Impaired heart rate variability in patients with mitral annular calcification: an observational study

Mitral annüler kalsifikasyonlu hastalarda azalmış kalp hızı değişkenliği: Gözlemsel bir çalışma

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ABSTRACT

Objective: The aim of the present study was to study time indices of heart rate variability (HRV) in patients with mitral annular calcification (MAC).

Methods: A cross-sectional observational study was performed. Fifty patients with echocardiographic evidence of MAC and 50 age- and gender-matched control subjects without echocardiographic evidence of MAC were included. All the study participants underwent 2-dimensional echocardiographic examinations and 24-hour Holter monitoring for HRV analysis. Student-t, Mann-Whitney U and Chi-square tests were used for statistical analysis.

Results: Hypertension and coronary artery disease were more common in the MAC group than in the control group. All HRV parameters including mean RR interval, SDNN, SDANN, SDNN index, pNN50 and RMSSD were reduced in the MAC group when compared with the control group ($p<0.05$ for all). In hypertensive subgroup, all HRV parameters except mean RR interval were diminished in patients with MAC when compared with those without MAC ($p<0.05$ for all). In non-hypertensive subgroup, all HRV parameters were also diminished in patients with MAC when compared with those without MAC. In the subgroup of patients with coronary artery disease, patients had lower HRV parameters except mean RR interval, pNN50 and RMSSD in comparison to those without MAC ($p<0.05$ for all). In the subgroup of patients without coronary artery disease, all HRV parameters were depressed in patients with MAC in comparison to those without MAC ($p<0.05$ for all).

Conclusion: Our findings indicate that MAC was associated with reduced heart rate variability which possibly reflects decreased parasympathetic tone with a predominant activity of the sympathetic tone. *(Anadolu Kardiyol Derg 2013; 13: 668-74)*

Key words: Mitral annular calcification, heart rate variability, autonomic dysfunction

ÖZET

Amaç: Çalışmanın amacı mitral annüler kalsifikasyonu (MAK) olan hastalarda kalp hızı değişkenliği (KHD) parametrelerini incelemektir.

Yöntemler: Çalışma enine kesitli gözlemsel amaçlı planlandı. Ekokardiyografik olarak MAK tespit edilmiş 50 hasta ile MAK'ı olmayan 50 hasta çalışmaya dahil edildi. Tüm hastalara iki boyutlu transtorasik ekokardiyografi ve KHD analizi için 24 saatlik Holter monitorizasyonu uygulandı. İstatistiksel analiz için Student-t, Mann-Whitney U ve Ki-kare testleri kullanıldı.

Bulgular: Hipertansiyon ve koroner arter hastalığı MAK grubunda kontrol grubuna göre daha yüksekti. Ortalama RR süresi, SDNN, SDANN, SDNN indeksi, pNN50 ve RMSSD'yi içeren tüm HRV parametreleri MAK grubunda kontrol grubuna göre azalmış saptandı ($p<0,05$). Hipertansiyon alt grubunda, ortalama RR süresi dışında diğer tüm HRV parametreleri MAK'lı hastalarda MAK'ı olmayanlara göre azalmış saptandı. Hipertansiyon olmayan hasta alt grubunda ise, tüm HRV parametreleri MAK'lı hastalarda MAK'ı olmayanlara göre yine azalmış saptandı ($p<0,05$). Koroner arter hastalığı olan hastaların alt grubunda, ortalama RR süresi, pNN50 ve RMSSD dışında tüm HRV parametreleri MAK'lı hastalarda MAK'ı olmayanlara göre düşük saptandı ($p<0,05$). Koroner arter hastalığı olmayan hastaların alt grubunda ise tüm HRV parametrelerinin MAK'lı hastalarda MAK'ı olmayanlara göre düşük olduğu saptandı ($p<0,05$).

Sonuç: Bu çalışmadaki bulgular MAK'lı hastalarda azalmış parasempatik aktiviteyle birlikte artmış sempatik aktivasyona işaret eden baskılanmış KHD'yi göstermektedir. *(Anadolu Kardiyol Derg 2013; 13: 668-74)*

Anahtar kelimeler: Mitral annüler kalsifikasyon, kalp hızı değişkenliği, otonomik disfonksiyon

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Introduction

Heart rate variability (HRV) is considered a sensitive method for the quantitative assessment of the autonomic control of the heart (1). A heart rate that is variable and responsive to demands is believed to bestow a survival advantage, whereas reduced HRV may be associated with poorer cardiovascular health and outcomes (2). In clinical cardiology, the analysis of HRV has recently been used to evaluate cardiac autonomic control both in normal subjects and in a wide variety of both cardiac and noncardiac disorders (3-10).

Previous data has demonstrated that presence of cardiac valve calcifications, which include mitral annular calcification (MAC) and aortic valve calcification (AVC), is independently associated with incident cardiovascular disease, cardiovascular death, and all-cause death suggesting that valve calcification is a marker of increased cardiovascular risk (11). The association of MAC with enhanced cardiovascular disease risk parallels the previously reported elevated cardiovascular risk observed with the presence of echocardiographic AVC (11).

In patients with AVC, HRV parameters have been demonstrated to be disturbed, however much less is known about HRV in patients with only MAC (12, 13). In this clinical setting, the demonstration of cardiac sympathovagal imbalance may have clinical implications since cardiac autonomic dysfunction might contribute to a higher mortality rate.

We hypothesized that patients with MAC have diminished HRV, which reflects cardiac autonomic dysfunction. It was therefore the aim of our study to examine HRV in patients with MAC.

Methods

Study design

This is an observational cross-sectional study.

Study population

We recruited subjects from the cardiology clinic of İnönü University Hospital in the city of Malatya between July 2009 and April 2010. Fifty consecutive patients in whom a diagnosis of MAC was made by transthoracic echocardiography (MAC group) and 50 age- and sex-matched patients without MAC (Control group) were included. Patients in the MAC and control groups were further divided into subgroups of hypertension (HT) and coronary artery disease (CAD). Patients were excluded if they had: rheumatic heart disease, congestive heart failure, acute coronary syndrome, cardiomyopathy, obstructive sleep apnea, BMI over 30 kg/m^2, prosthetic valves, renal failure, atrial fibrillation, cerebrovascular disease or thyroid disease, aortic valve sclerosis, frequent extrasystoles and a history of positive head-upright tilt testing. The study was approved by local Ethics Committee, and written informed consent was obtained from all patients.

Study protocol

The study population consisted of 50 patients with MAC, which was diagnosed by two-dimensional echocardiography. After diagnosis of MAC, baseline clinical, demographic and echocardiographic variables were recorded. After completing echocardiographic examination, 24-hour Holter recording for HRV analysis was done in each study participant. The control group consisted of 50 age- and sex-matched individuals without MAC on echocardiography. They also underwent the same protocol including two-dimensional echocardiographic examinations and 24-hour Holter recordings.

Study variables

Baseline clinical and demographic variables

The baseline variables of study were as following: age, gender and body mass index (BMI) was recorded and cardiovascular risk factors were determined: HT, CAD, diabetes mellitus (DM), hypercholesterolemia, smoking and cardiovascular medication use [beta-blockers, angiotensin-converting enzyme inhibitors (ACEI), angiotensin-II receptor blockers (ARB), calcium-channel blockers (CCB) and statins].

Definitions

CAD was defined as >50% luminal diameter narrowing of ≥1 coronary artery by angiography, history of coronary revascularization, an abnormal myocardial perfusion scan or dobutamine stress echocardiogram, regional left ventricular akinesia and/or dyskinesia on echocardiogram, or pathologic Q waves on 12-lead electrocardiogram. HT was defined as systolic blood pressure of 140 mmHg and above, diastolic blood pressure of 90 mmHg and above or ongoing antihypertensive medication (14). DM was defined as fasting serum glucose level > 126 mg/dL or ongoing diabetes medication (15). Hypercholesterolemia is defined as fasting serum total cholesterol level >200 mg/dL or ongoing lipid-lowering therapy (16). Smoking was defined as smoking ≥1 cigarette per day regularly for at least one year. BMI was calculated as weight in kilograms divided by the square of height in meters.

Transthoracic echocardiographic variables

Complete 2-dimensional and Doppler color flow measurements were performed in all patients with the 4-MHz transducer of a Philips ATL 5000 HD (Bothell, Washington). Interpretation of echocardiographic examinations was performed by one cardiologist blinded to clinical and demographic characteristics of the study population. MAC was defined as an intense echo-producing structure with highly reflective characteristics that was located at the junction of the atrioventricular groove and the posterior or anterior mitral leaflet on the parasternal long-axis, apical 4- or 2-chamber, or parasternal short-axis view. Measurements of left ventricular (LV) internal dimensions and wall thicknesses and of left atrium dimension were made

according to the recommendations of the American Society of Echocardiography (17). The LV mass index was calculated by the method described by Devereux and Reichek (18).

HRV indices

All patients underwent 24-hour electrocardiographic monitoring using a 6-channel digital Holter recorder (DMS 300-3A Digital Holter Recorder, California, USA) with built-in flash memory that was scanned by computer. All patients were asked to do their normal activities with their normal sleep-wake rhythm during ambulatory electrocardiographic monitoring. The tapes were analyzed by another cardiologist who was blinded to clinical and echocardiographic data of the study population. HRV measurements were obtained using a commercially available Holter software program (CardioScan 12.0, DMS, USA). After computerized primary analysis, all recordings were edited manually for eliminating from supraventricular, ventricular ectopy and artifacts. Recordings lasting for at least 22 hour and of sufficient quality for evaluation were included in the analysis. In case these criteria were not achieved, the recordings were repeated. One hundred recordings were assessed in the time domain parameters in accordance with the recommendations of the European Society of Cardiology and the North American Society of Pacing Electrophysiology (1).

The following time domain indices of HRV were measured in the 24-hour recordings: (1) The mean of all normal RR intervals in milliseconds (RR, msec); (2) the standard deviation of all normal-to-normal RR intervals (SDNN, msec); (3) the mean of the standard deviation of all normal to normal RR intervals for all 5-minute segments (SDNN index, msec); (4) the standard deviation of 5-minute mean RR intervals (SDANN, msec), (5) percentage of successive normal RR intervals exceeding 50 milliseconds (pNN50, %); and (6) the square root of the mean of the squares of the differences between successive normal-to-normal RR intervals (RMSSD, msec).

Table 1. Baseline characteristics of the study population

Variables	MAC group (n=50)	Control group (n=50)	*p
Age, years	61.7±8.5	59.0±7.9	0.1
Women/ men	27/23	26/24	0.8
BMI	26.2±2.6	25.5±2.3	0.1
Hypertension, n (%)	26 (52)	14 (28)	0.02
Coronary artery disease, n (%)	21 (42)	10 (20)	0.03
Diabetes mellitus, n (%)	13 (26)	9 (18)	0.4
Hypercholesterolemia, n (%)	16 (32)	11 (22)	0.3
Cigarette smoking, n (%)	9 (18)	7 (14)	0.8
Medication use, n (%)			
ACEI or ARB	17 (34)	12 (24)	0.7
β-blocker	9 (18)	7 (14)	0.4
CCB	6 (12)	9 (18)	0.5
Statin	11 (22)	10 (20)	0.8

Data are presented as mean±SD and number (percentage)
*unpaired Student's t-test and Chi-square test
ACEI - angiotensin-converting enzyme inhibitor, ARB - angiotensin receptor blocker, BMI - body mass index, CCB - calcium channel blocker, MAC - mitral annular calcification

Statistical analysis

Statistical analyses were performed with the use of SPSS software, version 17.0 (SPSS Inc. USA). The Kolmogorov-Smirnov test was used to evaluate whether the variables were normally distributed. Continuous variables were presented as means±SD or median with interquartile range (IQR) and categorical variables as frequency and percentage. Categorical data were analyzed using chi-square test or Fisher's exact test where appropriate. Continuous data were analyzed by Student's t-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. All p values were two-tailed, and values of less than 0.05 were considered to indicate statistical significance.

Results

Baseline characteristics

Baseline characteristics and clinical data of 50 patients with MAC (27 women and 23 men; mean age 61.7±8.5 years) and 50 control subjects without MAC (26 women/24 men; mean age 59±7.9 years) are summarized in Table 1. HT and CAD were more common in the MAC group than in the control group. There was no significant difference between the groups regarding age, gender, BMI, diabetes mellitus, hypercholesterolemia and cigarette smoking. In addition, the proportion of patients taking ACEI or ARB, beta-blocker, statin, CCB therapy did not differ significantly between the two groups.

Transthoracic echocardiographic findings

The two-dimensional and Doppler echocardiographic findings of the MAC and control group are shown in Table 2. No differences between groups were found in two-dimensional echocardiographic variables and LV mass index. However, left atrial diameter was significantly larger in the MAC group compared to the control group.

HRV findings

Data of the time domain analysis for the MAC and control groups are presented in Table 3. Mean RR interval, SDNN, SDANN and SDNN index were significantly lower in the MAC group compared to the control group ($p<0.05$ for all). Parameters of parasympathetic activity, pNN50 and RMSSD were depressed in patients with MAC ($p<0.05$ for all).

In addition, we evaluated HRV in patients with and without HT and CAD in the MAC and control group. As can be seen from Table 4, a decrease in HRV in hypertensive patients with

Table 2. Two-dimensional, pulse- and tissue-Doppler echocardiographic parameters of the study population

Variables	MAC group (n=50)	Control group (n=50)	*p
Ejection fraction, %	59.0±7.5	59±8	0.8
LVEDD, mm	47.2±3.3	46.3±3.2	0.2
LVESD, mm	31.9±4.5	31.6±3.2	0.6
Interventricular septum, mm	10.4±0.9	10.2±1.1	0.3
Posterior wall, mm	10.3±0.9	10.2±1.1	0.6
Aortic diameter, mm	21.9±2.4	21.6±1.6	0.4
Left atrial diameter, mm	36.7±4.7	32.7±3.2	< 0.0001
LV mass index, kg/m^2	113±15	109±17	0.2

Data are presented as mean±SD values
*unpaired Student's t-test
LV - left ventricle, LVEDD - left ventricular end-diastolic diameter, LVESD - left ventricular end-systolic diameter, MAC - mitral annular calcification

Table 3. HRV parameters in the MAC and control group

Variables	MAC group (n=50)	Control group (n=50)	*p
RR, msec	760±134	824±100	0.009
SDNN, msec	112±32	141±40	< 0.0001
SDANN, msec	100±32	129±39	< 0.0001
SDNN index, msec	44±13	62±21	< 0.0001
pNN50, %	5 (2-13)	10 (4-15)	0.02
RMSSD, msec	26.0 (19.5-36.2)	30.0 (23.5-38.0)	0.04

Data are presented as mean±SD values and median interquartile range (IQR)
*unpaired Student's t-test and Mann-Whitney U test
HRV - heart rate variability, MAC - mitral annular calcification, pNN50 - percentage of successive normal RR intervals exceeding 50 milliseconds, RMSSD - the square root of the mean of the squares of the differences between successive normal-to-normal RR intervals, RR - mean RR interval, SDANN - the standard deviation of 5-minute mean RR intervals, SDNN - the standard deviation of all normal-to-normal RR intervals, SDNN index - the mean of the standard deviation of all normal to normal RR intervals for all 5-minute segments

Table 4. Time-domain parameters of HRV in hypertensive and normotensive patients

Variables	HT (+)			HT (-)		
	MAC (+) n=26	MAC (-) n=14	*p	MAC (+) n=24	MAC (-) n=36	*p
RR, msec	789 (762-903)	826 (739-940)	0.08	798±82	844±72	0.03
SDNN, msec	102 (92-113)	123 (94-168)	0.04	116±32	146±38	< 0.0001
SDANN, msec	87 (79-104)	118 (76-160)	0.03	103±31	132±37	< 0.0001
SDNN index, msec	40 (33-51)	55 (37-88)	0.01	45±10	63±19	< 0.0001
pNN50, %	4.0 (1.7-7.0)	7.5 (3.2-15.7)	0.04	7.7±5.5	12.4±7	0.009
RMSSD, msec	22.0 (14.0-25.5)	27.5 (23.5-38.2)	0.01	27.4±12.2	36±12.7	0.01

Data are presented as mean±SD values and median interquartile range (IQR)
*unpaired Student's t-test and Mann-Whitney U test
HRV - heart rate variability, HT(+) - patients with hypertension, HT(-) - patients without hypertension, MAC(+) - patients with mitral annular calcification, MAC (-) - patients without mitral annular calcification, HRV abbreviations as in Table 3.

MAC was evident in SDNN, SDANN, SDNN index, pNN50 and RMSSD when compared with hypertensive patients without MAC. A similar decrease was also observed in mean RR interval, SDNN, SDANN, SDNN index, pNN50 and RMSSD in patients without hypertension in the MAC group when compared to those without hypertension in the control group ($p<0.05$ for all).

Similar comparisons performed in hypertensive and normotensive patients with and without MAC were also done in patients with and without CAD. As can be seen from Table 5, SDNN, SDANN and SDNN index were all depressed in patients with MAC when compared with those without MAC in the CAD (+) ($p<0.05$ for all) subgroup, whereas mean RR interval, pNN50 and RMSSD were not. All HRV parameters were reduced in patients with MAC when compared with those without MAC in the CAD (-) subgroup ($p<0.05$ for all).

Discussion

In the present study, we found diminished values for HRV time domain parameters in the study group, which might reflect impairment of parasympathetic activity, with a likely predominant activity of the adrenergic system in the diseased heart. We also found diminished values for HRV in HT and CAD subgroups with MAC, even in normotensive and CAD (-) patients without MAC.

Heart rate variability analysis has been proven to be a well-established and noninvasive tool for the investigation of the modulation of the autonomic nervous system (1).The modulation of heart rate is under complex regulatory mechanisms which involve the nervous, endocrine and cardiovascular systems (1). Alterations of autonomic equilibrium are encountered during ordinary activities but may also be associated with the onset of

Table 5. Time-domain parameters of HRV in patients with and without CAD

Variables	CAD (+)			CAD (-)		
	MAC (+) n=21	MAC (-) n=10	*p	MAC (+) n=29	MAC (-) n=40	*p
RR, msec	780 (702-806)	825 (770-905)	0.15	780±98	830±92	0.03
SDNN, msec	109 (79-123)	130 (114-174)	0.002	106±32	150±41	0.003
SDANN, msec	102 (69-108)	127 (97-162)	0.002	94±33	135±39	0.005
SDNN index, msec	42 (35-52)	54 (47-79)	0.03	44±14	63±22	< 0.0001
pNN50, %	5 (2-11)	9.0 (3.7-13.0)	0.19	6.7±5.2	12.1±10.5	0.03
RMSSD, msec	25.0 (17.5-31.0)	30 (22-33)	0.28	27.4±14.8	36±12.8	0.02

Data are presented as mean±SD values and median interquartile range (IQR)

*unpaired Student's t-test and Mann-Whitney U test

CAD (+) - patients with coronary artery disease, CAD (-) - patients without coronary artery disease, HRV - heart rate variability, MAC(+) - patients with mitral annular calcification, MAC (-) - patients without mitral annular calcification, HRV abbreviations as in Table 3.

disease. Alterations in cardiac autonomic control, as measured by heart rate variability, may define higher risk subgroups for cardiovascular morbidity and mortality (19). This has been documented in adult patients with hypertension (3, 4), diabetes (5), coronary artery disease (20), myocardial infarction (6), and chronic heart failure (6, 21). Whereas the exact mechanism is still open to debate, studies have shown that increasing age is associated with decreasing HRV (22). Also, previous investigations have found gender differences in 24-h HRV, with values for male subjects being significantly higher than those for age-matched female subjects (23). For these reasons, our study population consisted of age- and gender-matched subjects.

The time domain HRV parameters, SDNN, SDANN, and SDNN index were found to be decreased in the MAC group of our study population. The effect of short-term respiratory variations in heart rate which is assumed to be vagally mediated is very low, and depression of these parameters are thought to be due to enhanced sympathetic system activity (1). RMSSD and pNN50, which are supposed to be markers of parasympathetic activity were also decreased in patients with MAC. These findings constitute evidence for autonomic dysfunction characterized by sympatovagal imbalance in patients with MAC. The increased sympathetic activity and decreased parasympathetic tone could be detrimental and contribute to the higher cardiovascular mortality rate in patients with MAC (1, 24). Possible mechanisms by which MAC may impact HRV may be due to that MAC might cause restriction of mitral annular motion and dilatation necessary for normal valvular motion resulting in abnormal mitral inflow velocities (25), degenerative process within the mitral annulus associated with or accompanied by a "sclerodegenerative process" within the conduction system or within the intrinsic cardiac nerves and cardiac receptors (26, 27), and increased left atrial size (28, 29).

The association of CAD and HT with MAC, as noted in the present study, is consistent with the findings of previous studies (11, 30). However, there was no significant relationship between MAC and other traditional cardiovascular risk factors such as diabetes, hyperlipidemia, increased BMI and smoking. These findings are inconsistent with some of the previous data. While some investigators were finding that subjects with MAC had a higher prevalence of hypertension, diabetes, hyperlipidemia, increased BMI and smoking (31), others found no association between MAC, and diabetes and hyperlipidemia (32). Whereas, some researchers found significant relationships with CAD but not with smoking and gender (33). These differences with our study may be explained by (1) considerably younger population of our study than that seen in other studies, thus, some cardiac risk factors such as diabetes and hypercholesterolemia may not be overt at the time of study, (2) exclusion of patients from our study with renal failure, atrial fibrillation, congestive heart failure, valvular disease, cerebrovascular disease and those with a BMI over 30 kg/m^2, most of which were not excluded in most studies. Inclusion of those cardiac risk factors, especially those BMI >30 kg/m^2, may had resulted in higher prevalence of diabetes and hyperlipidemia in the MAC group of our study (3), Different modalities for the diagnosis of MAC such as two-dimensional echocardiography, M-mode echocardiography and CT scan with the use of different calcium-scoring systems and (4) inclusion of diverse racial and/or ethnical populations between studies.

Numerous studies have documented the association between cardiac autonomic function and hypertension and has found diminished values for time domain indices of HRV in this group of patients (3, 4). In our study, HRV parameters were lower in normotensive subjects with MAC than in those without MAC. Moreover, hypertensive patients with MAC displayed an even greater reduction in time domain parameters of HRV.

It has been demonstrated that the HRV is reduced in patients with ischemic heart disease (34, 35). In our study, a considerable reduction of several time domain HRV parameters was detected in CAD (+) patients with MAC. In addition, patients with MAC had lower HRV than those without MAC in the CAD (-) subgroup. This

finding also underscores the presence of MAC. It was found that RMSSD and pNN50, which are supposed to be quite robust parameters of vagally mediated HRV were similar in CAD (+) subgroup. But it may be due to relatively small number of patients in that group.

Study limitations

We recognize that our study has limitations that warrant consideration. First, the cross-sectional design does not allow us to infer causation between HRV parameters and MAC . Second, physical fitness and activity levels are known to have impact on HRV and we did not standardize these factors. Third, our study population is small. Lastly, we did not categorize MAC according to its severity. Further study is needed to examine the relationship between HRV and MAC.

Conclusion

Compared with patients without MAC, patients with MAC displayed decreased HRV in time domain parameters, possibly reflecting decreased parasympathetic tone with a predominant activity of the sympathetic tone, irrespectively of presence of HT and CAD.

Conflict of interest: None declared.

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References

1. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 1996; 93: 1043-65. [CrossRef]
2. Tsuji H, Venditti FJ Jr, Manders ES, Evans JC, Larson MG, Feldman CL, et al. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. Circulation 1994; 90: 878-83. [CrossRef]
3. Liao D, Cai J, Barnes RW, Tyroler HA, Rautaharju P, Holme I, et al. Association of cardiac autonomic function and the development of hypertension: the ARIC study. Am J Hypertens 1996; 9: 1147-56. [CrossRef]
4. Singh JP, Larson MG, Tsuji H, Evans JC, O'Donnell CJ, Levy D. Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. Hypertension 1998; 32: 293-7. [CrossRef]
5. Gerritsen J, Dekker JM, TenVoorde BJ, Kostense PJ, Heine RJ, Bouter LM, et al. Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn Study. Diabetes Care 2001; 24: 1793-8. [CrossRef]
6. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet 1998; 351: 478-84. [CrossRef]
7. Hayano J, Yamada M, Sakakibara Y, Fujinami T, Yokoyama K, Watanabe Y, et al. Short- and long-term effects of cigarette smoking on heart rate variability. Am J Cardiol 1990; 65: 84-8. [CrossRef]
8. Rossy LA, Thayer JF. Fitness and gender-related differences in heart period variability. Psychosom Med 1998; 60: 773-81.
9. Riva P, Martini G, Rabbia F, Milan A, Paglieri C, Chiandussi L, et al. Obesity and autonomic function in adolescence. Clin Exp Hypertens 2001; 23: 57-67. [CrossRef]
10. Hintsanen M, Elovainio M, Puttonen S, Kivimaki M, Koskinen T, Raitakari OT, et al. Effort-reward imbalance, heart rate, and heart rate variability: the Cardiovascular Risk in Young Finns Study. Int J Behav Med 2007; 14: 202-12. [CrossRef]
11. Fox CS, Vasan RS, Parise H, Levy D, O'Donnell CJ, D'Agostino RB, et al. Mitral annular calcification predicts cardiovascular morbidity and mortality: The Framingham Heart Study. Circulation 2003; 107: 1492-6. [CrossRef]
12. Vukasovic JL, Florenzano F, Adriazola P, Escobar E. Heart rate variability in severe aortic stenosis. J Heart Valve Dis 1999; 8: 143-8.
13. Arslan U, Özdemir M, Kocaman SA, Balcıoğlu S, Cemri M, Cengel A. Heart rate variability and heart rate turbulence in mild-to-moderate aortic stenosis. Europace 2008; 10: 1434-41. [CrossRef]
14. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003; 42: 1206-52. [CrossRef]
15. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2003; 26: 3160-7.
16. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285: 2486-97. [CrossRef]
17. Picard MH, Adams D, Bierig SM, Dent JM, Douglas PS, Gillam LD, et al. American Society of Echocardiography recommendations for quality echocardiography laboratory operations. J Am Soc Echocardiogr 2011; 24: 1-10. [CrossRef]
18. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man: Anatomic validation of the method. Circulation 1977; 55: 613-8. [CrossRef]
19. Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 1987; 59: 256-62. [CrossRef]
20. Forslund L, Björkander I, Ericson M, Held C, Kahan T, Rehnqvist N, et al. Prognostic implications of autonomic function assessed by analyses of catecholamines and heart rate variability in stable angina pectoris. Heart 2002; 87: 415-22. [CrossRef]
21. Casolo G, Balli E, Taddei T, Amuhasi J, Gori C. Decreased spontaneous heart rate variability in congestive heart failure. Am J Cardiol 1989; 64: 1162-7. [CrossRef]
22. Antelmi I, de Paula RS, Shinzato AR, Peres CA, Mansur AJ, Grupi CJ. Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. Am J Cardiol 2004; 93: 381-5. [CrossRef]

23. Umetani K, Singer DH, McCraty R, Atkinson M. Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. J Am Coll Cardiol 1998; 31: 593-601. [CrossRef]
24. Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J. Heart rate variability from 24 hour electrocardiography and the 2 year risk for sudden death. Circulation 1993; 88: 180-5. [CrossRef]
25. Labovitz AJ, Nelson JG, Windhorst DM, Kennedy HL, Williams GA. Frequency of mitral valve dysfunction from mitral annular calcium as detected by Doppler echocardiography. Am J Cardiol 1985; 55: 133-7. [CrossRef]
26. Nair CK, Runco V, Everson GT, Boghairi A, Mooss AN, Mohiuddin SM, et al. Conduction defects and mitral annulus calcification. Br Heart J 1980; 44: 162-7. [CrossRef]
27. Kriwisky M, Goldstein J, Gotsman MS. Accessory atrioventricular pathway, supra-, and infrahisian conduction impaired due to mitral annulus calcification. Clin Cardiol 1987; 10: 818-20. [CrossRef]
28. Nair CK, Thomson W, Ryschon K, Cook C, Hee TT, Sketch MH. Long-term follow-up of patients with echocardiographically detected mitral anular calcium and comparison with age- and sex-matched control subjects. Am J Cardiol 1989; 63: 465-70. [CrossRef]
29. Movahed MR, Saito Y, Ahmadi-Kashani M, Ebrahimi R. Mitral annulus calcification is associated with valvular and cardiac structural abnormalities. Cardiovasc Ultrasound 2007; 5: 14. [CrossRef]
30. Adler Y, Herz I, Vaturi M, Fusman R, Shohat-Zabarski R, Fink N, et al. Mitral annular calcium detected by transthoracic echocardiography is a marker for high prevalence and severity of coronary artery disease in patients undergoing coronary angiography. Am J Cardiol 1998; 82: 1183-6. [CrossRef]
31. Kanjanauthai S, Nasir K, Katz R, Rivera JJ, Takasu J, Blumenthal RS, et al. Relationships of mitral annular calcification to cardiovascular risk factors: the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2010; 213: 558-62. [CrossRef]
32. Allison MA, Cheung P, Criqui MH, Langer RD, Wright CM. Mitral and aortic annular calcification are highly associated with systemic calcified atherosclerosis. Circulation 2006; 113: 861-6. [CrossRef]
33. Adler Y, Koren A, Fink N, Tanne D, Fusman R, Assali A, et al. Association between mitral annulus calcification and carotid atherosclerotic disease. Stroke 1998; 29: 1833-7. [CrossRef]
34. Hayano J, Sakakibara Y, Yamada M, Ohte N, Fujinami T, Yokoyama K, et al. Decreased magnitude of heart rate spectral components in coronary artery disease: its relation to angiographic severity. Circulation 1990; 81: 1217-24. [CrossRef]
35. Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, Swenne CA, et al. Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. Atherosclerosis Risk In Communities. Circulation 2000; 102: 1239-44. [CrossRef]