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ORIGINAL ARTICLE / KLİNİK ÇALIŞMA

Plasma thiol and disulphide levels and their relationship with left ventricular systolic functions: A propensity score matching analysis

Plazma tiyol ve disülfit düzeylerinin sol ventrikül sistolik fonksiyonları ile ilişkisi: Eğilim skoru eşleşme analizi

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ABSTRACT

Objective: Left ventricular (LV) systolic function measured through LV ejection fraction (LVEF) has prognostic implications in patients with cardiac and non-cardiac conditions. The balance of thiol and disulphide levels reflects oxidative status in the body. In this study, we aimed to investigate the relationship between plasma thiol and disulphide levels, and LVEF calculated by transthoracic echocardiography (TTE).

Methods: This retrospective study included 1,048 patients referred for TTE examination and biochemical analyses, including plasma thiol and disulphide levels. After the application of exclusion criteria, the remaining 611 patients were included in the statistical analysis. Patients were classified into two groups, namely normal LVEF (n-LVEF) (n=446) and low LVEF (l-LVEF) (n=165) according to a cut-off level of LVEF 50%. To reduce sample selection bias and adjust for the influence of differences in patient characteristics on LVEF and oxidative status, 1:1 propensity score matching analysis was applied.

Results: Propensity score matching analysis yielded 125 patients in both groups with comparable demographics, medications, and blood parameters. Native thiol and total thiol levels were lower in l-LVEF patients than in n-LVEF patients ($p<0.001$ for both), whereas disulphide levels were higher in l-LVEF group ($p=0.008$). Native thiol ($r=0.384$, $p<0.001$), total thiol ($r=0.35$, $p<0.001$), and disulphide levels ($r=-0.129$, $p=0.004$) significantly correlated with LVEF.

Conclusion: Plasma thiol levels decrease and disulphide levels increase suggesting the presence of oxidative stress in patients with l-LVEF. Significant correlation between oxidative stress and LVEF sheds light about the possible pathogenetic role of thiol and disulphide in heart failure.

ÖZET

Amaç: Sol ventrikül ejeksiyon fraksiyonu (SVEF) aracılığı ile ölçülen sol ventrikül sistolik fonksiyonunun hem kalp hem de kalp dışı problemleri olan hastalarda prognostik etkileri vardır. Tiyol ve disülfit seviyelerinin dengesi vücuttaki oksidatif durumu yansıtmaktadır. Bu çalışma transtorasik ekokardiyografi (TTE) ile ölçülen SVEF ile plazma tiyol ve disülfit düzeyleri arasındaki ilişkinin araştırılmasını amaçladı.

Yöntemler: Bu retrospektif çalışmaya TTE incelemesi ve plazma tiyol ve disülfit seviyeleri dahil biyokimyasal analizler için yönlendirilen 1048 hasta dahil edilmiştir. Dışlanma kriterleri uygulandıktan sonra, geriye kalan 611 hasta istatistiksel analize dahil edildi. Hastalar SVEF 50% kesme seviyesine göre normal SVEF (n-SVEF) (n=446) ve düşük SVEF (d-SVEF) (n=165) olmak üzere iki gruba ayrıldı. Örneklerdeki seçim yanlılığını azaltmak, hastaların karakteristik özelliklerindeki farklılıkların SVEF ve oksidatif durum üzerindeki etkisini ayarlamak için 1:1 eğilim skoru eşleştirme analizi uygulandı.

Bulgular: Eğilim skoru eşleştirme analizi sonrası her iki grupta demografik, ilaçlar ve kan parametreleri açısından benzer 125 hasta elde edildi. Nativ tiyol ve toplam tiyol düzeyleri d-SVEF hastalarında n-SVEF hastalarına göre daha düşük iken (her ikisi için $p<0.001$), disülfit seviyeleri ise d-SVEF grubunda daha yüksek bulundu. ($p=0.008$). Nativ tiyol ($r=0.384$, $p<0.001$), toplam tiyol ($r=0.35$, $p<0.001$) ve disülfit seviyeleri ($r=-0.129$, $p=0.004$) SVEF ile anlamlı bir korelasyon gösterdi.

Sonuç: d-SVEF hastalarında oksidatif stresin varlığını düşündüren plazma tiyol seviyelerinde azalma ve disülfit seviyelerinde artış olmaktadır. Oksidatif stres ve SVEF arasındaki bu anlamlı ilişki, kalp yetmezliğinde tiyol ve disülfitin olası patogenetik rolü hakkında ışık tutabilir.

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Transthoracic echocardiography (TTE) is one of the most commonly used cardiac imaging tests in daily clinical practice.[1] It is an invaluable tool to determine left ventricular (LV) systolic functions, which has prognostic implications including death in patients with a variety of cardiac and non-cardiac conditions.[1-4] Left ventricular ejection fraction (LVEF) is the recommended parameter for evaluating and reporting LV systolic functions.[5] Deterioration in LV systolic functions, namely LV systolic dysfunction (LVSD) and the resulting heart failure (HF) is a progressive clinical condition in which the pumping function of the heart is impaired, and the heart cannot efficiently maintain blood flow for body needs.[3] Despite the improvements in treatment and follow-up strategies of patients with HF in the past decade, morbidity and mortality rates of HF are still high.[3,6,7] Various pathophysiological mechanisms, including inflammatory system activation and oxidative stress play a role during the initiation and progression of the disease.[8]

Thiols, which take part in prevention of oxidative stress in cells, are organic compounds containing sulfhydryl group. The plasma thiol pool is composed of albumin, protein thiols, and low molecular weight thiols, including cysteinylglycine, cysteine, homocysteine, glutathione, and γ-glutamylcysteine.[9,10] When oxidative stress occurs through reactive oxygen species (ROS), thiols carrying these sulfhydryl groups are oxidized and form reversible disulphide bonds. However, these disulphide bonds can again be reduced to thiol groups through antioxidative defense mechanisms, and this process is defined as dynamic thiol/disulphide homeostasis.[9-11] Increasing body of evidence suggests that abnormalities in this homeostasis play a role in the pathogenesis of various cardiovascular and non-cardiovascular diseases.[12-16] Although the contribution of oxidative stress in the pathogenesis of HF is well defined,[8,17,18] whether there is an association between LVEF and thiol/disulphide homeostasis is not known. In this retrospective study, we aimed to investigate the relationship between plasma thiol and disulphide levels and LVEF detected by TTE examination.

METHODS

Study population

The study included 1,048 consecutive patients who applied to the cardiology clinic and were referred for TTE examination between the years 2016 and 2019. Exclusion criteria were defined as follows: Acute decompensated and/or Class IV HF according to New York Heart Association Classification, acute coronary syndrome (ACS), acute aortic dissection, pulmonary embolism, active infection, chronic inflammatory and/or rheumatological disease, malignancy, hematological disease, moderate/severe renal insufficiency, hepatic failure, moderate/severe valvular disease, stroke, and antioxidant or nutritional therapy. Patients <18 years of age or patients with insufficient hospital data such as thiol and disulphide levels or TTE findings, and those lacking good imaging quality were also excluded from the analysis. Four hundred and thirty-seven patients who met the criteria mentioned above were excluded from the study, and the remaining 611 patients were included in the statistical analysis. The study protocol was approved by the Clinical Research Ethics Committee of Ankara City Hospital (Approval Date: June 25, 2020; Approval Number: E1-852/2020) and conformed with the principles defined in the Declaration of Helsinki.

Abbreviations:

ACS	*Acute coronary syndrome*
AF	*Atrial fibrillation*
AUC	*Area under the ROC curve*
CAD	*Coronary artery disease*
CI	*Confidence interval*
DM	*Diabetes mellitus*
ECG	*Electrocardiography*
HDL	*High-density lipoprotein*
HF	*Heart failure*
LDL	*Low density lipoprotein*
l-LVEF	*Low LVEF*
LV	*Left ventricular*
LVEF	*Left ventricular ejection fraction*
LVSD	*LV Systolic dysfunction*
MI	*Myocardial infarction*
n-LVEF	*Normal LVEF*
OR	*Odds ratio*
ROC	*Receiver operating characteristic*
ROS	*Reactive oxygen species*
TTE	*Transthoracic echocardiography*

Demographic, clinical, TTE, medication, and laboratory parameters of the study patients were obtained from previous hospital records. Diabetes mellitus (DM) was defined as the use of hypoglycemic agents or fasting glucose level ≥126 mg/dL and/or non-fasting glucose level ≥200 mg/dL. Hypertension was defined as using antihypertensive medication or mean office blood pressure measurements ≥140-90 mmHg at repeated measurements. Smoking was defined as currently smoking in the previous six months. Atrial fibrillation (AF) was diagnosed as irregular ventricular rate without P waves and the presence of fibrillatory waves in 12-lead electrocardiography (ECG) or ECG monitoring obtained at hospital admission

or follow-up ECGs or ambulatory ECG recordings. Coronary artery disease (CAD) was defined as a history of myocardial infarction (MI), percutaneous coronary intervention, coronary artery bypass grafting, or angiographically proven more than 30% obstruction in any epicardial coronary artery.

Biochemical analyses

Peripheral blood samples for the measurement of biochemical parameters were obtained from a cubital vein after a minimum of eight hours of overnight fasting, and analyses were performed with a Hitachi 747 autoanalyzer. Plasma samples were taken into EDTA/citrated tubes, and analyses were performed at the biochemistry laboratory of the hospital. Plasma concentrations of cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol were calculated by enzymatic chemical cleaning method using Cobas 6000 (Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was calculated after dextran sulfate magnesium precipitation. Low density lipoprotein (LDL) cholesterol was calculated by the Friedewald method.

Plasma thiol and disulphide levels were measured immediately after blood sampling through a spectrophotometric method developed recently by Erel and Neselioglu.[9] Briefly, disulphide bonds were reduced to form free functional thiol groups. An apparatus of Shimadzu UV-1800 spectrophotometer and a Cobas c501 automated analyzer (Roche, Mannheim, Germany) were used for the reduction analyses. Sodium borohydride was used as a tool for the reduction of dynamic disulphide bonds (–S–S) to functional native thiols (–SH). Formaldehyde was used to remove the remnants of sodium borohydride residues. Modified Ellman reagent was used to obtain total thiols. The amount of dynamic disulphide bonds was calculated as half of the difference between total thiol and native thiol.[9] Disulphide-to-native thiol, disulphide-to-total thiol, and native thiol-to-total thiol ratios were calculated.

Transthoracic echocardiography

TTE examinations were performed with the use of Philips iE33 xMatrix (Philips Healthcare, Inc., Andover, MA) with a 2.5 MHz phase array by one experienced research echocardiographer who was blinded to the study protocol and biochemical data of the participants. All the patients underwent comprehensive two-dimensional, M-mode, and Doppler echocardiographic examinations in the left lateral decubitus position. LVEF was calculated using the modified two-dimensional biplane Simpson's method. All echocardiographic procedures were compatible with the recommendations of the American Society of Echocardiography.[5]

After the echocardiographic examination, the participants were classified into two groups, namely normal LVEF (n-LVEF) and low LVEF (l-LVEF) according to a cut-off level of LVEF 50%. Patients with LVEF ≥ 50% were classified as n-LVEF, and l-LVEF was defined as patients with LVEF < 50%.[19]

Statistical analysis

All analyses were performed using SPSS for Macintosh, version 24.0 (IBM Corp.; Armonk, NY, USA). One-sample Kolmogorov-Smirnov test was used to test the distribution of numerical variables. The chi-squared test was applied for categorical variables and presented as percentages. Mann-Whitney U test was applied for abnormally distributed variables, and the results were given as median with interquartile range. The Student's t-test was applied to numerical data which conformed to the normal distribution, and the results were entered as mean and standard deviation. Propensity score matching analysis was performed to adjust for the influence of differences in patient characteristics on LVEF and oxidative status to reduce sample selection bias. Clinically important parameters and variables that are known to be associated with deteriorations in LVEF such as age, sex, DM, hypertension, smoking, AF, creatinine, hemoglobin, medications, and lipid profile including LDL, HDL and triglyceride were used as covariates to calculate propensity score for each patient. Patients with n-LVEF and l-LVEF were then matched by performing a 1:1 nearest-neighbor matching using a caliper width of 0.01 without replacement. After propensity score matching analysis, receiver operating characteristic (ROC) curve analysis was conducted to determine cut-off values of native and total thiol levels for predicting n-LVEF. The area under the ROC curve (AUC) was reported with 95% confidence interval (CI) in addition to sensitivity and specificity. For correlation analyses regarding LVEF and thiol and disulphide parameters, Spearman's correlation analysis was preferred because of non-normal distribution. During correlation analysis, the direction of

Table 1. Basal characteristics of study population before and after propensity score matching

Variables	Before propensity score matching (n=611)			After propensity score matching (n=250)		
	LVEF <50 (n=165)	LVEF ≥50 (n=446)	*p*	LVEF <50 (n=125)	LVEF ≥50 (n=125)	*p*
Age, years	68 (60-75)	61 (52-69)	<0.001	66.5±10.3	66.8±10.0	0.776
Sex (male), n (%)	129 (78)	256 (57)	<0.001	92 (73)	90 (72)	0.776
Diabetes mellitus, n (%)	69 (42)	127 (28)	0.002	48 (38)	49 (39)	0.897
Hypertension, n (%)	117 (71)	250 (56)	0.001	86 (69)	86 (69)	1.00
Smoking, n (%)	49 (30)	125 (28)	0.685	38 (30)	33 (26)	0.483
Atrial fibrillation, n (%)	39 (24)	83 (19)	0.168	26 (21)	29 (23)	0.671
History of CAD, n (%)	128 (78)	183 (41)	<0.001	100 (80)	64 (51)	<0.001
Drugs						
RAS blocker, n (%)	101 (61.2)	240 (53.8)	0.102	70 (56)	67 (54)	0.564
Beta blocker, n (%)	93 (56.4)	209 (46.9)	0.037	65 (52)	62 (50)	0.486
Diuretics, n (%)	104 (63.0)	240 (53.8)	0.041	71 (57)	74 (59)	0.856
Statins, n (%)	29 (17.6)	95 (21.3)	0.309	22 (18)	22 (18)	1.00
Acetylsalicylic acid, n (%)	56 (33.9)	167 (37.4)	0.420	43 (34)	45 (36)	0.962
Creatinine (mg/dL)	0.92 (0.77-1.10)	0.80 (0.69-0.94)	<0.001	0.90 (0.73-1.07)	0.87 (0.74-1.01)	0.609
Hemoglobin (mg/dL)	12.2±2.1	12.44±1.91	0.382	12.3 ±2.12	12.2±2.17	0.601
High density lipoprotein (mg/dL)	40 (33-48)	42 (49-35)	0.073	40 (34-50)	41 (33-48)	0.625
Low density lipoprotein (mg/dL)	99 (73-122)	110 (84-135)	0.002	103.1±36.6	99.7±39.6	0.481
Triglycerides (mg/dL)	127 (86-194)	145 (107-213)	0.002	132 (91-206)	133 (100-201)	0.658
Plasma thiol/disulphide parameters						
Native thiol (μmol/L)	358.3±72.8	418±62.4	<0.001	364.7±76.1	416.2±64.2	<0.001
Total thiol (μmol/L)	392.3±76.6	451.0±61.8	<0.001	401.3±78.6	445.1±64.2	<0.001
Disulphide (μmol/L)	17.0 (11.9-21.2)	15.6 (10.1-21.9)	0.319	17.6 (13.5-21.7)	14.5 (8.6-19.8)	0.008
Disulphide/native thiol	0.05 (0.03-0.06)	0.04 (0.02-0.05)	<0.001	0.05 (0.04-0.06)	0.03 (0.02-0.05)	<0.001
Disulphide/total thiol	0.04 (0.03-0.05)	0.04 (0.02-0.05)	<0.001	0.04 (0.03-0.06)	0.03 (0.02-0.05)	<0.001
Native thiol/total thiol	0.91 (0.89-0.94)	0.93 (0.90-0.96)	<0.001	0.91 (0.89-0.93)	0.94 (0.91-0.96)	<0.001
LVEF (%)	45 (40-48)	60 (55-65)	<0.001	45 (40-48)	60 (55-65)	<0.001

LVEF: left ventricular ejection fraction (%); RAS: renin-angiotensin-aldosterone receptor
Parameters are mean ± standard deviation or median (interquartile range), n (%). The Mann-Whitney U test was applied for non-normal distributed variables, whereas the Student's t-test was used for numerical data, which conformed to the normal distribution. P value < 0.05 was considered significant for statistical analyses.

the relationship was evaluated by the covariance coefficient, and the degree of the relationship was evaluated by the correlation r coefficient. Multivariate logistic regression analyses were performed in two separate models to assess whether decreased native thiol (Model 1) and decreased total thiol (Model 2) are associated with l-LVEF even in the presence of ischemic etiology. A two-tailed p value < 0.05 was considered as statistically significant.

RESULTS

Basal characteristics of the patients, including medications and blood parameters before and after propensity score matching are presented in Table 1. Patients were divided into two groups according to a cut-off level of LVEF 50% and defined as n-LVEF (n=446) and l-LVEF (n=165). After propensity score matching analysis, there remained 125 patients in both groups with comparable age, sex, DM, hypertension, smok-

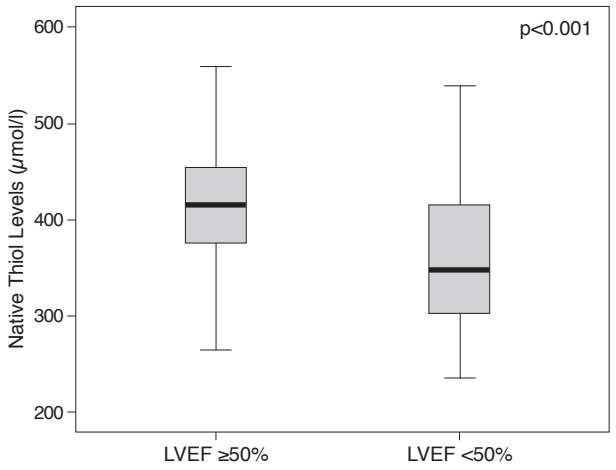


Figure 1. Comparison of plasma native thiol levels and left ventricular ejection fraction
LVEF: left ventricular ejection fraction.

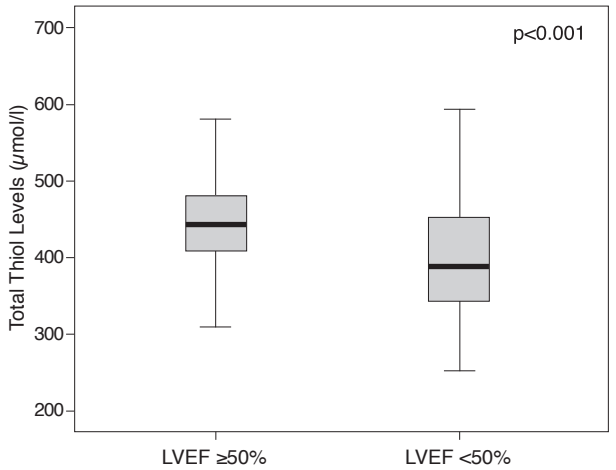


Figure 2. Comparison of plasma total thiol levels and left ventricular ejection fraction.
LVEF: left ventricular ejection fraction.

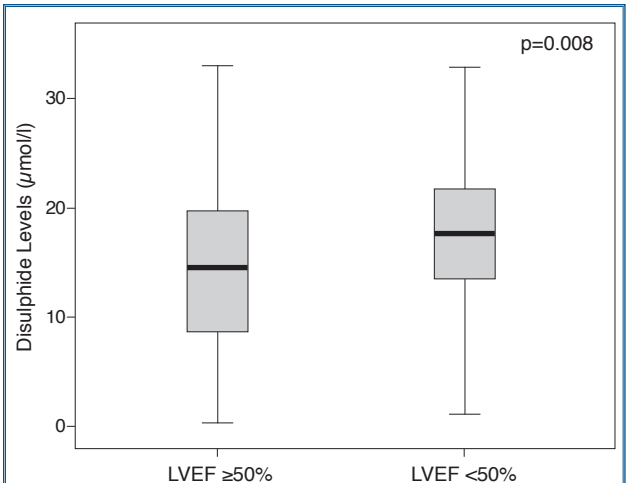


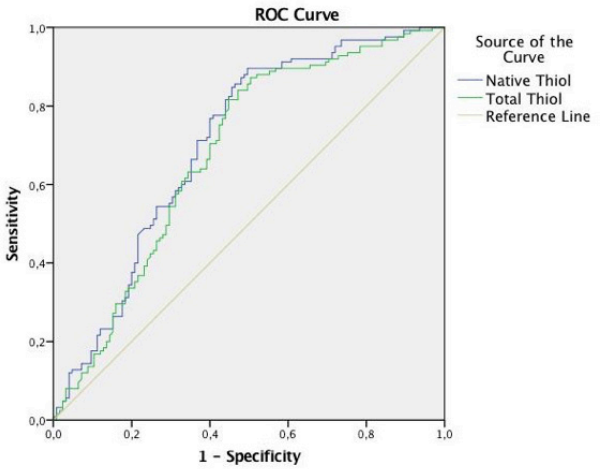
Figure 3. Comparison of disulphide levels and left ventricular ejection fraction.
LVEF: left ventricular ejection fraction.

ing, AF, and medications including renin-angiotensin-aldosterone system blocker, beta blocker, diuretics, statins, and acetylsalicylic acid. The percentage of patients with CAD was higher in the l-LVEF group than in the n-LVEF group (80% and 51%, respectively, p<0.001). Blood parameters including creatinine, hemoglobin, and lipid parameters were similar between the two groups. LVEF was significantly lower in the l-LVEF group than in the n-LVEF group (45% and 60%, respectively, p<0.001).

Plasma thiol and disulphide parameters of the study population before and after propensity score matching are given in Table 1. Native thiol and total thiol levels were significantly lower in patients with l-LVEF than in those with n-LVEF (364.7±76.1 and 416.2±64.2, p<0.001; 401.3±78.6, 445.1±64.2, p<0.001; respectively) (Figures 1 and 2), whereas disulphide levels were significantly higher in the l-LVEF group than in the n-LVEF group (17.6 [13.5-21.7], 14.5 [8.6-19.8], p=0.008, respectively) (Figure 3). Disulphide/native thiol and disulphide/total thiol levels were significantly higher in patients with l-LVEF (p<0.001 for both), and native thiol/total thiol level was significantly lower in the l-LVEF group (p<0.001). ROC curve analysis for native and total thiol levels to predict n-LVEF demonstrated an AUC value of 0.705 (95% CI: 0.640-0.770, p<0.001) for native thiol and AUC value of 0.681 (95% CI: 0.614-0.748, p<0.001) for total thiol. The cut-off value of native thiol (384) was associated with 71.4% sensitivity and 63.2% specificity. The cut-off value of total thiol (417) was associated with 70.6% sensitivity and 60.0% specificity (Figure 4).

Correlation analysis between plasma thiol and disulphide levels and LVEF are demonstrated in Table 2. Native thiol (r=0.384, p<0.001), total thiol (r=0.350, p<0.001) and native thiol/total thiol (r=0.256, p<0.001) significantly but moderately correlated positively with LVEF. However, disulphide (r=-0.129, p=0.004), disulphide/native thiol (r=-0.239, p<0.001) and disulphide/total thiol (r=-0.253, p<0.001) significantly but weakly correlated with LVEF in a negative manner.

Multivariate logistic regression analyses evaluating whether thiols are associated with l-LVEF even



	AUC	95% CI	P value	Sensitivity	Specifitiy
Native Thiol >384	0.75	0.640-0.770	<0.001	71.4%	63.2%
Total Thiol >417	0.681	0.614-0.748	<0.001	70.6%	60.0%

Figure 4. Receiver operating characteristic curve analysis of native and total thiol levels for prediction of left ventricular systolic functions.
ROC: receiver operating characteristic; AUC: area under the ROC curve; CI: confidence interval.

Table 2. Correlation analysis between plasma thiol-disulphide parameters and left ventricular ejection fraction

	Left ventricular ejection fraction (%)	
Co-variates	Correlation r coefficient*	*p*
Native thiol	0.384	<0.001
Total thiol	0.350	<0.001
Disulphide	-0.129	0.004
Disulphide/native thiol	-0.239	<0.001
Disulphide/total thiol	-0.253	<0.001
Native thiol/total thiol	0.256	<0.001

*Spearman's rho correlation analysis

in the presence of ischemic etiology in two separate models are presented in Table 3. In Model 1 including decreased native thiol and CAD, decreased native thiol emerged as an independent predictor of l-LVEF (Odds ratio [OR]: 4.63, 95% CI: 2.61-8.22, p<0.001). In Model 2 including decreased total thiol and CAD, decreased total thiol emerged as an independent predictor of l-LVEF (OR: 4.48, 95% CI: 2.44-8.19, p<0.001). Besides, CAD was an independent predictor of l-LVEF in both models (p<0.001 for both).

DISCUSSION

The results of this propensity-matched retrospective study indicated that plasma thiol levels significantly decrease and disulphide levels significantly increase in patients with l-LVEF suggesting a shift to oxidative status compared with patients with n-LVEF. Furthermore, LVEF significantly but moderately correlates with plasma thiol and weakly correlates with disulphide levels. To the best of our knowledge, this is the first study in the literature demonstrating an association between LVEF, and thiol and disulphide levels.

Oxidative stress occurs secondary to excess production of ROS relative to detoxification. ROS directly deteriorate contractile functions of cardiomyocytes through modification of proteins associated with excitation-contraction coupling. They also play role in cardiac remodeling by activating hypertrophic signaling, apoptotic pathways, and extracellular matrix remodeling. These cellular and molecular events occurring in myocardial tissue are directly involved in the initiation and progression of HF and make oxidative stress one of the key mechanisms in HF. Neuroendocrine system activation, which is the

Table 3. Multivariate logistic regression analyses for left ventricular ejection fraction

Model 1	OR	95% CI	*p*
Native thiol <384 (μmol/L)	4.63	2.61-8.22	<0.001
History of CAD	4.55	2.43-8.39	<0.001
Nagelkerke R square=0.260; -2 log likelihood=293.4; p<0.001			
Model 2			
Total thiol < 417 (μmol/L)	4.14	2.35–7.30	<0.001
History of CAD	4.48	2.44–8.19	<0.001
Nagelkerke R square=0.243; -2 log likelihood=297.5; p<0.001			

CAD: coronary artery disease; CI: confidence interval; OR: odds ratio

major mechanism that underlies the pathogenesis of HF, also contributes to oxidative stress in the myocardium.[8,20,21] Therefore, there has been a growing interest in the relationship between oxidative stress parameters and HF in recent decades. In this context, several biomarkers have been identified for diagnostic purposes, risk stratification, prognosis, and targeted therapy such as myeloperoxidase, biopyrrin, isoprostane, malondialdehyde, oxidized LDL, uric acid, α1-antitrypsin, and lectin-like oxidized LDL receptor-1.[22,23]

Thiols are antioxidant molecules that prevent detrimental effects of ROS in cells. Interaction of thiols and ROS causes oxidation of sulfhydryl groups of thiols forming reversible disulphide bonds, which is the first sign of radical-mediated protein oxidation.[9,24] Therefore, plasma and tissue levels of thiol groups may decrease during this interaction.[25] However, these disulphide bonds may again be reduced to form thiols by the cellular effects of various antioxidants. Thiol-disulphide homeostasis, which is the definition of these dynamic biochemical processes, could be determined in a unidirectional manner in the past through time consuming, expensive, and labor-intensive techniques. However, the novel method developed by Erel and Neselioglu[9] has led the researchers to evaluate both molecules individually and/or as a whole by an easy, inexpensive, and practical spectrophotometric assay. Therefore, plasma thiol and disulphide levels were measured using this novel method in this study.

A previous study demonstrated decreased serum thiol levels in patients with non-ST segment elevated ACS. Furthermore, native thiol levels independently predicted major adverse cardiac event within a six-month period.[12] An impaired thiol-disulphide balance suggesting a shift towards oxidative state was also shown in patients with pulmonary embolism.[14] A study performed by our group demonstrated significantly lower plasma thiol levels in patients with ascending aorta dilatation compared with that of the control group with normal ascending aorta diameter. Moreover, other oxidative stress parameters such as disulphide and ischemia-modified albumin levels and ferroxidase activity were found to be similar between the groups.[16] Similar to studies mentioned above, plasma thiol levels were significantly lower and disulphide levels were significantly higher in the l-LVEF group than in n-LVEF group in this study. LVEF also significantly correlated with plasma thiol and disulphide levels suggesting a negative correlation between LVEF and oxidative stress in the body.

MI, coronary ischemia, hypertension, valvular disease, and AF are the most common causes of HF.[26] It is known from previous studies that some of these risk factors are also associated with impaired thiol-disulphide balance.[12,27,28] In our study, the distribution of patients' basal characteristics were significantly different in terms of age, sex, DM, hypertension, and CAD. Moreover, beta blocker and diuretic usage, creatinine, LDL, and triglyceride levels were significantly different between the groups. Therefore, we chose to perform 1:1 propensity score matching analysis to adjust patient groups and minimize the possible effect of these variables on thiol and disulphide levels. Although the percentage of patients with CAD was higher in the l-LVEF group, there was no difference between the patient groups with ischemic and non-ischemic etiology in terms of plasma thiol and disulphide levels (unpublished data). Therefore, we chose not to include CAD into the propensity score matching as a covariate to perform analyses with adequate number of patients and allow to evaluate a true association between LVEF and thiol and disulphide levels. Thus, we performed multivariate regression analyses in two separate models to evaluate whether decreased native and total thiol levels are associated with l-LVEF even in the presence of CAD and found that both native and total thiol are significantly and independently associated with l-LVEF. Rajic et al.[17] have investigated whether oxidative stress markers including total thiol but not disulphide predict LVSD after acute MI treated with primary percutaneous coronary intervention. They found that thiol groups independently predicted LVSD defined as LVEF ≤40%. Moreover, thiol groups significantly correlated with LVEF. Similarly, Belch et al.[29] demonstrated significantly lower plasma thiol levels in patients with congestive HF compared with the control group and found a significant correlation between LVEF and plasma thiols in a study performed in the previous decades. However, there was not a defined cut-off level for LVEF in this study owing to lack of well-defined criteria and our limited understanding about the course of the disease and imaging modalities in those times.[29] In our study, we classified the patients according to a

cut-off level of LVEF 50%. In addition, we measured not only plasma thiol levels but also disulphide levels through a novel spectrophotometric method in a well-defined study population.

Limitations

Our study had several limitations relevant to the nature of retrospective studies. Patients were recruited from a single center, and association between oxidative stress and LVEF was evaluated in an observational fashion. Although we performed propensity score-matching analysis to exclude the possibility of bias, there could have been bias because of unmeasured baseline characteristics such as diastolic HF, natriuretic peptide levels, and inflammation related blood parameters. Current guidelines recommend stratification of patients with HF with a LVEF <50% in two groups, namely HF with mid-range LVEF (40%-49%) and reduced LVEF (< 40%).[3] However, we did not stratify patients according to this definition. In addition, we did not categorize the patients according to their clinically determined HF status. Furthermore, it would have been better if we had performed detailed measurements such as longitudinal strain, tissue Doppler echocardiography, or 3D echocardiography. However, we believe that these limitations should be the subject of well-designed future prospective studies.

Conclusion

Plasma thiol levels decrease and disulphide levels increase suggesting the presence of oxidative stress in patients with l-LVEF determined by TTE examination. Significant correlation between oxidative stress and LVEF sheds light about possible pathogenetic role of thiol and disulphide in HF.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethics Committee of Ankara City Hospital (Approval Date: June 25, 2020; Approval Number: E1-852/2020).

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