RESEARCH ARTICLE

Thiol disulphide homeostasis in patients with acute myocardial infarction (AMI)

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Abstract

Objective: To investigate novel oxidative stress marker thiol disulphide homeostasis in patients with acute myocardial infarction.

Methods: The case-control study was conducted at Yildirim Beyazit University, Ankara, Turkey, between October 26, 2015 and January 26, 2016. It comprised patients of ST elevation myocardial infarction, and healthy individuals. Troponin levels, native thiol, total thiol, and disulphide were compared among the groups.

Results: Of the 128 subjects, 98(76.5%) were patients and 30(23.43%) were controls. Disulphide levels were lower in the patients compared to the controls ($p<0.001$).As troponin levels increased, native thiol, total thiol and disulphide levels in patients decreased ($p<0.05$).

Conclusion: Native thiol and total thiol levels may be used as a novel oxidative stres marker in patients with acute myocardial infarction.

Keywords: Acute myocardial infarction, Thiol disulphide homeostasis, Oxidative stress.
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Introduction

The balance betweeen oxidant and anti-oxidant systems for normal cellular and tissue structures is very important.[1] The imbalance between pro-oxidants and anti-oxidants results in oxidative stress.[1-4] The cause of imbalance is the result of excessive production of reactive oxygen species (ROS). Another reason is the inefficient elimination of ROS by anti-oxidant mechanisms. In this case, various cellular and tissue structures are damaged and finally increases cardiovascular diseases.[5] Development and progression of atherosclerosis are closely related to oxidative stress. Plaque vulnerability increases and it accelerates coronary artery disease (CAD) in case of oxidative stress.[6,7] On the other hand, anti-oxidant system has a protective role against oxidative stress.[2-4] Thiols contain a sulphydryl group (-SH) which is composed of a hydrogen and sulfur atom attached to a carbon atom. These thiol molecules are an important part of anti-oxidant system in the body. In preventing oxidative stress in cells, they play a critical role.[1,4] Plasma thiol pool is composed of basically albumin and protein thiols, and on a lesser level, low-molecular-weight thiols, including cysteinylglycine, cysteine (Cys), homocysteine, glutathione and gama-glutamylcysteine.[2] The thiol-disulfide balance is maintained since oxygen molecules oxidises thiol groups of proteins and reversibly converts to disulfide bonds. Dynamic thiol-disulfide homeostasis, a recently-defined oxidative stress marker, is very important in cases including anti-oxidant defense, detoxification, apoptosis, regulation of enzyme activity, transcription and cellularsignal transduction mechanisms. The novel and automated method makes it possible to measure thiol-disulphide homeostasis levels one by one and cumulatively. This leads to optimal evaluation of thiol/disulphide homeostasis.[3-6.8,9] The current study was planned to investigate thiol-disulphide homeostasis in patients with acute myocardial infarction (AMI), and compare the results with healthy controls. Investigation of the correlation between these oxidative stress tests and troponin was also planned.

Patients and Methods

The case-control study was conducted at Yildirim Beyazit University, Yenimahalle Training and Research Hospital,

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Ankara, Turkey, between October 26, 2015 and January 26, 2016.

The sample size was calculated in the light of an earlier study.[6] By using data from that study, and applying 1:2 rule (1 healthy person to 2 patients), when accepted as power=0.90 and type I error=0.05, the required sample size was 20 controls and 40 patients.

Approval was obtained from the institutional ethics committee, written informed consent was taken from all the participants.

The patients were divided into three groups as the ones diagnosed with ST elevation myocardial infarction (STEMI), those diagnosed with non-ST elevation myocardial infarction (NSTEMI), and those with non-spesific chest pain. STEMI was diagnosed when patients had symptoms of AMI 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. Diagnosis of NSTEMI was based on increased troponin levels and presence of a characteristic chest pain that lasted for 20 minutes. The controls were healthy individuals selected randomly from among individuals 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, haematological disorders, severe renal or liver diseases, previous stroke, rheumatological diseases, or malignancy were excluded from the study.

At the time of diagnosis and before coronary angiography (CA), all patients were given 300mg acetyl salicylic acid ASA) per oral (PO). The patients were administered 600mg clopidogrel PO if they had STEMI. In NSTEMI patients, 300mg clopidogrel PO was administered if they were ≤75 years of age, 75mg clopidogrel PO if they were >75 years of age. Blood samples of AMI patients were obtained when they were admitted to the emergency department (ED) 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 the controls were put into plain tubes. Serum was separated after centrifugation at 1500g for 10 minutes, and stored at -80 C until analysis. Thiol-disulphide homeostasis was determined as described previously.[10] 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 (DTNB). 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.

Data were analysed using SPSS 11.5. Mann-Whitney U test was used for comparison between control and patient groups. Kruskall-Wallis one-way analysis of variance (ANOVA) test was used for comparisons between the control group and the 3 patient groups.

Mann-Whitney U test was used for between-groups comparisons with Bonferroni correction for multpiple comparisons in the case of determination of statistical differencess between groups. Additionally, relations of troponin levels between patient groups were analysed by using Spearman's rank correlation.

Mean ± standard deviations (SD) and median values were used for descriptive statistics.The results were accepted as statistically significant at $p<0.05$.

Results

Of the 128 subjects, 98(76.5%) were patients and 30(23.43%) were controls. Among the patients, there were 60(61.2%) males and 38(38.8%) females. Among the controls, there were 17(56.6%) males and 13(43.3%) females. Of the 98 patients, 43(43.8%) had STEMI, 35(35.7%) had NSTEMI and 20(20.4%) had non-spesific chest pain. Mean age of the patients was 59.57 ± 10.5years and that of the controls was 49.2 ± 10.6 years. Levels of native thiol, total thiol and disulphide were lower in the patientscompared to the controls ($p<0.001$)(Table-1).

Table-1: Native Thiol, Total Thiol and Disulphide Levels Between Control and Patient Groups.

	Control (n=30)	Patient (n=98)	P*
	Mean±SD (Median)	Mean±SD (Median)	
Native Thiol	398,64±82,43(403,40)	274,43±73,84(271,30)	**0,001**
Total Thiol	439,21±91,13(446,50)	303,22±814,31(294,95)	**0,001**
Disulphide	20,28±6,30(22,23)	12,38±6,96(13,70)	**0,001**

The difference was also significant between the control group and each of the three patient sub-groups (Table-2; Figure).

Native thiol, total thiol and disulphide levels in the control group were higher than STEMI and NSTEMI sub-groups ($p<0.05$), but there was no significant differencess in the non-specific pain group (Table-3).

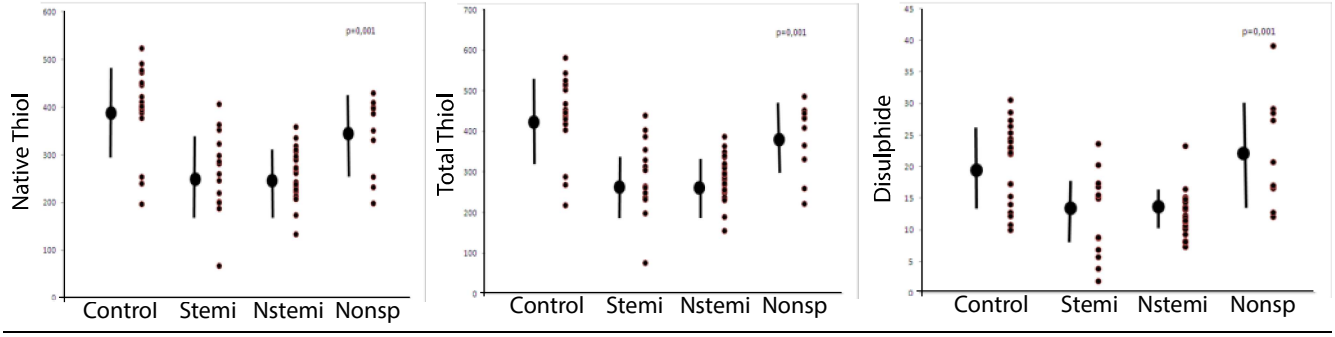

Figure: Native Thiol, Total Thiol and Disulphide Levels in groups.

Table-2: Native Thiol, Total Thiol and Disulphide Levels in between control and different patient groups (in total).

	Control (n=30) Mean±SD (Median)	STEMI (n=43) Mean±SD (Median)	NSTEMI (n=35) Mean±SD (Median)	Nonspecific (n=20) Mean±SD (Median)	P*
Native Thiol	398,64±82,43 (403,40)	254,10±79,75 (261,40)	258,73±51,50 (265,80)	340,09±82,75 (369,65)	0,001
Total Thiol	439,21±91,13 (446,50)	278,71±86,89 (265,40)	283,51±53,18 (282,50)	384,37±87,50 (421,10)	0,001
Disulphide	20,28±6,30 (22,23)	12,30±6,66 (15,10)	12,39±3,56 (12,10)	22,14±8,71 (19,03)	0,001

* :*Kruskall-Wallis Oneway ANOVA*
STEMI: ST-Elevation Myocardial Infarction
NSTEMI: Non-ST-elevation myocardial infarction.

Table-3: Results of Comparison Pairwise group for Native Thiol, Total Thiol and Disulphide*.

	Group	Stemi	Nstemi	Nonsp
Native Thiol	Control	**0,001**	**0,001**	0,048
	Stemi		0,914	0,030
	Nstemi			0,013
Total Thiol	Control	**0,001**	**0,001**	0,091
	Stemi		0,988	0,011
	Nstemi			**0,003**
Disulphide	Control	**0,004**	**0,001**	0,681
	Stemi		0,747	0,011
	Nstemi			**0,001**

* : Mann-Whitney U test with Bonferroni Correction (?* = 0,0083)
STEMI: ST-Elevation Myocardial Infarction
NSTEMI: Non-ST-elevation myocardial infarction

Table-4: Relations of troponin levels between patient groups*.

	Troponin I	
	r	p
Native Thiol	-0,424	0,003
Total Thiol	-0,453	0,002
Disulphide	-0,480	0,001

*: Spearman Rank Correlation Analysis.

If As troponin levels increased, native thiol, total thiol and disulphide levels in the patient group decreased ($p<0.05$) (Table-4).

Discussion

The prospective clinical study found that levels of native thiol and total thiol were lower in the patient group with respect to the control group. Disulphide levels were lower in the patient group compared to the control group. There were significant statistical differencess between the groups. These findings are very important. Thiol is an organic compound, and it contains an -SH group, which plays a critical role in prevention of oxidative stress in cells. The primary targets of ROS in proteins are-SH groups of sulfur containing amino acids (Cys, methionine, etc). When in the same environment with ROS, - SH groups are oxidised and form reversible disulphide bonds. But formed disulphide bonds may again be reduced to thiol groups by the cellular-reducing effects of some anti-oxidants, and thiol-disulphide homeostasis is kept by this mechanism. Loss of thiol groups is the basic molecular mechanism leading to structural and functional changes in proteins.[3,5] In the past, lower molecular weight thiol compounds could usually be measured. In the organism,

lower molecular weight thiols were only a small amount of the total thiol but thiols in albumin and other proteins formed large part of it. Subsequently, in previous studies it does not show accurate total thiol and disulfide amount in the body.[11,12] Dynamic thiol-disulfide homeostasis was first measured by a fully automated method developed by Erel and Neselioglu in 2014.[10] The current study determined native, total thiol and disulphide levels in MI patients by using this method which is an easy, safe, fast and inexpensive method. Various in vitro studies showed that abnormal thiol-disulphide homeostasis resulted in proliferation or apoptosis at the cellular level.[13,14] Similarly, in previous studies degenerative diseases, including chronic kidney disease, cardiovascular disease, chronic inflammation, autoimmunity, and hyperglycaemia, have been demonstrated to be associated with oxidative stress.[15,16] In different studies the relation between oxidative stress and diabetes and also risk of diabetes have been shown.[11] In a recent study, levels of native thiol and total thiol were low in pre-diabetic patients.[11] These results are in accordance with our outcomes. Since diabetes is a risk factor in CAD, great importance should be given to the rewiev of these results.

Other studies demonstrated that thiol-disulphide homeostasis declined in hypertension (HT) patients compared with the control group.[4,17] These findings support our results. HT is a risk factor for MI just as diabetes, additionally this case is meaningful. Based on this information, increased oxidative stress markers act synergistically with the standard risk factors of CAD. Furthermore, the onset of atherosclerotic disease increases oxidative stress.[3,5,7,18]

In another study , the serum native and total thiol levels were found to be significantly lower and disulphide levels were higher in preeclampsia when compared with healthy, uncomplicated pregnancies.[9] Furthermore, another recent study showed that thiol-disulfide homeostasis was found to be disturbed in trichloroethylene(TCE)exposed workers.[2] Another study determined that thiol-disulphide homeostasis in inflammatory bowel disease patients and examined the relation of disulphide-thiol balance with disease activity.[12] These studies used this new method.

Various stress factors for heart, such as pressure, volume overload and ischaemia, lead to the problems of myocardium via protein expression, changing in genes, and the oxidative effects. Oxidants interact with thiols which are a major extracellular and intracellular molecular anti-oxidant.[19] 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. Previously, many studies have shown that oxidative stress induces CAD via both excessive oxidative stress and inadequate defense mechanisms.[5-7] However, to the best of our knowledge, there are only a few studies in literature that investigated thiol-disulphide homeostasis as a novel marker of oxidative stress in patients with AMI, and compared the results with healthy controls.

Our study has several limitations. First one is inclusion of relatively small number of patients who were admitted to a single center. Another limitation was that the blood samples of the patients were taken at different fasting durations, but for healthy subjects blood samples were taken after a 12-hour fast. Time intervals between the onset of pain and admission to the ED were different in all the patients. It was not possible to standardise the cases.

Conclusion

Native thiol and total thiol levels, which are measured easily and done optionally by manual spectrophotometric assay, could be a valuable risk marker in AMI patients at admission to hospital. However, further studies are required.

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References

1. Erkus ME, AltiparmakIH, Akyuz AR, Demirbag R, Sezen Y, Gunebakmaz O, et al. The association between plasma thiol levels and left ventricular diastolic dysfunction in patient with hypertension. Scand J Clin Lab Invest. 2015; 75: 667-73.
2. Bal C, Büyüksekerci M, Koca C, Agis ER, Erdogan S, Baran P, et al. The compromise of dynamic disulfide/thiol homeostasisas a biomarker of oxidative stress in trichloroethylene exposure. Hum Exp Toxico. 2016; 35:915-20.
3. Ergin M, Cendek BD, Avsar AF, Erel O, Dynamic thiol-disulfide homeostasis in Hyperemesis gravidarum. J Perinatol. 2015; 35:788-92.
4. Atesl, Ozkayar N, Inan B, Yilmaz FM, Topcuoglu C, Neselioglu S, et al. Dynamic thiol/disulphide homeostasis in patients with newly diagnosed primary hypertension. J Am Soc Hypertens. 2016; 10:159-66.
5. Ozler S, Oztas E, Tokmak A, Ergin M, Isci E, Eren E, et al. The association of thiol/disulphide homeostasis and lipid accumulation index with cardiovascular risk factors in overweight adolescents

with polycystic ovary syndrome.ClinEndocrinol (Oxf). 2016; 84:516-23.

6. Kundi H, AtesI, Kiziltunc E, Cetin M, CicekciogluH, Neselioglu S, et al, A novel oxidative stress marker in acute myocardial infarction; thiol/ disulphide homeostasis. Am J Emerg Med. 2015; 33:1567-71.
7. Kundi H, Erel O, Balun A, Çiçekçioglu H, Cetin M, Kiziltunc E, et al. Association of Thiol / Disulphide Ratio with Syntax score in Patients with NSTEMI. Scand J Clin Lab Invest.2015; 49:95-100.
8. Yilmaz OH, Bal C, NeseliogluS,Büyüksekerci M, Gündüzöz M, Eren F, et al. Thiol/Disulphide Homeostasis in Asphalt Workers. Arch Environ Occup Health. 2016; 71:268-72.
9. Ozler S, Erel O, Oztas E, Ersoy AO, Ergin M, Sucak A, et al. Serum Thiol/Disulphide Homeostasis in Preeclampsia. Hypertens Pregnancy. 2015; 34:474-85.
10. Erel O, Neselioglu, A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014; 47:326-32.
11. AtesI, Kaplan M, Inan B, Alisik M, Erel O, Yilmaz N, et al. How does thiol/disulfide homeostasis change in prediabetic patients? Diabetes Res ClinPract. 2015; 110:166-71.
12. Yuksel M, AtesI, Kaplan M, Alis?ik M, Erel O, Saygili F, et al. The dynamic thiol/disulphide homeostasis in inflammatory bowel disease and its relation with disease activity and pathogenesis. Int J Colorectal Dis. 2016; 31:1229-31.
13. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med. 2010; 48:749-62.
14. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol. 2006; 71:551-64.
15. AtesI, Kaplan M, Yuksel M, Mese D, Alisik M, Erel O, et al. Determination of thiol/disulphide homeostasis in type 1 diabetes mellitus and the factors associated with thiol oxidation. Endocrine. 2016; 51:47-51.
16. S. Tangvarasittichai, Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes. 2015; 6: 456-80.
17. AtesI, Ozkayar N, Altay M, Yilmaz FM, Topcuoglu C, Alisik M, et al. Is disulphide/thiol ratio related to blood pressure in masked hypertension? Clin Exp Hypertens. 2016; 38:150-4.
18. Elnakish MT, Hassanain HH, Janssen PM, Angelos MG, Khan Me, Emerging role of oxidative stress in metabolic syndrome and cardiovascular diseases: important role of Rac/NADPH oxidase. J Pathol. 2013; 231: 290-300.
19. KrannerI, Birti S, Anderson KM, Pritchard HW. Glutathione half-cell reduction potential: a universal stress marker and modulator of programmed cell death? Free RadicBiol Med. 2006; 40:2155-65.