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A Novel Oxidative Stress Marker In Acute Myocardial Infarction; Thiol/Disulphide Homeostasis

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Title Page

Original Article

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Running Head: A Novel Marker in AMI patients

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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, LDL, 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 LVEF, and troponin, HDL, native thiol, total thiol, and disulphide levels, as well as disulphide/native thiol and disulphide/total thiol ratios (p<0.05). Stepwise logistic regression model indicated that HDL (OR=0.923: p<0.001) and disulphide levels (OR=0.548: p<0.001), and disulphide/total thiol ratio (OR=0.356: p<0.001) were significantly and independently related to AMI. The cut-off 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 pathophysiological role of thiol/disulphide homeostasis in AMI.
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 myocardial infarction (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]. Also we investigated that the correlation between thiol disulphide ratio with syntax score in non-ST myocardial infarction (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 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 acute myocardial infarction (AMI), and compare the results with healthy controls.

MATERIAL AND METHODS

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

The patients with AMI were divided into two groups as the ones diagnosed with ST elevation myocardial infarction (STEMI), and the ones diagnosed with NSTEMI. STEMI was diagnosed when patients had symptoms of acute myocardial infarction lasting 30 minutes accompanied by >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 check-up, 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 (CA), all patients were given 300 mg acetyl salicylic acid po. The patients were administered 600 mg clopidogrel po if they had STEMI. In NSTEMI patients were administered 300 mg clopidogrel po if they were ≤ 75 years of age, 75 mg clopidogrel po if they were > 75 years of age. CA was performed in all AMI patients with standard Judkins technique through the femoral artery, using 6 Fr catheters (Massachusetts, Expo, Boston Scientific Corporation) 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’s 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 DTNB [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 (SPSS) 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 ± standard deviation, 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 non-normally distributed numerical variables. Categorical variables were compared with Chi-square and Fisher’s Exact Chi-square 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 cut-off 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.

RESULTS

The demographic characteristics of the patients and the controls are shown in Table 1. The gender (p=0.467), smoking counts (p=0.205), mean body mass index (BMI) (p=0.436), and mean age (p=0.335) 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. LVEF was significantly smaller in patients with AMI (58 ± 7% vs 47 ±11%, p<0.001) (Table 1).

The mean HDL level was lower in AMI patients when compared to the controls (51 ± 12 mg/dL vs 40 ± 12 mg/dL, p=0.001). There were no differences for the levels of other serum lipids between the study and the control groups (p>0.05). Native thiol (345 ± 45 µmol/L vs 241 ± 69 µmol/L, p<0.001), total thiol (376 ± 48 µmol/L vs 269 ± 72 µmol/L, p<0.001), and disulphide (15 ± 4 µmol/L vs 14 ± 6 µmol/L, p=0.035) levels were lower in AMI patients when compared to the controls (Figures 1 and 2). Mean disulphide/ native thiol (4 ± 1 vs 7 ± 4%, p<0.001) and mean disulphide/ total thiol ratios (4 ± 1 vs 7 ± 2%, p<0.001) (Figure 3), and median peak troponin I level (0.006 vs 27 mg/dL, p<0.001) were higher in patients with AMI (Table 1).

Also we correlated that native thiol and total thiol levels decreased with increasing age (r=-0.619, p<0.001) and BMI (r=-0.387, p<0.001). On the other hand, native thiol and total thiol levels increased as HDL level (r=0.215: p=0.006) and LVEF (r= 0.303: p=0.014) increased.
Disulphide level, disulphide/ native thiol ratio, and disulphide/ total thiol ratio decreased with increasing age (r=-0.362, p=0.038). Disulphide level, disulphide/ native thiol ratio, and disulphide/ total thiol ratio increased as peak Troponin I level increased (r= 0.313, p=0.008).

Comparison of STEMI and NSTEMI patients did not reveal any differences for thiol (237 ± 66 vs 244 ± 71 µmol/L, p=0.691), total thiol (262 ± 68 vs 272 ± 74 µmol/L, p=0.604), and disulphide (13 ± 5 vs 14 ± 6 µmol/L, p=0.373) levels, or disulphide/ native thiol (6 ± 4 ve 7 ± 4%, p=0.706) and disulphide/ total thiol rates (5 ± 3 vs 6 ± 3: p=0.712%). There were no correlations among the oxidative stress levels and ratios, and the culprit lesions.

Stepwise logistic regression model showed that HDL level (OR=0.923, p<0.001), disulphide level (OR=0.548, p<0.001) and disulphide / total thiol ratio (OR=0.350: p<0.001) were significantly and independently associated with AMI (Table 2).

Lastly, ROC analysis was performed to determine the cut-off value of disulphide / total thiol ratio to predict AMI. The cut-off value of disulphide / total thiol ratio on admission to predict an AMI in all population was 4.3, with a sensitivity of 70% and a specificity of 69% (area under the curve 0.790, p < 0.001; Figure 4). The positive and negative predictive values were 53% and 82%, respectively.

**DISCUSSION**

In this study, we found significant differences between AMI and the control groups for thiol/ disulphide homeostasis, and the results of our study indicated that HDL level, disulphide level, and disulphide / total thiol rate were independently associated for AMI. To the best of our knowledge, this is the first study that investigated thiol/ disulphide homeostasis as a novel marker of oxidative stress in patients with AMI, and compared the results with healthy controls.

Injury due to ROS is prevented by enzymatic or non-enzymatic antioxidant mechanisms, namely superoxide dismutase, catalase, and glutathione S- trans enzyme systems, and
important biological thiols such as glutathione, cysteine, homocysteine, N-acetyl cysteine, and gamma glutamine. Thiol is an organic compound, and it contains an –SH group which plays a critical role in prevention of oxidative stress in cells.

–SH groups of sulfur containing amino acids (cysteine, methionine, etc.) in proteins are the primary targets of ROS. When in the same environment with ROS, –SH groups are oxidized, and form reversible disulphide bonds. This is the first sign of radical-mediated protein oxidation [15]. Loss of thiol groups is the main molecular mechanism resulting in structural and functional alterations in proteins [16]. Antioxidants, and particularly thiol groups attempting to prevent devastating effects of free radicals, may not preserve their plasma and tissue levels during those interactions [17]. However, formed disulphide bonds may again be reduced to thiol groups by the cellular reducing effects of some antioxidants, and thiol/disulphide homeostasis is maintained in this way.

Various in vitro studies showed that abnormal thiol/disulphide homeostasis resulted in proliferation or apoptosis at the cellular level [18, 19]. Thiol/disulphide homeostasis could be measured unilaterally in the past [20], however the levels of both substances may be measured separately and additively, and may be evaluated both individually and in total with the method developed by Erel and Neselioglu [14].

The relationship between oxidative stress and CAD has attracted clinical interest for a long time, and it has been shown that both excessive oxidative stress and inadequate defense can induce early onset of severe CAD [21]. Increased oxidative stress markers act synergistically with the standard risk factors of CAD [22, 23]. Oxidative stress starts as result of an impaired balance between antioxidant defense and ROS. The onset of atherosclerotic disease increases oxidative stress [5-7].

In the light of all aforementioned studies and the studies that indicated the pathophysiological role of the oxidative stress in AMI, we hypothesized that there might be an impaired thiol/disulphide homeostasis in AMI patients when compared to the controls. We found that native
thiol, total thiol, and disulphide levels were lower while mean disulphide/ native thiol and mean disulphide/ total thiol ratios were higher in patients with AMI when compared to the controls. There were significant positive correlations between peak troponin I level and disulphide level, and disulphide/ native thiol and disulphide/ total thiol ratios. In addition, there was a positive significant correlation between LVEF and thiol levels. Our findings have suggested that a low thiol level and high disulphide/ native thiol and disulphide/ total thiol ratios are higher AMI patients on admission to hospital when compared to the control group. Our results also indicated that HDL and disulphide levels, and disulphide / total thiol ratio were independent risk factors for AMI.

Our study has several limitations. First one is inclusion of relatively small number of patients who admitted to a single center. The second limitation of our study is lack of longitudinal follow up of the patients to determine whether alterations of thiol/ disulphide homeostasis could be predictive risk factors for AMI. Finally, our findings were not compared with other clinical risk factors such as GRACE, TIMI score and oxidative stress markers such as lipid hydroperoxide, total antioxidant status, total oxidant status, oxidative stress index, paraoxonase and arylesterase.

CONCLUSIONS

To the best of our knowledge, our study is the first one that investigated thiol/ disulphide homeostasis in patients with AMI, and compared the results with healthy controls. Our results indicated that thiol/ disulphide homeostasis could be a good biochemical risk marker in AMI patients at admission to hospital, because this novel test is an easily available, relatively cheap and optionally manual spectrophotometric assay. Further longitudinal studies on a larger patient population are needed to determine whether alterations in thiol/ disulphide homeostasis could be predictive risk factors for MI.
ACKNOWLEDGMENTS

None

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This research received no grant from any funding agency in the public, commercial or not-for-profit sectors.

DECLARATION OF CONFLICTING INTERESTS

The Authors declare that there is no conflict of interest.

REFERENCES


FIGURE LEGENDS

Figure 1: Native and total thiol levels showed statistically significant differences between AMI and the control groups.

Figure 2: Disulphide levels were significantly higher in the control group compared to AMI group.

Figure 3: Disulphide / native thiol and disulphide / total thiol ratios showed significant differences between the controls and patients with myocardial infarction.

Figure 4: The receiver operating characteristic (ROC) curve of disulphide / total thiol ratio for the prediction of AMI
Figure 1.
Figure 3.
Figure 4.

ROC Curve

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTR (%)</td>
<td>4.3</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>70</td>
</tr>
<tr>
<td>Specificity</td>
<td>69</td>
</tr>
<tr>
<td>AUC</td>
<td>0.790</td>
</tr>
<tr>
<td>95% CI (lower)</td>
<td>0.741</td>
</tr>
<tr>
<td>95% CI (upper)</td>
<td>0.838</td>
</tr>
</tbody>
</table>

DTR: Disulphide / Total Thiol Ratio
Table 1. Demographic and clinical characteristics of the AMI patients and the control group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=150)</th>
<th>AMI (n=300)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male =n (%)</td>
<td>82 (54.6)</td>
<td>180 (60)</td>
<td>0.467</td>
</tr>
<tr>
<td>Female =n (%)</td>
<td>68 (45.4)</td>
<td>120 (40)</td>
<td>0.556</td>
</tr>
<tr>
<td>Age, Mean ± SD</td>
<td>53 ± 9</td>
<td>52 ± 13</td>
<td>0.335</td>
</tr>
<tr>
<td>BMI, Mean ± SD</td>
<td>27 ± 4</td>
<td>28 ± 5</td>
<td>0.436</td>
</tr>
<tr>
<td>Smoking =n (%)</td>
<td>62 (41.3)</td>
<td>134 (44.6)</td>
<td>0.205</td>
</tr>
<tr>
<td>Type of AMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STEMI =n (%)</td>
<td>-</td>
<td>150 (50)</td>
<td>-</td>
</tr>
<tr>
<td>NSTEMI =n (%)</td>
<td>-</td>
<td>150 (50)</td>
<td></td>
</tr>
<tr>
<td>Culprit Lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD =n (%)</td>
<td>-</td>
<td>180 (60)</td>
<td>-</td>
</tr>
<tr>
<td>CX =n (%)</td>
<td>-</td>
<td>68 (23)</td>
<td></td>
</tr>
<tr>
<td>RCA =n (%)</td>
<td>-</td>
<td>52 (17)</td>
<td></td>
</tr>
<tr>
<td>LVEF, %, Mean ± SD</td>
<td>58 ± 7</td>
<td>47 ±11</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Peak Troponin I, mg/dL</td>
<td>0.006 (0.003-0.009)</td>
<td>29 (0.2-64.8)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Triglyceride, mg/dL,</td>
<td>118 (17-600)</td>
<td>127 (25-637)</td>
<td>0.583</td>
</tr>
<tr>
<td>Total Cholesterol mg/dL,</td>
<td>211 ± 53</td>
<td>196 ± 40</td>
<td>0.803</td>
</tr>
<tr>
<td>LDL, mg/dL, Mean ± SD</td>
<td>131 ± 44</td>
<td>124 ± 41</td>
<td>0.255</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>p-value</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>51 ± 12</td>
<td>40 ± 12</td>
<td>0.001*</td>
</tr>
<tr>
<td>Native thiol, µmol/L</td>
<td>345 ± 45</td>
<td>241 ± 68</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total thiol, µmol/L</td>
<td>376 ± 48</td>
<td>269 ± 72</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Disulphide, µmol/L</td>
<td>16 ± 4</td>
<td>14 ± 6</td>
<td>0.035*</td>
</tr>
<tr>
<td>Disulphide/Native thiol %,</td>
<td>4 ± 1</td>
<td>7 ± 4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disulphide/Total thiol %,</td>
<td>4 ± 1</td>
<td>7 ± 2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
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</tbody>
</table>


*p<0.05 is considered significant for statistical analyses.
Table 2: Significant predictors of AMI in stepwise multivariable logistic regression analysis

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>HDL</td>
<td>0.923</td>
<td>0.884</td>
<td>0.965</td>
</tr>
<tr>
<td>Disulphide</td>
<td>0.548</td>
<td>0.437</td>
<td>0.686</td>
</tr>
<tr>
<td>Disulphide / Total thiol</td>
<td>0.356</td>
<td>0.238</td>
<td>0.532</td>
</tr>
</tbody>
</table>

Nagelkerke $R^2 = 0.620$: p<0.001*

HDL: High density lipoprotein, OR: Odds ratio, CI: Confidence interval.

Age, body mass index, gender, lipid levels, native thiol, total thiol, disulphide, disulphide / native thiol and disulphide / total thiol were included in stepwise regression analysis as probable predictors.

*p<0.05 is considered significant for statistical analyses.