Serum Thiol/Disulfide Homeostasis and Endocan Levels in Patients Who Underwent Diagnostic Exercise Electrocardiography Test

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ABSTRACT

Introduction: Inflammation and oxidative stress are associated with increased risk of cardiovascular diseases. Parameters derived from exercise electrocardiography (ECG) test, such as Duke treadmill score and heart rate recovery (HRR), have prognostic importance and can be used to predict cardiovascular risk. Endocan and thiol/disulfide homeostasis are emerging biomarkers that reflect the inflammatory and oxidative status. The aim of this study was to investigate serum thiol/disulfide homeostasis and endocan levels in an exercise ECG testing patient cohort and to evaluate the association of these markers with Duke treadmill score and HRR.

Patients and Methods: Patients who underwent diagnostic exercise ECG test were divided into two groups according to Duke treadmill score and HRR. Serum endocan levels and thiol/disulfide homeostasis were compared between high and low/intermediate Duke treadmill score groups and blunted and normal HRR groups.

Results: Serum endocan levels were similar between the groups according to Duke treadmill score and HRR. Thiol/disulfide ratio was significantly lower in patients with blunted HRR than in those with normal HRR. Serum native thiol levels, total thiol levels, and total thiol/disulfide ratio were significantly elevated in patients with a high Duke treadmill score than in those with low/intermediate Duke treadmill score. Native thiol levels and total thiol levels were significantly correlated with Duke treadmill score. Multivariate regression analysis revealed that hypertension and diabetes were independent predictors of blunted HRR, whereas hypertension and low endocan levels were those of low/intermediate Duke treadmill score.

Conclusion: Serum thiol levels are correlated with Duke treadmill score, and low endocan levels are associated with low/intermediate Duke treadmill score.

Key Words: Inflammation; oxidative stress; Duke score; heart rate recovery; thiol/disulfide homeostasis; endocan

Tanısal Egzersiz Elektrokardiyografi Testi Yapılan Hastalarda Serum Tiyol Disulfid Dengesi ve Endokan Düzeyleri

ÖZET

Giriş: İnflamasyon ve oksidatif stres artmış kardiyovasküler risk ile ilişkilidir. Duke koşu bandı skoru ve kalp hızı toparlanması (KHT) gibi egzersiz elektrokardiyografi (EKG) testinden türetilen parametreler prognostik öneme sahiptir ve kardiyovasküler risk tahmini için kullanılabilir. Endokan ve tiyol/disülfid dengesi, inflamatuvar ve oksidatif durumu yansıtan yeni biyolojik belirteçlerdir. Bu çalışmanın amacı, bir egzersiz EKG testi yapılan hastalarda serum tiyol/disülfid dengesi ve endokan düzeylerini araştırmak ve bu belirteçlerin Duke treadmill skoru ve KHT ile ilişkisini değerlendirmektir.

Hastalar ve Yöntem: Tanısal egzersiz EKG testi yapılan hastalar Duke skoru ve KHT'ye göre iki gruba ayrıldı. Yüksek Duke skoru-orta/düşük Duke skoru grupları ile künt ve normal KHT grupları arasında serum endokan düzeyleri ve tiyol/disülfid dengesi karşılaştırıldı.

Bulgular: Serum endokan düzeyleri, Duke treadmill skoru ve KHT'ye göre gruplar arasında benzerdi. Tiyol /disülfid oranı, azalmış KHT'li hastalarda normal KHT'li hastalara göre anlamlı derecede düşüktü. Yüksek/ Duke skoru olan hastalarda, düşük/orta Duke skoru olan hastalara göre, serum nativ tiyol düzeyleri, total tiyol düzeyleri ve total tiyol/disülfid oranı anlamlı olarak yüksekti. Doğal tiyol düzeyleri ve total tiyol düzeyleri Duke skoru ile anlamlı korelasyon göstermekteydi. Çok değişkenli regresyon analizinde, azalmış KHT'inin bağımsız öngördürücüleri hipertansiyon ve diyabet varlığı; düşük/orta Duke skorunun bağımsız öngördürücüleri ise hipertansiyon varlığı ve düşük endokan düzeyleridir.

Sonuç: Serum tiyol düzeyleri Duke skoru ile ilişkilidir ve düşük endokan seviyeleri düşük/orta Duke skoru ile ilişkilidir.

Anahtar Kelimeler: İnflamasyon; oksidatif stres; Duke skoru; tiyol disülfid dengesi; endokan

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INTRODUCTION

Exercise electrocardiography (ECG) test is frequently used for diagnostic and prognostic purposes in clinical cardiology. As its diagnostic sensitivity and specificity is relatively low, stand-alone testing for coronary artery disease (CAD) diagnosis is reserved for patients with intermediate risk for CAD. Besides diagnostic usage, exercise ECG can also give prognostic information. Duke treadmill score and heart rate recovery (HRR) are two prognostic variables derived from exercise ECG[1]. Inflammation and oxidative stress are associated with atherogenesis and increased sympathetic activity. Biomarkers that reflect the oxidative and inflammatory status can be used for determining the individuals at a risk of future cardiovascular events. Endocan as a marker of endothelial dysfunction and inflammation and thiol/disulfide homeostasis as a marker of oxidative status are emerging biomarkers in this field. Serum thiols are a class of organic sulfur derivatives, and they constitute an important part of the antioxidant defense system. Reactive oxygen substrates can damage the DNA, lipids, and proteins. Under oxidative stress, sulfhydryl compounds of thiols form disulfide bonds when interacting with an oxidant molecule and neutralize the molecule to a less toxic form. This homeostasis between thiols and disulfide bonds can represent oxidative status of the organism[2-6]. Endocan is a soluble proteoglycan and is secreted by activated endothelial cells[7]. It plays an important role in the regulation of cell adhesion, inflammatory processes, and tumor progression[8]. Recent studies have demonstrated the association of these molecules with some cardiovascular diseases and diseases with an increased cardiovascular risk[9-14].

Exercise ECG can give prognostic information for future cardiovascular events. Endocan and thiol/disulfide homeostasis are emerging biomarkers in patients with an increased risk of cardiovascular diseases. No date stating the association between prognostic findings of exercise ECG and these biomarkers is available. Therefore, we aimed to investigate the serum thiol/disulfide homeostasis and endocan levels in an exercise ECG testing patient cohort and to evaluate the association of these markers with prognostic indicators such as Duke treadmill score and HRR.

PATIENTS and METHODS

This study was conducted in October and November 2015. The study protocol was approved by the local ethical committee, and written informed consent was obtained from all participants. All participants were admitted to our outpatient clinic owing to chest pain and an intermediate risk of pretest probability for coronary artery disease according to their age, sex, and symptoms; therefore, we decided to perform exercise ECG. Medical histories and cardiovascular risk factors were recorded. Patients who use B blockers or nondihydropyridine calcium channel blocker were advised to discontinue the pills at least 2 days before the test. All blood samples for endocan, thiol, and disulfide analysis were obtained before the exercise ECG test. The samples were centrifuged at 1500 g for 10 minutes. Serum was stored at -80°C, and all samples were simultaneously processed. Serum lipid parameters, creatinine levels, hemogram parameters were obtained from the local laboratory records. Thiol-disulfide homeostasis were determined as described before [15]. Endocan levels were processed using ELISA kits (Boster, Wuhan, China). The exercise ECG testing was performed with the standard Bruce protocol. Baseline heart rate, maximum heart rate, age-predicted heart rate, and blood pressure response were recorded. HRR at first minute and second minute, chronotropic response index (CRI), and Duke treadmill score were calculated as described before [16]. A HRR < 12 beats/minute at the first minute or < 22 beats/minute at the second minute was accepted as blunted HRR. Patients with Duke treadmill score < 5 were included into the low/intermediate Duke treadmill score group, and these patients were accepted to have high/intermediate risk to future cardiovascular events. Patients with Duke treadmill score ≥ 5 were included in the high Duke treadmill score group and were accepted to have low risk for future cardiovascular events. A CRI > 0.8 was accepted as normal[16,17]. Changes in ST segment were interpreted by a physician who was blind to the study.

Statistical Analysis

Normality distribution of the continuous variables was tested using Kolmogorov-Smirnov test. Results were presented as mean ± standard deviation for normally distributed variables and as median (interquartile range 25-75) for abnormally distributed variables. Statistical comparisons between continuous variables were performed with independent samples t test or Mann-Whitney U test in accordance with normality test results. Statistical comparisons of categorical variables were performed using Chi-square test or Fisher's exact test. For the multivariate analysis, the possible factors identified using univariate analysis were further involved in the logistic regression analysis. Spearman's Rho was used for demonstrating the correlation between Duke treadmill score and thiol levels. SPSS 17.0 software for Windows (SPSS Inc. Chicago, IL) was used for data analysis. A p value < 0.05 was considered statistically significant.

RESULTS

In October and November 2015, we performed diagnostic exercise ECG test on 360 patients, 87 of which agreed to participate in the study. Sixty-six of the exercise ECG recordings were interpreted as normal. Twelve patients were further evaluated with a myocardial perfusion scintigraphy imaging; all were normal. Nine patients underwent coronary angiography; 1 of these was diagnosed with normal coronary arteries and the other 8 patients were diagnosed with obstructive CAD, and all of these underwent successfully revascularization.

Table 1. Baseline demographic features and laboratory parameters of the study population

	Overall (n= 87)	Normal HRR (n= 67)	Blunted HRR (n= 20)	p	Duke treadmill Score ≥ 5# (n= 71)	Duke treadmill Score < 5# (n= 16)	p
Age, years	50 ± 12	49 ± 10	54 ± 13	0.068	49 ± 11	56 ± 12	**0.034**
Male, n (%)	46 (52.9)	37 (55.2)	9 (45)	0.42	36 (50.7)	10 (62.5)	0.39
Hypertension, n (%)	24 (27.6)	14 (20.9)	10 (50)	**0.011**	15 (21.1)	9 (56.3)	**0.010**
Smoking, n (%)	42 (48.2)	32 (47.7)	10 (50)	0.21	32 (45.1)	10 (62.5)	0.34
Diabetes mellitus, n (%)	17 (19.5)	9 (13.4)	8 (40)	**0.020**	12 (16.9)	5 (31.3)	0.29
Family history for CAD, n (%)	15 (17.2)	10 (14.9)	5 (25)	0.13	13 (18.3)	2 (12.5)	0.72
B blocker n (%)	8 (9.2)	4 (6)	4 (20)	0.078	6 (8.5)	2 (12.5)	0.63
ACEI/ARB n (%)	20 (23)	12 (17.9)	8 (40)	0.066	14 (19.7)	6 (37.5)	0.18
Statin n (%)	9 (10.3)	6 (9)	3 (15)	0.42	7 (9.9)	2 (12.5)	0.66
Creatinine, mg/dL	0.88 ± 0.13	0.89 ± 0.13	0.88 ± 0.14	0.79	0.88 ± 0.13	0.91 ± 0.12	0.31
Total cholesterol, mg/dL	205 ± 34	203 ± 36	211 ± 27	0.35	206 ± 36	202.0 ± 37	0.67
HDL, mg/dL	47 ± 9	46 ± 9	48 ± 8	0.23	47 ± 9	42 ± 6	**0.035**
LDL, mg/dL	130 ± 31	130 ± 33	131 ± 28	0.93	131 ± 32	127 ± 28	0.60
Triglyceride, mg/dL	148 (99-165)	148 (132-190)	144 (97-153)	0.19	139 (86-165)	148 (99-228)	0.13
Hemoglobin, g/dL	14.5 ± 1.3	14.6 ± 1.2	14.2 ± 1.7	0.25	14.4 ± 1.4	14.7 ± 0.6	0.24
White blood cell count, $*10^3$	4.4 (3.6-5.7)	7.4 (6.7-9.3)	6.8 (6-9)	0.37	7.6 (6.6-8.8)	7.3 (6.5-10.4)	0.48
Platelet count, $*10^3$	253.1 ± 71.3	253.1 ± 69.5	254.6 ± 79.9	0.98	255.1 ± 71.0	244.1 ± 70.1	0.57
BMI (kg/m^2)	27.8 ± 4.1	27.5 ± 3.9	28.9 ± 4.6	0.19	27.3 ± 3.9	30.0 ± 4.5	**0.021**
Heart rate recovery (2. minute) (beat)	35 (24-54)	39 (30-58)	17 (16.5-19)	**< 0.001**	35 (27-57)	26 (18-44)	0.11
Chronotropic response index	0.98 (0.84-1.14)	1.02 (0.86-1.20)	0.90 (0.76-0.99)	**0.01**	0.99 (0.84-1.13)	0.96 (0.83-1.22)	0.98
Duke treadmill score	11 (6-14)	12 (6-15)	7.5 (2.5-11)	0.10	12 (9-15)	-1 (-3-2)	**< 0.001**
Endocan pg/mL	269.5 (203.9-344.5)	267.6 (210.2-364.6)	271.0 (198.7-301.8)	0.44	282.3 (217.1-364.6)	208.2 (184.3-314.6)	0.051
Native thiol μmol/L	272.9 ± 42.3	276.9 ± 41.3	259.4 ± 44.1	0.10	278.4 ± 40.8	248.2 ± 41.3	**0.009**
Total thiol μmol/L	302.7 ± 43.5	306.2 ± 42.1	290.9 ± 46.9	0.16	307.7 ± 41.8	280.3 ± 44.8	**0.022**
Disulfide μmol/L	14.7 (11.8-17.2)	14.5 (11.4-16.8)	15.4 (12.6-17.6)	0.21	14.9 (11.7-17.2)	14.2 (12.8-18.4)	0.55
Total thiol/Disulfide ratio	19.9 (17.2-24.8)	20.2 (18.3-26.8)	19.0 (16.1-21.6)	**0.043**	20.2 (18.3-26.1)	16.9 (15.8-22.4)	**0.032**

ACEI: Angiotensin converting enzyme inhibitor, ARB: Angiotensin 2 receptor blocker, BMI: Body mass index, HDL: High density lipoprotein, HRR: Heart rate recovery, LDL: Low density lipoprotein. # Duke treadmill score < 5 refers to low/intermediate Duke treadmill score group, and these patients were accepted to have high/intermediate risk for future cardiovascular events. Duke treadmill score ≥ 5 refers to high Duke treadmill score group, and these patients were accepted to have low risk for future cardiovascular events.

Baseline characteristics of the study population according to high/intermediate or low risk Duke scores and normal or blunted HRR are shown in Table 1. Patients with blunted HRR were significantly more diabetic and more hypertensive than those with normal HRR. CRI was significantly decreased in patients with blunted HRR. Serum endocan levels, native thiol levels, total thiol levels, and disulfide levels were similar between the groups; however, total thiol/disulfide ratio was significantly lower in patients with blunted HRR than those with normal HRR (Table 1). Patients with intermediate/high risk Duke treadmill

scores were significantly elder, more hypertensive, had lower HDL levels, and increased body mass index than those with low risk Duke treadmill scores. Serum native thiol levels, total thiol levels, and total thiol/disulfide ratio were significantly higher in patients with a low risk Duke treadmill score (Table 1). Serum endocan levels were decreased in patients with intermediate/high risk Duke treadmill score, but these were not statistically significant (Table 1). Correlation analysis revealed a significant association between Duke treadmill score with native thiol and that with total thiol (Figure 1 and 2, p= 0.021 and 0.024, respectively; Spearman's Rho= 0.248 and 0.234, respectively). There was a positive but insignificant correlation between Duke treadmill score and total thiol/disulfide ratio (Spearman's Rho= 0.192, p= 0.075). Endocan levels were not correlated with Duke treadmill score or HRR (p= 0.63 and 0.17, respectively; Spearman's Rho= 0.148 and 0.234, respectively). Serum thiols were not correlated with HRR (Native Thiol p= 0.87, Spearman's Rho= –0.017 and Total Thiol p= 0.61, Spearman's Rho= –0.055). We separately performed univariate and multivariate logistic regression analysis to predict blunted HRR and low/intermediate Duke treadmill score. Multivariate logistic regression with forward LR revealed that hypertension and diabetes were independent predictors of blunted HRR hypertension and low endocan levels were independent predictors of low/intermediate Duke treadmill score (Table 2,3).

DISCUSSION

In this study, we evaluated the thiol/disulfide homeostasis and endocan levels with prognostic findings of diagnostic exercise ECG test. There are two main findings of this study. First is the positive correlation between serum thiols and Duke treadmill score, and second is low endocan level as one of the predictor of intermediate/high risk Duke treadmill score.

Table 2. Regression analysis showing the predictors of blunted heart rate recovery

	Univariate model				Multivariate model			
		95% CI				95% CI		
	OR	Lower	Upper	p	OR	Lower	Upper	p
Age	1.041	0.996	1.088	0.073				
Hypertension	3.786	1.317	10.883	0.013	3.136	1.044	9.419	0.042
Diabetes	4.296	1.378	13.397	0.012	3.492	1.068	11.420	0.039
B blocker use	3.937	0.887	17.481	0.072				
ACEI/ARB Use	3.056	1.026	9.097	0.045				
Native thiol	0.990	0.977	1.002	0.109				
Total thiol Disulfide ratio	0.897	0.811	0.992	0.035				

ACEI: Angiotensin converting enzyme inhibitor, ARB: Angiotensin II receptor blocker, CI: Confidence interval, OR: Odds ratio.

Table 3. Regression analysis showing the predictors of low/intermediate Duke treadmill score

	Univariate model				Multivariate model			
		95% CI				95% CI		
	OR	Lower	Upper	p	OR	Lower	Upper	p
Age	1.052	1.003	1.103	0.039				
Hypertension	4.800	1.535	15.013	0.007	6.002	1.772	20.336	0.004
HDL	0.922	0.855	0.996	0.038				
BMI	1.178	1.018	1.364	0.028				
Endocan	0.994	0.987	1.000	0.062	0.993	0.986	0.999	0.033
Native thiol	0.981	0.966	0.996	0.013				
Total thiol	0.984	0.970	0.998	0.027				
Total thiol/ Disulfide ratio	0.898	0.805	1.003	0.056				

BMI: Body mass index, HDL: High density lipoprotein, CI: Confidence interval, OR: Odds ratio.

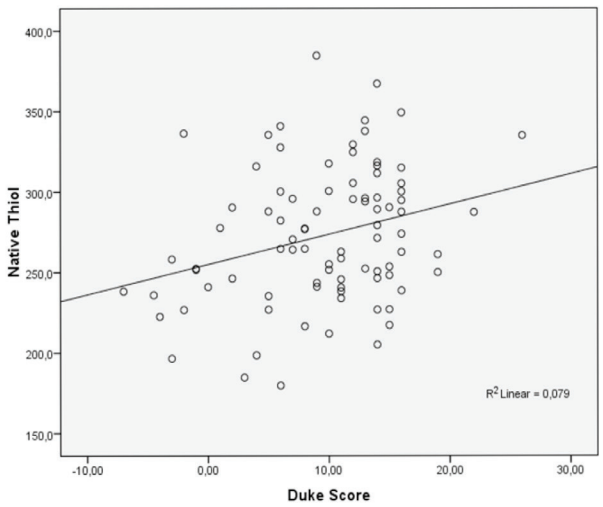

Figure 1. Correlation analysis between native thiol levels and Duke treadmill score.

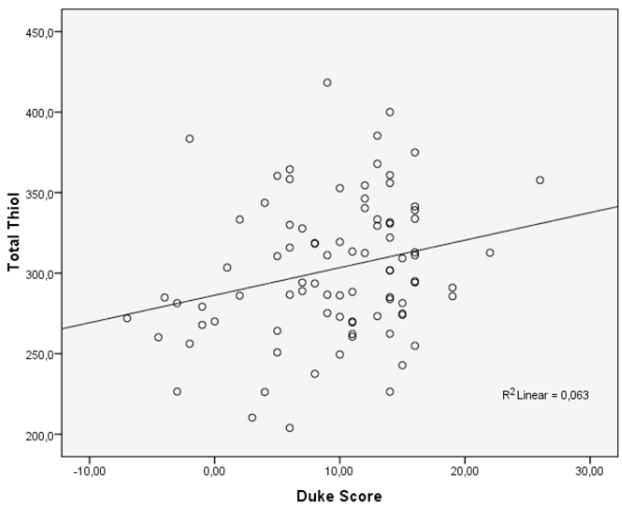

Figure 2. Correlation analysis between total thiol levels and Duke treadmill score.

Exercise ECG can provide important prognostic information besides its diagnostic utilization. The prognostic values of chronotropic response, heart rate recovery, and Duke treadmill score were demonstrated[18-20]. Duke treadmill score reflects atherosclerotic burden and HRR reflects sympathetic activity. It was previously demonstrated that abnormal HRR has increased the risk of ventricular arrhythmia or sudden death[21,22].Oxidative stress, endothelial dysfunction, and inflammation are closely associated with increased cardiovascular risk, atherogenesis, and increased sympathetic activity[23,24]. Previous studies demonstrated the association of high-sensitive CRP levels with Duke treadmill score and HRR[25,26]. Based on this scientific knowledge, we evaluated two emerging endothelial dysfunction, inflammation and oxidative stress markers in exercise ECG testing patient cohort. In this study, we found no association between endocan levels and HRR, and in contrast, low endocan levels are one of the independent predictors of low/intermediate Duke treadmill score.

Recent studies suggest that endocan may reflect endothelial dysfunction and inflammation for various disease states[27-29]. Kundi et al. demonstrated that endocan levels were significantly correlated with Syntax score and high-sensitive C-reactive protein[30]. In another study, endocan levels were found to be correlated with carotid intima media thickness and flow mediated dilation in obstructive sleep apnea patients[31]. All these studies suggest and support the possible association of endocan with inflammation and atherogenesis, but our findings are different. We started this study with the hypothesis that endocan levels should be associated with blunted HRR and low Duke treadmill score, but our findings did not support the hypothesis. When compared with the abovementioned endocan studies, the median endocan levels of our study are significantly lower (approximately lower than those of half or one third of other studies). Thus, it can be concluded that inflammatory status of our study cohort is lower than those of other studies. Our study population comprised patients with intermediate risk of pretest probability for CAD according to their age, sex, and symptoms, and it can be concluded that endocan is an unsuitable marker for cardiovascular risk prediction in this patient group. In fact we found that low endocan level is one of the independent predictor low/intermediate Duke treadmill score. Further studies are required to verify this finding. In contrast to endocan, we found that there was a deterioration trend in thiol/disulfide homeostasis both in patients with high/intermediate risk Duke score and those with blunted HRR. Serum thiol levels were significantly correlated with Duke treadmill score. Thiols are important elements for oxidation reactions, and under oxidative stress, they form disulfide bonds, which can be broken to form thiols again[32]. This dynamic thiol/disulfide homeostasis plays an important role in antioxidant defense system. Previously, we have demonstrated the alterations of thiol/disulfide homeostasis in patients with acute myocardial infarction. In another study, we showed that there is a negative correlation between thiol/disulfide ratio and Syntax score[11,33]. The significant correlation between CRP and serum thiols have been demonstrated in a recent study, and with this study we first demonstrated that serum thiols are significantly correlated with Duke treadmill score[34].

Study Limitations

The most important limitation of this study is the small sample size. The study population comprised patients with intermediate risk of pretest probability according to their age, sex, and symptoms. The other important limitation is the cross-sectional nature of the study. We did not evaluated the association between these biomarkers and cardiovascular outcomes, of course a study

designed for evaluating the association of these biomarkers both with exercise ECG parameters and cardiovascular outcomes provide more precise results. Before the enrollment no patient was diagnosed with coronary artery disease or heart failure. Patients with coronary artery disease or heart failure may exhibit more dramatic results. We did not process high-sensitive CRP levels. If these were studied, definite conclusions about the unfavorable findings of endocan levels could have been established.

CONCLUSION

Serum thiols are correlated with Duke treadmill score, and low endocan levels are associated with low/intermediate Duke treadmill score.

CONFLICT of INTEREST

The authors reported no conflict of interest related to this article.

REFERENCESBUTIONS

Concept/Design: EK, BI, HK, HÇ
Analysis/Interpretation: EK, BI, CT,
Data Acquisition: BI, MÇ, BG, EÖ
Writting: EK, BI, EÖ, MÇ, HK
Critical Revision: HÇ, CT
Final Approval: All of authors

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