



# Association of coronary artery disease severity and disulphide/native thiol ratio

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## ABSTRACT

**Objective:** Oxidative stress is among the major components of cardiovascular disease pathogenesis. Thiols play a significant role in prevention of oxidative stress in the cell. The purpose of this study is to investigate the relationship between the severity of coronary artery disease and disulphide/native thiol ratio, also determine if this ratio can be used as a marker of oxidative stress in this population.

**Methods:** A total number of 107 patients with angiographically established coronary artery disease and 26 control subjects with normal coronary arteries were enrolled. The mean Gensini score of patients were calculated (mean=30) and a score of 29 or below was considered as mild and a score of 30 or higher coronary artery disease as severe. Serum total, native thiol was measured and the disulphide and disulphide/native thiol ratio were calculated as described by Erel&Neselioglu.

**Results:** Patients with mild and severe coronary artery disease had significantly lower native thiol levels and higher disulphide/native thiol ratio levels when compared to the control subjects. Also severe disease's disulphide/native thiol ratio were higher than mild.

**Conclusion:** The increased disulphide/native thiol ratio related with the severity of coronary artery disease, may reflect the augmented oxidative stress in coronary artery disease.

**Keywords:** coronary artery disease, disulphide/native thiol ratio, oxidative stress

## INTRODUCTION

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in the world with increasing incidence in recent years. The half of the mortality from cardiovascular diseases mainly arises from coronary artery disease (CAD) (1, 2).

The pathogenesis of CAD includes endothelial dysfunction, inflammation, oxidative stress and vascular calcification which together lead to plaque formation with eventual rupture, ending up with a cardiovascular event (3, 4).

Oxidative stress is a phenomenon that occurs when cell's antioxidant capacity is overwhelmed by the accumulated reactive oxygen species (ROS) in the cell (5). Current literature clearly describes the relationship between oxidative stress and CAD, and therefore exercise and diet have been suggested to combat oxidative stress. Moreover, reduction of oxidative stress with antioxidant agents may help in prevention and/or management of CVD (6).

Thiols, the organic compounds containing sulfhydryl group (-SH) groups, play a significant role in prevention of oxidative stress in the cell. The thiol groups, which are found in the amino acid components of proteins, get oxidized by free radicals forming reversible disulphide bonds. This represents the initial step of protein oxidation which opposes the harmful effects of ROS. The disulphide bonds can be reduced to thiol groups with the aid of various antioxidants to maintain an equilibrium in both directions (7). Disulphide/native thiol Ratio (DTR) is a novel marker that is used as a measure of thiol and disulphide homeostasis. An increased DTR is shown to participate in the pathogenesis of CVD, diabetes, cancer and renal insufficiency (8).

Coronary artery disease is a chronic disease that begins years before a cardiovascular event. Thus, identification of the risk is of paramount importance for the primary prevention of CVD. Despite efforts to perform an early diagnosis and to stop the progression of CVD, currently there is no effective method. Therefore, novel reliable markers and methods are necessary for early detection of CVD in order to control the progression of disease. As a novel marker of oxidative stress, DTR is evaluated in several diseases. To the best of our knowledge, this is the first study to evaluate DTR in CAD patients.

Since DTR is a novel, noninvasive and easy method, this study purposed to reveal the relationship between DTR and CAD severity determined by Gensini score. Therefore, we aimed to explore the utility of DTR as a marker of oxidative stress in this population.

## MATERIALS AND METHODS

### Ethical Permission

The present study was approved by the local Ethics Committee of Namik Kemal University. The study complied with the Declaration of Helsinki and informed consent was obtained from all groups.

### Study Design, Eligibility Criteria, and Analyses

In this study 133 consecutive patients (82 men and 51 women) with stable angina pectoris who underwent coronary angiography for suspected CAD at Namik Kemal University Faculty of Medicine were enrolled. The exclusion criteria included the following; presence of acute coronary syndromes,

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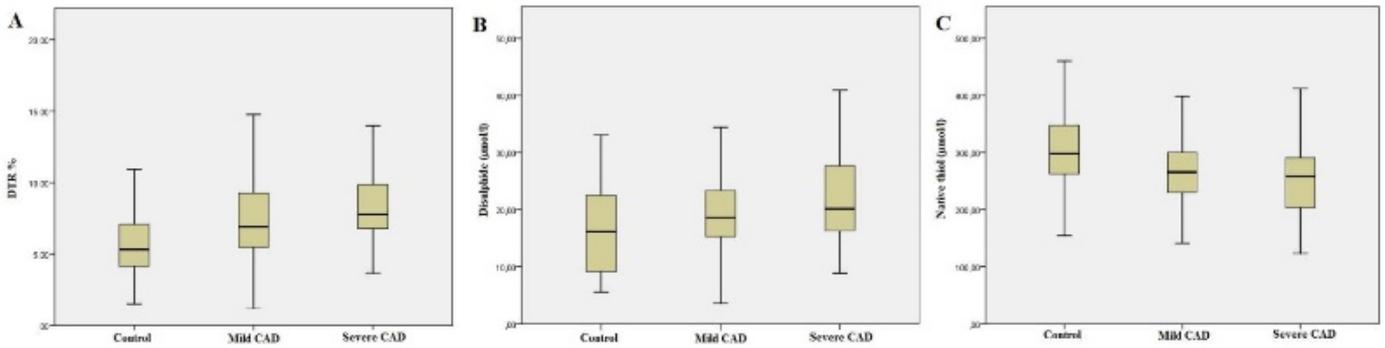


Figure 1: Inter-group comparisons of DTR, disulphide and native thiol measurements

pregnancy, hypertension, type 1 or type 2 diabetes mellitus, malignancy, systemic diseases, acute or chronic inflammation. Located in patients group and healthy controls serum fasting glucose, cholesterol measuring the levels, created the standard form was recorded. This form of arterial blood pressure, demographic data from patients and pharmacological agents used were added. Data of these patients were collected.

Three interventional cardiologists blinded to the clinical data of the patients, calculated the Gensini scores which is used as a measure of severity of the CAD. There was no inconsistency between the interventional cardiologists who evaluated the Gensini score. The subjects were categorized into three groups; patients with severe CAD (Gensini score  $\geq 30$ ), patients with mild CAD (Gensini score  $<30$ ) and control group (9).

The blood samples, which were collected into ethylene diamine tetra acetic acid (EDTA) treated tubes prior to coronary angiography, were centrifuged at 1500 g for 10 minutes and were stored at  $-800\text{ C}$ .

### Biochemical Measurements

Uric acid, creatinine, fasting blood glucose (FBG), total cholesterol, high density lipoprotein cholesterol (HDL-C), triglycerides concentrations were measured by using the commercial kits in the Cobas e6000- E501 instrument (Roche Diagnostics, Japan), and low-density lipoprotein cholesterol (LDL-C) values were calculated using the Friedewald formula ( $[\text{LDL-C}] = [\text{Total C}] - [\text{HDL-C}] - [\text{triglycerides}/5]$ ) (10).

Thiol parameters were measured by the method described earlier by Erel&Neselioglu. The analyser used was Roche, Cobas 501, Mannheim, Germany (200). Native thiol (-SH) and total thiol (-SH+ -S-S-) were measured directly. The Serum disulphide content was calculated by the formula: (Serum total thiol-Serum native thiol)/2. The ratios of disulphide/native thiol (DTR) and disulphide/total thiol were calculated (8).

### Statistical Analysis

Statistical analysis of the data was carried out by SPSS 17.0 software for Windows. The continuous variables were expressed in mean $\pm$ standard deviation or median (minimum-maximum) values, the categorical variables were expressed as percentage.

Homogeneity of the groups data were analyzed by Shapiro Wilk test and two by two comparisons were made with Student t test in normally distributed variables and with Mann Whitney U test in variables without normal distribution. The correlation

analysis was made by Pearson test.  $P$  value  $<0.05$  was considered statistically significant.

### RESULTS

A total number of 107 patients with angiographically established CAD and 26 control subjects with normal coronary arteries were enrolled in this study ( $n=133$ ).

The mean age of severe CAD patients was  $58.3\pm 12.21$  and mild CAD was  $61.3\pm 10.4$  while the mean age of control group was  $58.3\pm 12.21$  (Table 1).

Table 1: Baseline clinical and biochemical characteristics of the study population, and univariate analyses

		Control; n=26	Mild CAD; n=54	Severe CAD; n=53
Age	(Year)	58.3 $\pm$ 12.21	61.3 $\pm$ 10.4	58.3 $\pm$ 12.21
Male/Female	%	73.07 / 26.92	75.9 / 24.07	77.35 / 22.64
SBP	(mm-Hg)	117.7 $\pm$ 9.61	121.2 $\pm$ 11.81	125.5 $\pm$ 11.34
DBP	(mm-Hg)	83.0 $\pm$ 3.45	82.6 $\pm$ 6.80	81.7 $\pm$ 5.60
FBG	(mg/dL)	93.6 $\pm$ 8.7	95.3 $\pm$ 7.2	97.7 $\pm$ 8.1
Creatinine	(mg/dL)	0.77 $\pm$ 0.24	0.87 $\pm$ 0.24	0.95 $\pm$ 0.25
TC	(mg/dL)	172 $\pm$ 26.9	186 $\pm$ 29.4	206 $\pm$ 21.34
TG $\Omega$	(mg/dL)	144 (53 -306)	141 (45 -359)	179 (79 - 458)
HDL	(mg/dL)	47.5 $\pm$ 11.1	45.5 $\pm$ 13.3	45.0 $\pm$ 14.3
LDL	(mg/dL)	96.4 $\pm$ 26.4	113.2 $\pm$ 22.9	124.4 $\pm$ 24.15
Uric acid	(mg/L)	4.43 $\pm$ 1.41	5.12 $\pm$ 1.52	8.9 $\pm$ 1.51

Unless otherwise noted, data are presented as mean  $\pm$  standard deviation,  $\Omega$  median (min-max); SBP: systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; TC: Total cholesterol; TG: Triglycerides; HDL: High density lipoprotein; LDL: Low density lipoprotein, CAD: Coronary artery disease

The mean Gensini score in CAD patients was 30. When the patients with mild CAD was compared to control subjects, the level of native thiol was significantly lower ( $p=0,034$ ) and moreover, the level of disulphide to thiol ratio (DTR) was significantly higher ( $p=0,021$ ). Similarly, when patients with severe CAD was compared to control subjects, the native thiol levels were significantly lower ( $p=0,014$ ), DTR and disulphide levels were significantly higher ( $p=0,001$  and  $p=0,031$  respectively) (Figure 1).

When the patients with severe CAD were compared to patients with mild CAD, DTR level was significantly higher ( $p=0,038$ ). Although, the native thiol levels were also lower in patients with severe CAD when compared to mild CAD, it was not statistically significant ( $p=0,091$ ) (Table 2).

There was a negative correlation between the native and total thiol levels and uric acid levels in all groups. ( $p=0.014$ ,  $r=-0.255$  and  $p=0.011$ ,  $r=-0.263$  respectively).

Table 2: Laboratory findings of the study patients

	Control; n=26		$p^*$	Mild CAD; n=54		$p\Omega$	Severe CAD; n=53		$p\text{B}$
Native thiol ( $\mu\text{mol/l}$ )	296,70	154,70-459,90	0,034	268,18	141,00-398,50	0,014	250,85	123,50-412,00	0,091
Total thiol ( $\mu\text{mol/l}$ )	331,10	170,70-508,40	0,072	304,21	201,80-416,70	0,060	295,16	141,20-468,20	0,204
Disulphide ( $\mu\text{mol/l}$ )	17,20	5,55-33,05	0,146	19,43	3,65-34,40	0,031	21,90	8,85-40,90	0,114
DTR (%)	5,72	1,49-10,93	0,021	7,46	1,16-14,76	0,001	8,62	3,68-15,05	0,038
Disulphide/total thiol (%)	4,95	1,44-8,44	0,021	6,31	1,16-11,40	0,001	7,38	3,42-13,79	0,031
Native thiol/total thiol (%)	89,32	71,41-97,11	0,021	86,24	58,36-99,68	0,001	84,57	58,37-93,15	0,065

All results expressed as median, minimum-maximum.

\*Comparison of control and mild coronary artery

$\Omega$  Comparison of control and severe coronary artery disease

B Comparison of mild and severe coronary artery disease

## DISCUSSION

Reactive oxygen species have deleterious effects on cardiovascular system. They have destructive influence on cell membrane by causing protein and lipid peroxidation. The balance between antioxidant mechanisms and oxidative stress should be ensured to avoid tissue damage. In cardiovascular physiology, intracellular antioxidants like superoxide dismutase (SOD), catalyze, glutathione peroxidase and glutathione reductase or lipolytic and proteolytic enzymes like protease and phospholipase provide protection. Studies in cell culture proved that every insult causing an increase in ROS, results in a decrease in high energy phosphates, reduction in contractile reserve and functional anomalies (11, 12).

Atherosclerotic risk factors like hyperlipidemia, diabetes, hypertension, smoking and aging increase ROS levels in endothelium, vascular smooth muscle and adventitial layers of vasculature. ROS are shown to initiate and aggravate the process of atherogenesis (13). If the antioxidant mechanisms fail, the secretion of proatherogenic and proinflammatory cytokines, oxidation of LDL, proliferation of vascular smooth muscle and endothelial dysfunction are followed by rapid progression of atherosclerotic plaques to overt CAD. A decreased activity of antioxidant enzymes like SOD in human coronary arteries, prove the close relationship between atherosclerosis and oxidative stress. (12-15).

Cellular thiol redox state and reactive oxygen species (ROS) are major mediators of various processes of cell like, apoptosis, growth, differentiation. Enormous oxidative stress or ROS production is related to CVD. The heart is equipped with strong antioxidant systems in the prevention of ROS-induced disorders. Thioredoxin-fold family's proteins are major players of defense against oxidative stress. Thioredoxin and glutaredoxin activities are very important for the progression and severity of several CVD. These proteins are antioxidants, also they regulate apoptotic signaling molecules like apoptosis signal-regulating kinase 1 and Ras or transcription factors like NF- $\kappa$ B (16).

Recent studies have showed that the thiol groups decrease the oxidative damage. Ghibu et al have reported that the alpha lipoic acid containing diet may have a role in primary prevention of cardiovascular diseases (17). In another study, in which CAD patients undergoing coronary bypass grafting, N-acetylcysteine was administered in their pulmonary artery and the value of oxidative stress on reperfusion was decreased also improvement in recovery of cardiac function was proven (18).

Ates et al reported that DTR were higher in masked hypertension patients than in the control group and a positive correlation of diastolic blood pressure and systolic blood

pressure was observed with DTR (19). Another study conducted in patients with left ventricular diastolic dysfunction, showed that plasma thiol is an independent predictor for the presence of left ventricular diastolic dysfunction. This shows that thiol groups also play a role in the pathogenesis of diastolic function. Increased thiol levels may provide protection against the evolution to diastolic dysfunction (20).

In a study of 300 patients with acute myocardial infarction, Kundi et al reported that ROS has inhibitory effects on antioxidant mechanisms like CAT, SOD and GSH-Px enzyme systems and thiol group containing biological agents like GSH, Cys, homocysteine NAC and glutamin. They also found lower levels of thiol and disulphide levels and higher DTR levels in patients when compared to control subjects (21).

In another research, Kundi et al was to evaluate the association of native thiol/disulfide ratio (TDR) and coronary atherosclerosis severity as assessed by the Syntax score, in non-ST elevation myocardial infarction patients. TDR was significantly decreased in patients with high Syntax scores (22).

In our study, we found that the native thiol levels were significantly lower while the disulphide and DTR levels were significantly higher in both severe and mild CAD patients when compared to control subjects. This reflects the increased oxidative stress in CAD patients and is in concordance with previous studies addressing oxidative stress in CAD. We have also shown that, with increasing severity of CAD, the DTR increases. We believe that not native/total thiol or disulphide but DTR is an accurate measure of oxidative stress in CAD.

The medical treatment of oxidative stress may slow the progression of cardiovascular diseases down. The oxidative markers like SOD, MDA, GSH-Px and CAT cannot be used in routine practice due to lack of standardization. The method we used in this study is a novel standardized and practical way of determining the oxidative stress in clinical practice. This useful, standardized and a novel method may help in the management of CVD.

## LIMITATIONS

There are some limitations in the present study. First of all this was a cross sectional study and sample size is limited. Second, coronary angiography could detect only major lesions in the coronary arteria because it is a visually method. Prospective designed with larger sample size studies are needed to verify our findings.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

1. WHO, The World Health Report 2002. Reducing Risks, Promoting Healthy Life. Geneva: World Health Organization; 2002.
2. Murray CJ, Lopez AD. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet*. 1997;349(9063):1436-42.
3. Wu J, Xia S, Kalionis B, Sun T. The Role of Oxidative Stress and Inflammation in Cardiovascular Aging. *Biomed Res Int*. 2014;(2014):615312.
4. Heusch G, Libby P, Gersh B, et al. Cardiovascular remodeling in coronary artery disease and heart failure. *Lancet*. 2014;383(9932):1933-43.
5. Silva JP, Gomes, AC, Coutinho OP. Oxidative DNA damage protection and repair by polyphenolic compounds in PC12 cells. *Eur J Pharmacol*. 2008; 601(1): 50-60.
6. Lee S, Park Y, Zuidema M.Y, Hannink M, Zhang C. Effects of interventions on oxidative stress and inflammation of cardiovascular diseases. *World J Cardiol*. 2011;3(1):18-24.
7. Dean RT, Fu S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. *Biochem J*. 1997;324(1):1-18.
8. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem*. 2014;47(18):326 -32.
9. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol*. 1983;51(3):606.
10. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6):499-502.

11. Singal PK, Khaper N, Palace V, et al. The role of oxidative stress in the genesis of heart disease. *CardioVasc Res.* 1998;40(3):426-32.
12. Hazini A, Cemek M, Işıldak İ, et al. Investigation of ischemia modified albumin, oxidant and antioxidant markers in acute myocardial infarction. *Postepy Kardiol Interwencyjne J.* 2015;11(4):298-303.
13. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. *Am J Cardiol.* 2003;91(3):7-11.
14. Luft FC. Somatic DNA oxidative damage and coronary disease. *J Mol Med.* 2005;83(4):241-3.
15. Heinecke JW. Oxidativestress: new approaches to diagnosis and prognosis in atherosclerosis. *Am J Cardiol.* 2003;9(3):12-6.
16. Berndt C, Lillig C. H, Holmgren A. Thiol-based mechanisms of the thioredoxin and glutaredoxin systems: implications for diseases in the cardiovascular system. *Am J Physiol Heart Circ Physiol.* 2007;292(3):1227-36.
17. Ghibu S, Richard C, Vergely C, Zeller M, Cottin Y, Rochette L. Antioxidant Properties of an Endogenous Thiol: Alpha-lipoic Acid, Useful in the Prevention of Cardiovascular Diseases *Journal of Cardiovascular Pharmacology.* 2009;54(5):391-8.
18. Ferrari R, Ceconi C, Curello S, et al. Oxygen free radicals and myocardial damage: protective role of thiol-containing agents. *Am J Med.* 1991;91(3):95-105.
19. Ateş I, Ozkayar N, Altay M, et al. Is disulphide/thiol ratio related to blood pressure in masked hypertension? *Clin Exp Hypertens.* 2016;2(38):150-4.
20. Erkus ME, Altıparmak IH, Akyuz AR, et al. The association between plasma thiol levels and left ventricular diastolic dysfunction in patient with hypertension. *Scand J Clin Lab Invest.* 2015;75(8):667-73.
21. Kundi H, Ates I, Kiziltunc E, et al. A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. *Am J Emerg Med.* 2015;33(11):1567-71.
22. Kundi H, Erel O, Balun A, et al. Association of thiol/disulfide ratio with syntax score in patients with NSTEMI. *Scand Cardiovasc J.* 2015;49(2):95-100.



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