# Serum Thiol-disulphide Homeostasis and Endocan Levels In Patients Who Underwent Diagnostic Exercise Electrocardiography Test

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## ABSTRACT

**Introduction:** Inflammation and oxidative stress is associated with increased cardiovascular risk. Parameters derived from exercise electrocardiography(ECG) test like Duke treadmill score and heart rate recovery(HRR) have prognostic importance and can be used for cardiovascular risk prediction. Endocan and thiol/disulphide homeostasis are emerging biomarkers which reflect the inflammatory and oxidative status. The aim of this study is to investigate serum thiol/disulphide 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.

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

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

**Conclusion:** Serum thiols levels are correlated with Duke score and low endocan levels are associated with low/intermediate Duke score.

**Keywords:** Inflammation, oxidative stress, Duke score, thiol/disulphide homeostasis, endocan

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

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## 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, inflamatuar 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 Metod:** 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 ön gördürücüleri 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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## 1- Introduction

Exercise electrocardiographic (ECG) test is frequently used for diagnostic and prognostic purposes in clinical cardiology. As it's 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. Exercise ECG can give prognostic information besides it's diagnostic usage. Duke treadmill score and heart rate recovery (HRR) are two prognostic variable derived from exercise ECG (1). Inflammation and oxidative stress are associated with atherogenesis and increased sympathetic activity. Biomarkers which reflect the oxidative and inflammatory status can be used for determining the risky individuals for future cardiovascular events. Endocan ''as a marker of endothelial dysfunction and inflammation'' and thiol/disulphide 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 DNA, lipids and proteins and under oxidative stress sulfhydryl compounds of thiols form disulphide bounds when interacting with an oxidant molecule and neutralize the molecule to a less toxic form. This homeostasis between thiols and disulphide bounds can represent oxidative status of the organism (2-6). Endocan is a soluble proteoglycan and can be secreted by activated endothelial cells (7). Endocan has an important role in 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/disulphide homeostasis are emerging biomarkers in patients with an increased cardiovascular risk. There is no data if there is any association between prognostic findings of exercise ECG and these biomarkers. So we aimed to investigate the serum thiol/disulphide 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.

## 2- Patients and Methods

This study was conducted in October and November 2015. The study protocol was approved by the local ethical committee and an informed consent form was obtained from all of the participants. All of the patients were admitted to our outpatient clinic with chest pain and have an intermediate risk

of pretest probability for coronary artery disease according to their age, sex and symptoms, for this reason it was decided to perform exercise ECG. Medical histories and cardiovascular risk factors were recorded. Patients who use a B blocker or a nondihydropyridine calcium channel blocker were advised to discontinue the pills at least two days before the test. All blood samples for endocan, thiol and disulphide 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 processed simultaneously. Serum lipid parameters, creatinine levels, hemogram parameters were obtained from the local laboratory records. Thiol-disulphide homeostasis were determined as described before (15). Endocan levels were processed with 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 was recorded. HRR at first minute and second minute, chronotropic response index (CRI) and Duke treadmill score were calculated as described (16). A HRR less than 12 beats/minute at the first minute or less than 22 beats/minute at the second minute was accepted as blunted HRR. Patients with Duke score <5 were included into the low/intermediate Duke score group and these patients were accepted that they have high/intermediate risk for future cardiovascular events. Patients with Duke score ≥5 were included into the high Duke score group and these patients were accepted that they have low risk for future cardiovascular events. A CRI >0.8 was accepted as normal (16, 17). ST segment changes was interpreted by a physician who was blind to the study.

*Statistical analysis:* Normality distribution of the continuous variables were 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 were used for demonstrating the correlation between Duke score and thiol levels. SPSS 17.0 software for Windows (SPSS Inc. Chicago, IL) was used for analysis of data. A *P* value <0.05 was considered statistically significant.

**3- Results**

In October and November 2015, we performed a total of 360 diagnostic exercise ECG test. Eighty seven patients agreed to participate to the study. Sixty six of the exercise ECG recordings were interpreted as normal. Twelve patients were further evaluated with a myocardial perfusion scintigraphy imaging that all were normal. Nine patients underwent coronary angiography, 1 of these were diagnosed with normal coronary arteries and the other 8 patients diagnosed with obstructive CAD and all of them successfully revascularizated.

Baseline characteristics of the study population according to high/intermediate risk 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 patients with normal HRR. CRI was significantly decreased in patients with blunted HRR. Serum endocan levels, native thiol levels, total thiol levels and disulphide levels were similar between the groups however total thiol/disulphide ratio was significantly lower in patients with blunted HRR than the patients with normal HRR (Table 1). Patients with intermediate/high risk Duke score were significantly elder, more hypertensive, have lower HDL levels and have increased body mass index than patients with low risk Duke score. Serum native thiol levels, total thiol levels and total thiol/disulphide ratio were significantly higher in patients with a low risk Duke score (Table 1). Serum endocan levels were decreased in patients with intermediate/high risk Duke score but this was not statistically significant (Table 1). Correlation analysis revealed a significant association between Duke score with native thiol and 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 score and total thiol/disulphide ratio (Spearman's Rho=0.192, p=0.075). Endocan levels were not correlated with Duke 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 too (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 score. Multivariate logistic regression with forward LR revealed that hypertension and diabetes were the independent predictors of blunted HRR (Table 2); hypertension and low endocan levels were independent predictors of low/intermediate Duke score (Table 3).

**4- Discussion**

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

Exercise ECG can provide important prognostic information besides it's diagnostic utilization and the prognostic value of chronotropic response, heart rate recovery, Duke treadmill score were demonstrated (18-20). Duke score reflects atherosclerotic burden and HRR reflects sympathetic activity. It was previously demonstrated that abnormal HRR have 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). With the basis of 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 that there was no association between endocan levels with HRR and in contrast low endocan levels were one of the independent predictors of low/intermediate Duke score. Recent studies suggested 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 of these studies suggest and support the possible association of endocan with inflammation and atherogenesis but our findings are not in the same direction. We started this study with the hypothesis that endocan levels should be associated with blunted HRR and low Duke score but our findings were opposite of our hypothesis. When we looked at the endocan studies that we mentioned above our median endocan levels are significantly lower than other studies (about half or one third of other studies). This can be concluded as inflammatory status of our study cohort is lower than other studies. Our study population was composed of patients with intermediate risk of pretest probability for CAD according to their age, sex and symptoms and at least we can conclude that endocan is not a suitable marker for cardiovascular risk prediction in this patient group. In fact we found that low endocan levels is one of the independent predictor low/intermediate

Duke score. This finding must be verified with more and bigger studies. In contrast to endocan, we found that there was a deterioration trend in thiol/disulphide homeostasis both in high/intermediate risk Duke score patients and in patients with blunted HRR. Serum thiol levels were significantly correlated with Duke score. Thiols are important elements for oxidation reactions and under oxidative stress, they form disulphide bounds which can be reduced to thiols again. (32). This dynamic thiol/disulphide homeostasis plays an important role in antioxidant defense system. We previously demonstrated the alterations of thiol/disulphide homeostasis in patients with acute myocardial infarction and in another study we showed that there was a negative correlation between thiol/disulphide ratio and Syntax score (11, 33). The significant correlation between CRP and Serum thiols have been demonstrated in a recent study (34) and with this study we firstly demonstrated that Serum thiols are significantly correlated with Duke treadmill score.

*Study Limitations*

The most important limitation of this study is the small sample size. The study population was composed of 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 non of these patients were diagnosed with coronary artery disease or heart failure. Patients with coronary artery disease or heart failure may exhibit more dramatic results. We did not processed high sensitive CRP levels. If we were studied CRP levels we could make firm conclusions about the unfavorable findings of endocan levels.

*Conclusion*

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

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**TABLES**

***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 Score ≥5ˣ (n=71)** | **Duke Score <5ˣ (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, gr/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, *10³* | 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, *10³* | 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/m²)* | 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** |
| *Disulphide 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/Disulphide 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 score <5 refers to low/intermediate Duke score group and these patients were accepted that they have high/intermediate risk for future cardiovascular events. Duke score ≥5 refers to high Duke score group and these patients were accepted that they have low risk for future cardiovascular events

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

| | Univariate Model | | | | Multivariate Model | | | |
|---|---|---|---|---|---|---|---|---|
| | OR | 95% CI | | p | OR | 95% CI | | p |
| | | Lower | Upper | | | Lower | Upper | |
| 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 Disulphide 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 score.

| | Univariate Model | | | | Multivariate Model | | | |
|---|---|---|---|---|---|---|---|---|
| | OR | 95% CI | | p | OR | 95% CI | | p |
| | | Lower | Upper | | | Lower | Upper | |
| 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 Disulphide Ratio | 0.898 | 0.805 | 1.003 | 0.056 | | | | |

BMI: body mass index, HDL: high density lipoprotein, CI: confidence interval, OR: odds ratio

**FIGURE LEGEND**

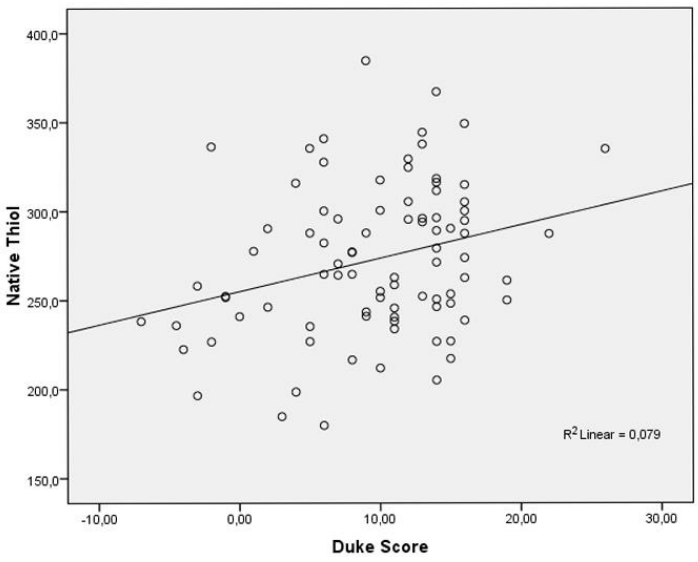


Figure 1: Correlation analysis between native thiol levels and Duke score.

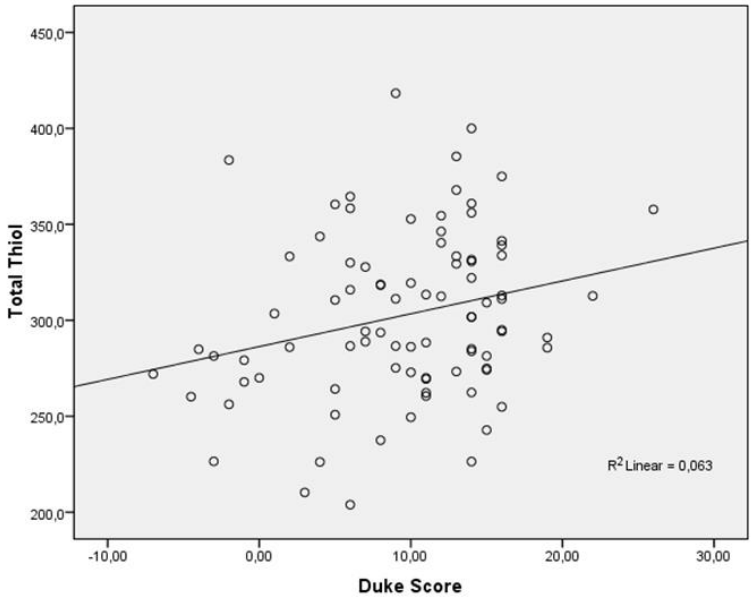


Figure 2: Correlation analysis between total thiol levels and Duke score.

**Declaration of Interest:** No competing interest declared.