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Original Contribution

A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis☆,☆☆,★

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ABSTRACT

Background: The aim of this study was to investigate a novel oxidative stress marker (thiol/disulphide homeostasis) in patients with acute myocardial infarction (AMI) and compare the results with healthy controls for the first time in literature.

Methods: A total of 450 participants including 300 patients with AMI and 150 healthy individuals were included in the study. Left ventricular ejection fraction, body mass index, peak troponin I levels, triglyceride, total cholesterol, low-density lipoprotein, high-density lipoprotein (HDL), native thiol, total thiol, and disulphide as well as disulphide/native thiol and disulphide/total thiol ratios were compared between the groups.

Results: There were significant differences between AMI patients and the controls for left ventricular ejection fraction and troponin, HDL, native thiol, total thiol, and disulphide levels as well as disulphide/native thiol and disulphide/total thiol ratios ($P < .05$). Stepwise logistic regression model indicated that HDL (odds ratio [OR] = 0.923, $P < .001$) and disulphide levels (OR = 0.548, $P < .001$) and disulphide/total thiol ratio (OR = 0.356, $P < .001$) were significantly and independently related to AMI. The cutoff value of disulphide/total thiol ratio percentage on admission to predict AMI in all population was 4.3, with a sensitivity of 70% and a specificity of 69%.

Conclusion: Thiol/disulphide homeostasis may be used as a novel oxidative stress marker in patients with AMI because it is readily available, easily calculated, and relatively cheap. Further studies are needed to confirm the pathophysiologic role of thiol/disulphide homeostasis in AMI.

1. Introduction

Oxidative stress is the major mechanism in development and progression of atherosclerosis [1]. It has been accepted that oxidative stress occurs due to an imbalance between antioxidants and reactive oxygen species (ROS), promotes coronary artery disease (CAD), and increases plaque vulnerability [2]. Previous studies showed that oxidative stress markers increased after MI [3,4], and a strong correlation was demonstrated between oxidative stress and CAD [5-7].

Thiols are a class of organic compounds that contain a sulfhydryl group (−SH), which is composed of a hydrogen and a sulfur atom attached to a carbon atom [8]. Plasma thiol pool is largely formed by albumin and protein thiols and, to a lesser extent, by low-molecular-weight thiols such as cysteinylglycine, cysteine (Cys), homocysteine, glutathione, and γ-glutamylcysteine [9]. Thiols can undergo oxidation reaction via oxidants and form disulphide bonds [10]. Oxidation of Cys residues can lead to reversible formation of mixed disulphides between low-molecular-mass thiols and protein thiol groups when oxidative stress increases. Those disulphide bonds can be reduced back to thiol groups; therefore, thiol-disulphide homeostasis is maintained [11].

It has been reported that thiol oxidation offers an alternative mechanism by which oxidative stress could contribute to disease with little or no dependence upon free radicals [12]. It was previously shown that lipid peroxidation increased after thrombolysis in patients with MI [3], and the pathogenesis of cardiovascular diseases involved an abnormal thiol-disulphide homeostasis [12]. Furthermore, we investigated the correlation between thiol disulphide ratio with syntax score in non–ST-elevation MI (NSTEMI) patients [13]. To the best of our knowledge, no studies up to date investigated thiol/disulphide homeostasis as a novel oxidative stress marker in patients with acute myocardial infarction (AMI) and compared the results with healthy controls.

The aim of this study was to investigate a novel, easily calculated, readily available, and relatively cheap oxidative stress marker, thiol/disulphide homeostasis, in patients with AMI and compare the results with healthy controls.

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2. Material and methods

A total of 450 participants were enrolled in the study including 300 patients with AMI (180 males and 120 females) as the study group and 150 healthy subjects (82 males and 68 females) as the controls.

The patients with AMI were divided into 2 groups as the ones diagnosed with STEMI and the ones diagnosed with NSTEMI. ST-elevation MI was diagnosed when patients had symptoms of AMI lasting 30 minutes accompanied by greater than 1 mm (0.1 mV) ST-segment elevation in 2 consecutive leads and later confirmed by increase in troponin I. On the other hand, diagnosis of NSTEMI was based on increased troponin levels and presence of a characteristic chest pain that lasted for 20 minutes. The controls were the healthy individuals selected randomly among the people who admitted to hospital for checkup and did not have any known systemic diseases and did not use any medications. Patients with active infectious or inflammatory diseases, hematologic disorders, severe renal or liver diseases, previous stroke, rheumatologic diseases, or malignancy were excluded from the study.

At the time of diagnosis and before coronary angiography, all patients were given 300-mg acetyl salicylic acid PO. The patients were administered 600-mg clopidogrel orally if they had STEMI. Non-STEMI patients were administered 300-mg clopidogrel orally if they were age 75 years or younger and 75-mg clopidogrel orally if they were age older than 75 years. Coronary angiography was performed in all AMI patients with standard Judkins technique through the femoral artery, using 6F catheters (Expo; Boston Scientific Corporation, Boston, MA) and Siemens Axiom Sensis XP device.

Transthoracic echocardiography was performed in all participants. In AMI group, it was performed within 48 hours of hospital admission. Left ventricular ejection fraction (LVEF) was calculated using Simpson method.

The blood samples of AMI patients were obtained in coronary intensive care unit just before the coronary angiography, and the blood samples of the controls were obtained in the morning, after a fasting period of 12 hours. Blood samples collected from the patients and controls were put into plain tubes. Serum was separated after centrifugation at 1500*g* for 10 minutes and stored at −80°C until analysis.

Thiol/disulphide homeostasis was determined as described previously [14]. Briefly, reducible disulphide bonds were first reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native ones were detected after reaction with 5, 5′-dithiobis-(2-nitrobenzoic) acid. Half of the difference between total and native thiols provided the dynamic disulphide amount (−S-S). After the determination of native thiol (−SH) and disulphide (−S-S) amount, native thiol/disulphide ratio (−S-S−/−SH) was calculated.

Statistical Package for Social Sciences for Windows version 22 (IBM SPSS Inc, Chicago, IL) was used for statistical analysis of data. Kolmogorov-Smirnov test was used to analyze the distribution pattern of the variables. Normally distributed numerical variables were presented as mean ± SD, and the ones not normally distributed were presented as median (minimum − maximum). Categorical variables were presented as number and percent. Intergroup comparisons of normally distributed numerical variables were done with Student *t* and ANOVA tests. Mann-Whitney *U* and Kruskall-Wallis H tests were used for intergroup comparisons of nonnormally distributed numerical variables. Categorical variables were compared with χ^2 and Fisher exact χ^2 tests. The relations among the numerical variables were analyzed with Pearson and Spearman correlation analysis. Stepwise multivariable logistic regression analysis was used to determine independent significant risk factors. The receiver operating characteristics (ROC) curve was used to show the sensitivity and specificity of disulphide/total thiol ratio, optimal cutoff value for predicting AMI.

Ankara Numune Education and Research Hospital's Local Ethics Committee approved the study protocol, and all participants provided their written informed consents.

3. Results

The demographic characteristics of the patients and the controls are shown in Table 1. The sex ($P = .467$), smoking counts ($P = .205$), mean body mass index (BMI) ($P = .436$), and mean age ($P = .335$) of the patients and the controls were not different significantly. Among AMI patients, 50% had STEMI, and 50% had NSTEMI. Left anterior descending artery was occluded in 60%, circumflex artery was occluded in 23%, and right coronary artery was occluded in 17% of the patients AMI. Left ventricular ejection fraction was significantly smaller in patients with AMI (58% ± 7% vs 47% ± 11%, $P < .001$) (Table 1).

The mean high-density lipoprotein (HDL) level was lower in AMI patients when compared with the controls (51 ± 12 vs 40 ± 12 mg/dL, $P = .001$). There were no differences for the levels of other serum lipids between the study and the control groups ($P > .05$). Native thiol (345 ± 45 vs 241 ± 69 μmol/L, $P < .001$), total thiol (376 ± 48 vs 269 ± 72 μmol/L, $P < .001$), and disulphide (15 ± 4 vs 14 ± 6 μmol/L, $P = .035$) levels were lower in AMI patients when compared with the controls (Figs. 1 and 2). Mean disulphide/native thiol (4% ± 1% vs 7% ± 4%, $P < .001$) and mean disulphide/total thiol ratios (4% ± 1% vs 7% ± 2%, $P < .001$) (Fig. 3) and median peak troponin I level (0.006 vs 27 mg/dL, $P < .001$) were higher in patients with AMI (Table 1).

Furthermore, we correlated that native thiol and total thiol levels decreased with increasing age ($r = -0.619$, $P < .001$) and BMI ($r = -0.387$, $P < .001$). On the other hand, native thiol and total thiol levels increased as HDL level ($r = 0.215$, $P = .006$) and LVEF ($r = 0.303$, $P = .014$) increased. Disulphide level, disulphide/native thiol ratio, and disulphide/total thiol ratio decreased with increasing age ($r = -0.362$, $P = .038$). Disulphide level, disulphide/native thiol

Table 1
Demographic and clinical characteristics of the AMI patients and the control group

Variables	Control (n = 150)	AMI (n = 300)	*P*
Sex			
Male, n (%)	82 (54.6)	180 (60)	.467
Female, n (%)	68 (45.4)	120 (40)	.556
Age, mean ± SD	53 ± 9	52 ± 13	.335
BMI, mean ± SD	27 ± 4	28 ± 5	.436
Smoking, n (%)	62 (41.3)	134 (44.6)	.205
Type of AMI			
STEMI, n (%)	–	150 (50)	–
NSTEMI, n (%)	–	150 (50)	
Culprit lesion			
LAD, n (%)	–	180 (60)	–
CX, n (%)	–	68 (23)	
RCA, n (%)	–	52 (17)	
LVEF, %, mean ± SD	58 ± 7	47 ±11	<.001*
Peak troponin I, mg/dL	0.006 (0.003-0.009)	29 (0.2-64.8)	<.001*
Median (IQR)			
TG, mg/dL	118 (17-600)	127 (25-637)	.583
Median (IQR)			
Total cholesterol mg/dL	211 ± 53	196 ± 40	.803
Mean ± SD			
LDL, mg/dL, mean ± SD	131 ± 44	124 ± 41	.255
HDL, mg/dL, mean ± SD	51 ± 12	40 ± 12	.001*
Native thiol, μmol/L, mean ± SD	345 ± 45	241 ± 68	<.001*
Total thiol, μmol/L, mean ± SD	376 ± 48	269 ± 72	<.001*
Disulphide, μmol/L, mean ± SD	16 ± 4	14 ± 6	.035*
Disulphide/native thiol %, mean ± SD	4 ± 1	7 ± 4	<.001*
Disulphide/total thiol %, mean ± SD	4 ± 1	7 ± 2	<.001*

Abbreviations: LAD, left anterior descending artery; CX, circumflex artery; RCA, right coronary artery; IQR, interquartile range; TG, triglyceride; LDL, low-density lipoprotein.
* $P < .05$ is considered significant for statistical analyses.