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ARAŞTIRMA / RESEARCH

Increased thiol/disulphide ratio in patients with ST elevation-acute coronary syndromes

ST elevasyonlu akut koroner sendromlu hastalarda artmış tiyol/disülfid oranı

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Abstract

Purpose: The aim of this study was to compare thiol/disulphide haemostasis levels between healthy volunteers and the patients who were admitted to emergency with the complaint of ST Elevation myocardial infarction (STEMI).

Materials and Methods: This case-control study was carried out in the Department of Emergency Medicine. The participants consisted of 48 healthy volunteers and 48 hospitalized patients with STEMI. The thiol / disulphide level was evaluated in each study group. The association of Thiol levels (native/total) was evaluated in patients with STEMI.

Results: No significant difference was found between the patients with STEMI and healthy volunteers regarding their age or gender. The disulphide (SS) levels were similar in both groups. The total thiol (TT) and native thiol (SH) levels were much lower and the SS/TT levels were much higher in the patients with STEMI when compared with the volunteers.

Conclusion: This study revealed that the oxidant/antioxidant ratio was shifted to the oxidative side in patients with STEMI. An abnormal thiol/disulphide state might be considered as an important factor in the pathogenesis and in monitoring the treatment response. The thiol resources may be used for diagnosis of STEMI.

Keywords: STEMI, Acute Coronary Syndrome, oxidative stress, thiol / disulphide, haemostasis

Öz

Amaç: Bu çalışmanın amacı, sağlıklı gönüllüler ile ST elevasyonlu miyokart enfarktüsü (STEMI) şikayeti ile acil servise kabul edilen hastalar arasında tiyol / disülfit hemostaz düzeylerini karşılaştırmaktır.

Gereç ve Yöntem: Bu vaka kontrol çalışması Acil Tıp Anabilim Dalı'nda yapılmıştır. Katılımcılar, 48 sağlıklı gönüllüden ve 48 hastanede yatan STEMI hastalarından oluşmaktadır. Her çalışma grubunda tiyol / disülfit seviyesi değerlendirildi. STEMI'li hastalarda Tiyol düzeylerinin (doğal/toplam) birlikteliği değerlendirildi.

Bulgular: STEMI'li hastalar ile sağlıklı gönüllüler arasında yaşlarına ve cinsiyetlerine göre anlamlı bir fark bulunmadı. Disülfit (SS) seviyelerinin her iki grupta da benzer olduğu saptandı. Gönüllülere kıyaslandığında STEMI hastalarında total tiyol (TT) ve doğal tiyol (SH) seviyelerinin çok daha düşük ve SS/TT seviyelerinin ise daha yüksek olduğu saptandı.

Sonuç: Bu çalışma, STEMI hastalarında oksidan/antioksidan oranının oksidatif tarafa kaydığını ortaya çıkarmıştır. Anormal bir tiyol/disülfit dengesi, patogenezde ve tedavi yanıtının izlenmesinde önemli bir faktör olarak düşünülebilir. Tiyol kaynakları, STEMI'nin teşhisi için kullanılabilir.

Anahtar Kelimeler: STEMI, Akut Koroner Sendrom, oksidatif stres, tiyol / disülfit, hemostaz

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INTRODUCTION

Oxidative stress is the main mechanism in the development and acceleration of atherosclerosis[1]. It has been accepted that oxidative stress occurs due to the imbalance of antioxidants and reactive oxygen species (ROS) and that it triggers coronary artery diseases (CAD) and increases the formation of plaques[2].

The occurrence of oxidative stress depends on the antioxidant defence mechanisms, and plasma thiols are strong antioxidants which remove the free radicals physiologically[3]. The most abundant form of thiol found in human cells is the glutathione protecting the optimal redox status for cellular functions[4,5]. Normal levels of reduced glutathione are necessary to protect cells from the harmful effects of oxidants[6,7]. Many enzymes such as catalase, superoxide dismutase and glutathione peroxidase play an important role in the defence mechanisms of antioxidant[6].

Known also as a mercaptan, a thiol is composed of a sulphur atom and a hydrogen atom connected to a carbon atom and it is a functional sulfhydryl group[8]. Dynamic thiol / disulphide homeostasis plays a crucial role in antioxidant defence, detoxification, apoptosis, enzyme regulation, transcription and in the mechanisms of cellular signal transduction[9,10]. The levels of thiol / disulphide homeostasis are measured one by one or cumulatively via a contemporary and automatic method[11]. In this way, the situation could be evaluated thoroughly.

This study aims to investigate the correlation between the clinical and laboratory parameters and the thiol / disulphide homeostasis having a vital role for STEMI patients.

MATERIALS AND METHODS

The study was conducted in the Department of Emergency Medicine of our hospital between February 2015 and March 2016. Approval for the study was obtained from the İzmir Katip Celebi University Atatürk Training and Research Hospital ,Clinical Research Ethics Committee (2016/142). Helsinki Declaration was followed as the guideline in this study.

Our study prospectively included 100 patients who were admitted to our Department of Emergency Medicine with chest pain and volunteers. Two patients with STEMI were excluded from the study due to laboratory error and two volunteers were excluded from the study because it was later discovered that they did not meet the exclusion criteria.

Following the results of clinical, electrocardiographic, laboratory, and imaging studies, 48 of these patients were diagnosed with STEMI and the remaining 48 were defined as volunteers (with no history of chronic illness or regular drug use). Individuals meeting the criteria for recruitment were included in the study.

The exclusion criteria of our study included the lack of patient consent, being >18 years old, significant valvular heart disease or valve surgery history, chronic renal failure, acute and chronic infection, autoimmune disease, recent surgery, malignancy, vitamin or antioxidant support, malnutrition, and steroid and nonsteroidal anti-inflammatory treatment. The age, the gender, the mean heart rate, and the arterial blood pressure of patients were recorded at the time of hospitalisation.

Measurement of biochemical markers

The complete blood count, troponin levels, liver and kidney function tests, and the bleeding profile of all patients were routinely studied at admission. Thiol/disulphide homeostasis was measured with a recently developed method by Erel and Neselioglu [11]. Reducible disulphide bonds were reduced in order to compose free functional thiol groups. Unused reductant sodium borohydride was used up and extracted with formaldehyde, and all thiol groups - native and reduced - were determined after their reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid. Half of the difference between native and total thiols ensured the dynamic disulphide quantity (–S-S). After the detection of the native thiol (–SH) and disulphide (–S-S) amounts, the ratio of disulphide-to-native-thiol (–S-S–/–SH) ratio was calculated [11].

Statistical analysis

The IBM-Statistical Package for the Social Sciences (IBM-SPSS, version 21.0) program was used to analyze the data. In order to measure the differences in the biochemical parameters of the patients and the control population, the Student's *t*-test was used. Pearson's correlation and Spearman's rho tests were used to examine the correlations among the variables. Univariate and multivariate logistic

regression analysis was used to detect whether there was any relationship between the degrees of STEMI and serum thiol/disulphide homeostasis parameters. The data collected were examined using 95% confidence levels, and the *p*- values<0.05 were considered as significant.

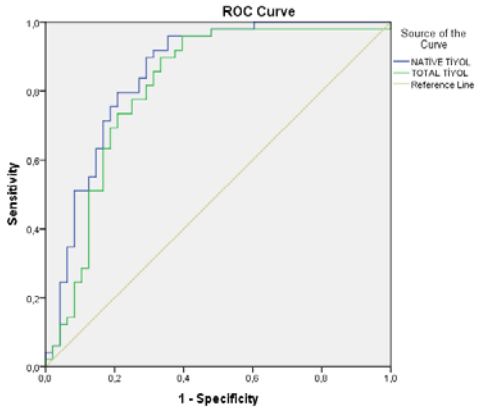

Figure 1. ROC analysis of native thiol, total thiol and disulphide

RESULTS

The demographic features of the study and control group are displayed in Table 1. Regarding their gender, no significant difference has been detected statistically between the patient and the control group (p=0.131). There was no significant relationship between STEMI and control group. (p>0.05). When the vascular structures with lesion were analysed in the patient group, the following values were detected; LAD occlusion 79.1%, RCA occlusion 47.9% and Cx occlusion 29.1%. No significant difference has been found relating to the lesion findings.

When the average systolic and diastolic blood pressure of study and control group was analysed, no significant statistical difference was found. (p>0.05) (Table 1). The levels of native thiol (284,66 ± 29,10/ L against 241,86 ± 31,56 µmol / L, p <0.001) and the levels of total thiol (329,58 ± 33,83 µmol / L against 289,05 ± 36,53 µmol / L, p <0.001) was lower in STEMI patients compared to the control group and was statically significant. (Table 1 p_{nat}<0.001 ve p_{tot}<0.001).

Table 1. The comparison of the demographic and laboratory test values between STEMI and Control group

Parameter	Control (n=48)	STEMI (n=48)	P value
Gender			
Female=n(%)	19 (39.5)	12 (25)	.318
Male=n(%)	29 (60.5)	36 (75)	
Age Average ± SD**	47.80 ± 17.03	60.38 ± 12.68	.386
Vascular Lesion***			
LAD=n(%)	-	38 (79.1)	.167
RCA=n(%)	-	23 (47.9)	.237
Cx=n(%)	-	14 (29.1)	.190
Systolik Blood Pressure	141.67 ± 23.91	136.92 ± 19.55	.284
Diastolic Blood Pressure	77.29 ± 11.41	79.71 ± 11.86	.305
Native Thiol µmol / L	284.66 ± 29.10	241.86 ± 31.56	<0.001*
Total Thiol µmol / L	329.58 ± 33.83	289.05 ± 36.53	<0.001*
Disulphide µmol / L	25.21 ± 1.49	24.87 ± 1.41	.256
% Disulphide /Native Thiol	8.91 ± 0.73	10.39 ± 0.92	<0.001*
% Disulphide /Total Thiol	7.69 ± 0.51	8.67 ± 0.64	<0.001*
%Native/Total Thiol	86.41 ± 2.81	83.66 ± 2.20	<0.001*

*p<0.05 is considered significant for statistical analyses. **: Standard deviation; ***: More than one vascular occlusion have been observed at the same time. CX: Circumflex artery LAD: Left anterior descending artery, STEMI: ST elevation myocardial infarction, RCA: Right coronary artery, SD: Standard deviation

Although the level of disulphide was higher in the control group with 25,21 ± 1,49 µmol / L than that of the STEMI patients with 24,87 ± 1,41 µmol / L, this was not statistically significant. (p = 0.256). While the average disulphide /native thiol (8,91 ± 0,73 against 10,39 ± 0,92, p <0.001) and the average

disulphide / total thiol rates (7,69 ± 0,51 against 8,67 ± 0,64, p <0.001) were found higher in STEMI patients; the average native thiol/ total thiol rates (86,41 ± 2,81 against 83,66 ± 2,20, p <0.001) were found higher in the control group than that of in STEMI patients (Table 1). Finally, in the STEMI patient group, ROC analysis of Native Thiol and Total Thiol values was used to determine both specificity, sensitivity and cut off values in determining STEMI. Native Thiol had a sensitivity of 95.9%, specificity of 74.6% (area under the curve (AUC) 0.853 and p <0.001) for 239.68, which was taken as the cut off value. In total thiol, sensitivity was 91.8%, specificity was 82.5% (area under the curve (AUC) 0.812 and p <0.001) for 285.67 cut off values. When these values were evaluated, high sensitivity and specificity of Native Thiol and Total Thiol suggested that it might be a test for evaluating STEMI patients (Figure 1, table 2).

Table 2. The results of data analysis of the ROC curve

Parameter	AUC	Standard deviation	p*	95% CI		Sensitivity	Specificity	Cut off
				Lower	Upper Bound			
Native Tiyol	.853	.040	<0.001*	.775	.932	95.9	74.6	239.68
Total Tiyol	.812	.047	<0.001*	.720	.903	91.8	82.5	285.67

*p<0.05 is considered significant for statistical analyses.

DISCUSSION

Acute coronary syndrome is the most common and fatal complication of CAD. In these cases, the initiator is either an atherosclerotic plaque rupture or an intracoronary thrombosis related to an erosion[12,13]. During the process of CAD development, the accumulation of oxidized lipid particles in the subendothelial cells contributes to the development of the atheromatosis plaque. The oxidative stress in the microenvironment of the plaque causes more oxidation in the lipid molecules and leads to formation of free radicals[14,15]. The imbalance caused by the formation of increased free radicals and the inefficiency of antioxidant mechanisms results in oxidative stress [8]. Previous studies have shown that the oxidative stress indicators increase after a myocardial infection and that there is a strong correlation between oxidative stress and CAD[3,16-19]. Increased oxidative stress indicators have a synergistic effect on KAH's standard risk factors [20,21]. Oxidative stress begins as a result of the deterioration between the antioxidant defence and the oxygen types. The atherosclerotic disease onset increases the oxidative stress[6,18,19].

In their recent studies Erel and Neselioğlu have shown that the levels of plasma disulphide is higher in patients with diabetes, obesity, pneumonia and degenerative diseases such as smoking, and that it is lower in patients with multiple myeloma, bladder cancer, colon cancer and proliferative diseases such as kidney cancer[11]. Tests on animals have shown that exogenous H2S heals myocardial functions and significantly decreases myocardial injuries and the rate of mortality in rats with myocardial ischemi-reperfusion injury[22,23].

Koprivica et al.[24] conducted research on the role of oxidative stress in the development of endothelial dysfunction and atherosclerotic disease, and also on the relationship between the oxidative stress and various types of ACS. As a result, they found that the lipid peroxidation index was meaningfully higher and that nitric oxide, hydrogen peroxide, superoxide dismutase activity and catalase activity were meaningfully low in the patients diagnosed with ACS[24]. Dynamic thiol disulphide homeostasis plays a significant role in detoxification, antioxidant protection, signal transduction, transcription factors, enzymatic activities, apoptosis and in the organisation of cellular signal mechanisms[10,25]. It has been reported that the status of abnormal thiol-disulphide homeostasis has a role in the pathogenesis of various diseases such as cardiovascular diseases and diabetes[7,26]. Recently new colorimetric methods have been used to detect thiol/disulphide homeostasis in the research of neurological diseases such Parkinson, Alzheimer, migraine, seizure, and epilepsy and of many diseases which are believed to have a role in immunological and inflammatory mechanisms[11]. The balance of normal thiol/disulphide is associated with many diseases and it shows that especially in the active period antioxidant systems are affected by inflammatory diseases[10,27].

In two different studies done by Kundi et al., it was shown that oxidative stress increased on patients

with acute MI and NSTEMI by using thiol/disulphide homeostasis parameters [16,28]. In their study, Sivri et al. evaluated the thiol disulphide homeostasis in the patients with Non-STEMI and they found that native thiol, total thiol, and native thiol/total thiol values were statistically significantly lower and disulphide/native thiol and disulphide/total thiol values were statistically significantly higher in the NSTE-ACS group when compared to the control group [29]. In another study done by Kundi et al. it was detected that native thiol, total thiol and disulphide levels were lower and averagedisulphide/native thiol and average disulphide/total thiol rates were higher when compared to the rates of the control group [16,28]. The results we have found show that the levels of disulphide/native thiol and the rate of disulphide/total thiol in our patients increase and become independent risk factors for AMI, which is concordant with the results of other studies. The fact that the data of thiol disulphide homeostasis received from both Non-STEMI and STEMI patients is covalent strongly supports the idea that it can be used in patients with ACS as a marker.

The findings in our study must be interpreted with some limitations. First of all, this is a monocentric and a small scaled study. Secondly, no comparison has been made with other oxidative stress indicators such as myeloperoxidase, paraoxonase and ischemia-modified albumin as these haven't taken place in the analysis. This study must be supported by large scale and multicentric studies.

In conclusion, serum thiol levels (both native and total) were decreased and disulphide/native thiol, disulphide/total thiol ratios were increased in patients with STE-ACS. These results suggest that dynamic thiol/disulphide homeostasis may be used in the diagnosis of STE-ACS patients. However, its prognostic performance needs to be confirmed in the future.

Yazar Katkıları: Çalışma konsepti/Tasarımı: FET; Veri toplama: SN; Veri analizi ve yorumlama: FT; Yazı taslağı:ZK, PYA, FET; İçeriğin eleştirel incelenmesi: PYA; Son onay ve sorumluluk: FET, ZK, PYA, UP, MÇ, FT, SA, SE, SN; Teknik ve malzeme desteği: SA, SE; Süpervizyon: ZK, FET; Fon sağlama (mevcut ise): yok.
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