/HF (r = -0.672, $p$ = 0.001) and RMSDD (r = 0.654, $p$ = 0.001) (Figure 1). In addition, Vc moderately correlated with PNN50 (r = 0.525, $p$ = 0.001), HF (r = 0.499, $p$ = 0.001) and LFnu (r = -0.488, $p$ = 0.001). There were significant correlations of R% with PNN50 (r = -0.432, $p$ = 0.003) and RMSDD (r = -0.422, $p$ = 0.004) and LF/HF (r = 0.340, $p$ = 0.024) (Figure 2).

Effects of age, gender, smoking status, BMI and DP variables on HRV indices were examined in a multivariate linear regression analysis. When parasympathetic indices of HRV were se-

**Table I.** Heart variability parameters of participants.

| Variable | Participants (n = 44) |
|---|---|
| SDNN, ms | 158.2 ± 39.3 |
| SDANN, ms | 145.3 ± 38.9 |
| RMSDD, ms | 41.8 ± 18.3 |
| PNN50 | 13.6 ± 9.6 |
| LF, ms$^2$ | 1127.7 ± 588.1 |
| HF, ms$^2$ | 463.8 ± 254.5 |
| LFnu, | 66.6 ± 10.3 |
| HFnu | 23.4 ± 11.3 |
| LF/HF | 2.9 ± 1.7 |

Numeric variables with a normal distribution were presented as the mean ± standard deviation. Please, refer text for details of HRV indices.

**Table V.** Dynamic pupillometry measurements of participants and correlations with HRV indices.

| Variable | Participants (n = 44) | Correlation coefficient (r) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | SDNN | SDANN | RMSDD | PNN50 | LF | HF | LF/HF | LFnu | HFnu |
| R0, mm | 4.40 ± 0.86 | ns | ns | ns | ns | ns | ns | ns | 0.317* | ns |
| R1, mm | 4.90 ± 0.80 | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| R2, mm | 3.36 ± 0.76 | ns | ns | -0.318* | ns | ns | ns | ns | ns | ns |
| R% | 0.75 (0.60-0.92) | ns | ns | -0.422** | -0.432** | ns | ns | 0.340* | ns | ns |
| Lc, ms | 267 (133-300) | -0.314* | ns | -0.306* | ns | ns | -0.299* | 0.331* | ns | -0.301* |
| Ld, ms | 817.4 ± 70.6 | ns | ns | 0.334* | 0.348* | ns | ns | -0.301* | ns | ns |
| Dc, ms | 533 (333-767) | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Dd, ms | 1607.5 ± 113.9 | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Ac, mm | 1.54 ± 0.27 | 0.346* | 0.324* | 0.495** | 0.506** | ns | 0.397** | -0.493** | -0.361* | ns |
| Vc, mm/s | 4.96 ± 1.15 | 0.379* | 0.343* | 0.654** | 0.525** | -0.316 | 0.499** | -0.672** | -0.488** | 0.342* |
| Vd, mm/s | 1.75 ± 0.38 | 0.368* | 0.372* | 0.468** | 0.438** | ns | ns | -0.317* | ns | ns |

Numeric variables with a normal distribution were presented as the mean ± standart deviation. Abbreviations: Ac, amplitude of contraction; Lc, latency of contraction; Ld, latency of dilatation; Dc, duration of contraction; Dd, Duration of dilatation; Vc, velocity of contraction; Vd, velocity of dilatation. ** $p < 0.01$ (2-tailed): * $p < 0.05$ (2-tailed).

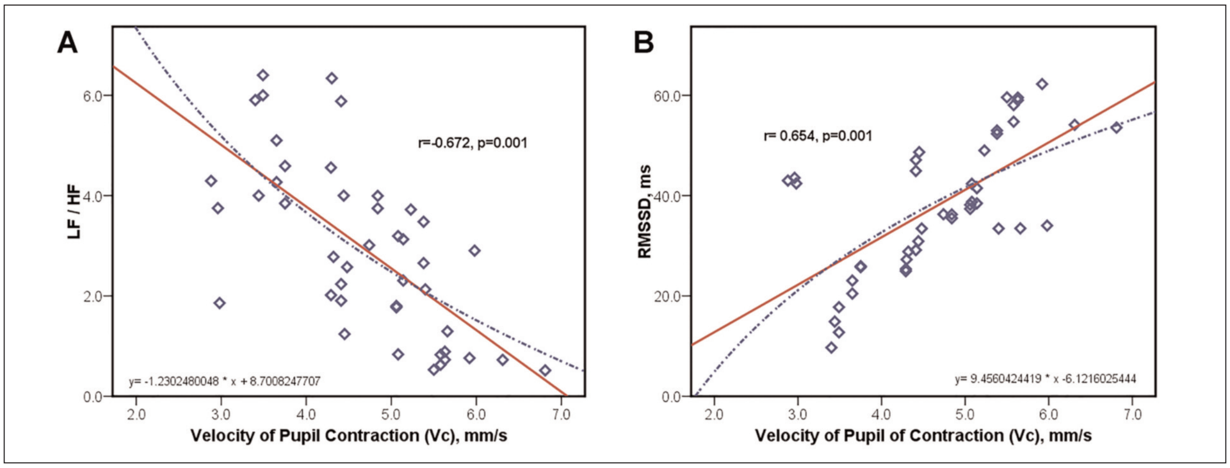


**Figure 1.** Correlation analysis of velocity of pupil contraction (Vc) with *(A)* LF/HF and *(B)* RMSDD. r = correlation .

lected as a dependent variable; Vc (β = 0.647, $p$ = 0.011) and Ac (β = 0.320, $p$ = 0.013) were found to be significant predictors of RMSSD. Notably, Vc (β = 0.578, $p$ = 0.036) was found to be the only significant predictor of PNN50. When sympathetic indices of HRV were chosen as a dependent variable; Vc (β = -0.617, $p$ = 0.008) and R% (β = 0.309, $p$ = 0.038) were found to be significant predictors of LF/HF.

## Discussion

The main findings of this study are as follows: (1) pupillary autonomic functions assessed by DP correlates with cardiac autonomic functions evaluated by HRV, (2) among the measured DP parameters, Vc and Ac were found to be predictors of parasympathetic indices of cardiac autonomic functions, (3) Vc and R% were found as independent predictors for sympathetic indices of cardiac autonomic functions. To the best of our knowledge, it is the first study that evaluated the utility of DP as a predictor of cardiac autonomic activity assessed by HRV. DP indices were found to be correlated and associated with HRV parameters.

Sympathetic and parasympathetic limbs play an important role in modulating both intrinsic and actual HR[2,10]. Parasympathetic activity reduces the HR via vagal nerve. In contrast, sympathetic activity accelerates the HR via circulating catecholamines and neural release of norepinephrine[23,24]. Likewise, ANS have a modulatory role on dynamic change in pupil size. Both sphincter and dilator muscles of the iris receive

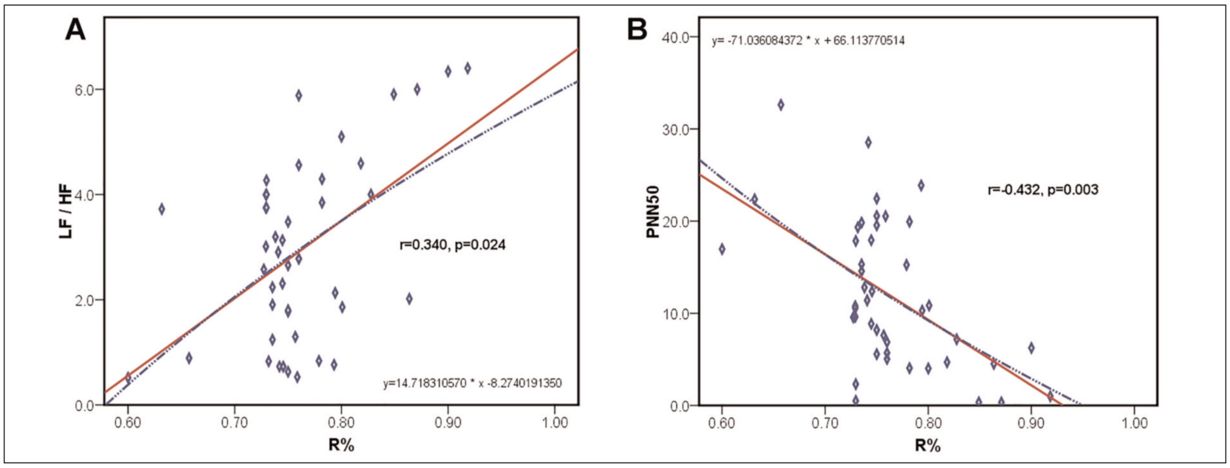


**Figure 2.** Correlation analysis of velocity of R2/R0 (R%) with *(A)* LF/HF and *(B)* PNN50. r = correlation coefficient.

dual innervations from the two branches of the ANS[15,16]. Parasympathetic fibers supply the iris sphincter and cause reduction of pupil size by contraction of the muscle. Meanwhile, the sphincter had sympathetic innervations which are capable of inhibiting pupil contraction by relaxation of the sphincter muscle. This inhibition is primarily via beta-adrenergic receptors. Pupil dilation via sympathetic inhibition of the sphincter is about one-third of the maximum physiological dilation. On the other side, the sympathetic innervation causes contraction of iris dilator muscle via alpha-adrenergic receptors which ends up with an increase in pupil diameter[16,17]. DP is a non-invasive, standardized and fully automated system for assessing ANS activity of pupils. Characteristic V-shaped light response recorded during DP is divided into 3 parts: the first part reflects parasympathetic activation; the second part consists both sympathetic and the parasympathetic activity; and the third one signifies sympathetic activity alone[15-17] (Figure 3). From the first part; Ac and Vc are indicators of parasympathetic activity. In accordance with this data, we found that Ac and Vc have significant positive correlations with RMSDD and PNN50. Importantly, Vc was found to independently predict parasympathetic HRV indicators of cardiac autonomic activity. Therefore, Vc can be used as an indicator of parasympathetic modulation on the heart. Furthermore, Vc is an independent predictor of sympathetic HRV indices.

On the other hand, among DP indices, it has been known that R0, R% and Lc are mainly under sympathetic control and can be used as an indicator of pupillary autonomic activity[15]. Complying

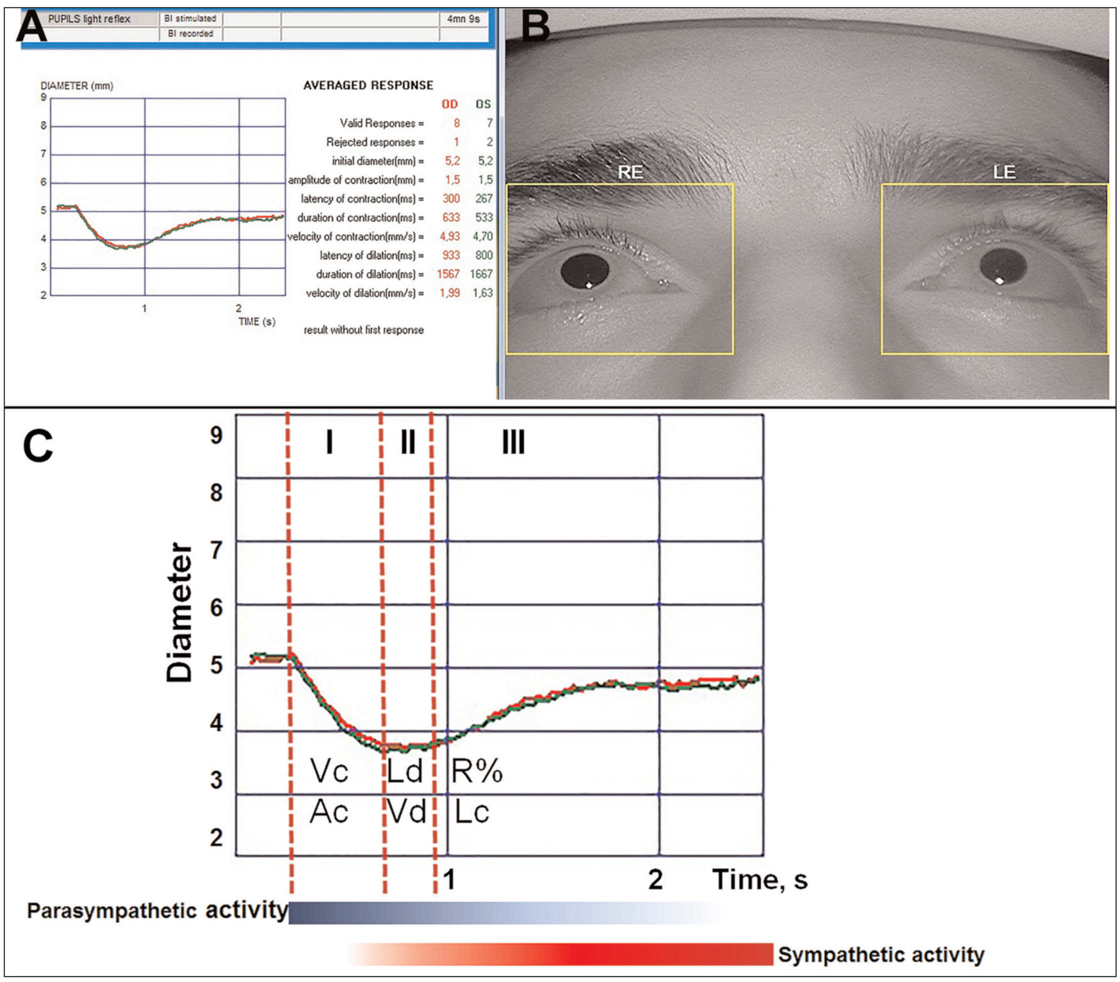


**Figure 3.** ***A,*** Dynamic pupillometry analysis ***(A)*** and ***(B)*** automatically definition of pupillary contour by DP device are shown. Characteristic V-shaped response of dynamic pupillometry ***(C)***. Ac, amplitude of contraction; Lc, latency of contraction; Ld, latency of dilatation; Vc, velocity of contraction; Vd, velocity of dilatation.

with this, we found that R% and Lc have a positive correlation with LF/HF and negative correlation with parasympathetic HRV indicators. Furthermore, R% was found to be an independent predictor of LF/HF. Consequently, R% can be used as indicator of sympathetic modulation on the heart. Remaining parameters, Ld and Vd carried the effect of both sympathetic and parasympathetic activity. There was also a significant positive correlation between Vd and Ld particularly with RMSDD and PNN50, in our analysis.

## Conclusions

The pupillary autonomic functions assessed by DP correlates with cardiac autonomic functions evaluated by HRV. Among the DP parameters analyzed, Vc was found to be a significant independent predictor of the parasympathetic limb of the cardiac autonomic functions. R% was found to be a significant independent predictor of the sympathetic limb of the cardiac autonomic functions. Considering the physiological mechanisms and results, Vc can be used as an index of parasympathetic activity, and R% can be used as an index of sympathetic modulation on the heart. Further studies should be performed for a better understanding of these two autonomic systems interrelations and application of DP indices in CAUD and different clinical settings in cardiology.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

## References

1) Okutucu S, Aytemir K, Evranos B, Aksoy H, Sabanov C, Karakulak UN, Kaya EB, Kabakci G, Tokgozoglu L, Ozkutlu H, Oto A. Cardiac resynchronization therapy improves exercise heart rate recovery in patients with heart failure. Europace 2011; 13: 526-532.
2) Aksoy H, Okutucu S, Aytemir K, Kaya EB, Evranos B, Kabakci G, Tokgozoglu L, Ozkutlu H, Oto A. Baseline aortic pre-ejection interval predicts reverse remodeling and clinical improvement after cardiac resynchronization therapy. Cardiol J 2011; 18: 639-647.
3) Gillum RF, Makuc DM, Feldman JJ. Pulse rate, coronary heart disease, and death: the NHANES I epidemiologic follow-up study. Am Heart J 1991; 121: 172-177.
4) Shaper AG, Wannamethee G, Macfarlane PW, Walker M. Heart rate, ischaemic heart disease, and sudden cardiac death in middle-aged British men. Br Heart J 1993; 70:49-55.
5) Al-Trad BA, Faris MA, Al-Smadi M, Bashir A, Mansi M, Alaraj M, Al-Hazimi A. Cardiac autonomic dysfunction in young obese males is not associated with disturbances in pituitary-thyroid axis hormones. Eur Rev Med Pharmacol Sci 2015; 19: 1689-1695.
6) Okutucu S, Oto A. Risk stratification in nonischemic dilated cardiomyopathy: current perspectives. Cardiol J 2010; 17: 219-229.
7) Ozgur S, Ceylan O, Senocak F, Orun UA, Dogan V, Yilmaz O, Keskin M, Aycan Z, Okutucu S, Karademir S. An evaluation of heart rate variability and its modifying factors in children with type 1 diabetes. Cardiol Young 2014; 24: 872-879.
8) Kaya EB, Okutucu S, Aksoy H, Karakulak UN, Tulumen E, Ozdemir O, Inanici F, Aytemir K, Kabakci G, Tokgozoglu L, Ozkutlu H, Oto A. Evaluation of cardiac autonomic functions in patients with ankylosing spondylitis via heart rate recovery and heart rate variability. Clin Res Cardiol 2010; 99: 803-808.
9) Okutucu S, Karakulak UN, Kabakci G. Circadian blood pressure pattern and cardiac autonomic functions: different aspects of same pathophysiology. Anadolu Kardiyol Derg 2011; 11: 168-173.
10) Okutucu S, Karakulak UN, Aytemir K, Oto A. Heart rate recovery: a practical clinical indicator of abnormal cardiac autonomic function. Expert Rev Cardiovasc Ther 2011; 9: 1417-1430.
11) Almeida-Santos MA, Barreto-Filho JA, Oliveira JL, Reis FP, da Cunha Oliveira CC, Sousa AC. Aging, heart rate variability and patterns of autonomic regulation of the heart. Arch Gerontol Geriatr 2016; 63: 1-8.
12) Ganan-Calvo AM, Fajardo-Lopez J. Universal structures of normal and pathological heart rate variability. Sci Rep 2016; 6: 21749.
13) Martinez-Ricarte F, Castro A, Poca MA, Sahuquillo J, Exposito L, Arribas M, Aparicio J. Infrared pupillometry. Basic principles and their application in the non-invasive monitoring of neurocritical patients. Neurologia 2013; 28: 41-51.
14) Koike Y, Kawabe T, Nishihara K, Iwane N, Hano T. Cilnidipine but not amlodipine suppresses sympathetic activation elicited by isometric exercise in hypertensive patients. Clin Exp Hypertens 2015; 37: 531-535.
15) Keivanidou A, Fotiou D, Arnaoutoglou C, Arnaoutoglou M, Fotiou F, Karlovasitou A. Evaluation of autonomic imbalance in patients with heart failure: a preliminary study of pupillomotor function. Cardiol J 2010; 17: 65-72.
16) Monaco A, Cattaneo R, Mesin L, Fiorucci E, Pietropaoli D. Evaluation of autonomic nervous

system in sleep apnea patients using pupillometry under occlusal stress: a pilot study. Cranio 2014; 32: 139-147.

17) Monaco A, Cattaneo R, Mesin L, Ciarrocchi I, Sgolastra F, Pietropaoli D. Dysregulation of the autonomous nervous system in patients with temporomandibular disorder: a pupillometric study. PLoS One 2012; 7: e45424.

18) Piha SJ, Halonen JP. Infrared pupillometry in the assessment of autonomic function. Diabetes Res Clin Pract 1994; 26: 61-66.

19) Fotiou DF, Brozou CG, Haidich AB, Tsiptsios D, Nakou M, Kabitsi A, Giantselidis C, Fotiou F. Pupil reaction to light in Alzheimer's disease: evaluation of pupil size changes and mobility. Aging Clin Exp Res 2007; 19: 364-371.

20) Tsiptsios D, Fotiou DF, Haidich AB, Brozou GC, Nakou M, Giantselidis C, Karlovasitou A, Fotiou F. Evaluation of pupil mobility in patients with myasthenia gravis. Electromyogr Clin Neurophysiol 2008; 48: 209-218.

21) Matei D, Constantinescu V, Corciova C, Ignat B, Matei R, Popescu CD. Autonomic impairment in patients with migraine. Eur Rev Med Pharmacol Sci 2015; 19: 3922-3927.

22) Russo V, Rago A, Nigro G. The importance of selection criteria for the evaluation of Heart Rate Variability in women. Eur Rev Med Pharmacol Sci 2016; 20: 582-583.

23) Landstrom AP, Sun JJ, Ray RS, Wehrens XH. It's not the heart: autonomic nervous system predisposition to lethal ventricular arrhythmias. Heart Rhythm 2015; 12: 2294-2295.

24) Florea VG, Cohn JN. The autonomic nervous system and heart failure. Circ Res 2014; 114: 1815-1826.