THE ROLE AND VALUE OF OXIDATIVE STRESS IN THE DIAGNOSIS AND PATHOGENESIS OF STEMI PATIENTS

STEMİ HASTALARININ TANISINDA VE PATOGENEZİNDE OKSİDATİF STRESİN ROLÜ VE DEĞERİ

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ÖZET

Amaç: Akut ST elevasyonlu miyokard infarktüste (STEMİ) diğer tanısal belirteçlerin yanısıra oksidatif stress parametrelerinin nasıl etkilendiğini araştırarak, oksidatif stres parametrelerinin akut STEMİ hastalarında kullanılabilirliğini tartışmak istedik.

Materyal ve Metod: EKG ve kardiyak enzim sonuçları ile STEMİ tanısı doğrulanan 31 hasta retrospektif olarak çalışmaya dahil edilmiştir. 35 sağlıklı gönüllü kontrol grubu olarak alınmıştır. Acil servise başvuru anında, oksidatif stres parametrelerini ölçmek için periferal venöz kan örneği alındı. Serum antioksidan durumu, STEMİ hastaları ve sağlıklı gönüllülerde total antioksidan durum (TAS) ve tiyol (T-SH) düzeyi ölçülerek, serum oksidatif durumu total oksidatif durum (TOS) ölçülerek değerlendirildi ve ardından oksidatif stres indeksi (OSI) hesaplandı. Bulgular: Hastaların 29'u (%93.5)erkek, 2'si (%6.5) kadındı. Yaş ortalaması 54.90 ± 12.23 yıldı. Kontrol grubu ile karşılaştırıldığında TOS, TAS ve OSI düzeyleri hasta grubunda yüksekti ve bu sonuçlar istatistiksel olarak anlamlı olup; sırasıyla, 15.02±10.82 μmol H2O2 equivalent/l vs. 8.13±2.71 μmol H2O2 equivalent/l, p=0.001; 2310.95±549.80 μmol Trolox equivalent/l vs. 2065.71±280.98 μmol Trolox equivalent/l p=0.031; 0.65±0.40 arbitrary unit vs. 0.39±0.10 arbitrary unit, p=0.001). Ayrıca T-SH düzeyi hasta grubunda kontrol grubuna göre düşüktü (sırasıyla, 282.00±183.91 μmol/l . 472.11±307.15 μmol/l p=0.013).

Sonuç: Bu çalışmada STEMİ hastalarında T-SH düzeyleri, TOS ve OSI düzeylerinde değişiklikler olması, patogenezde oksidatif stresin rol aldığını düşündürmektedir. Bu testlerin, tanıda, mevcut testlerin yanısıra yol gösterebileceği ileri sürülebilir. Ancak daha kapsamlı ve iyi dizayn edilmiş ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Oksidatif stress, ST yükseklikli myokard infarktüsü, total oksidan durum, total antioksidan durum, tiyol (TS-H)

ABSRACT

Objective: The objective of the study was to evaluate the usage of oxidative stress parameters in acute ST-elevation myocardial infarction (STEMI) beside other diagnostic markers, by determination of how oxidative stress parameters were affected.

Materials and Methods: A total of 31 consecutive patients with STEMI were diagnosed by electrocardiography and cardiac enzyme results were included in the study. Thirty five volunteers were included as the control group. On admission to the emergency department, peripheral venous blood sample was taken for measuring oxidative stress parameters. Serum antioxidative status was evaluated by measuring Total Antioxidant Status (TAS) and Thiol (T-SH) levels in patients with ST-elevation myocardial infarction and in healthy individuals. Serum oxidative status was evaluated by measuring Total Oxidant Status (TOS), then Oxidative Stress Index (OSI) was calculated.

Results: The male/female ratio of the patients was 29/2. Mean age was 54.90±12.23 years. TOS, TAS and OSI levels increased in the patient group compared to the control group (respectively, 15.02±10.82 μmol H2O2 equivalent/l vs. 8.13±2.71 μmol H2O2 equivalent/l, p=0.001; 2310.95±549.80 μmol Trolox equivalent/l vs. 2065.71±280.98 μmol Trolox equivalent/l p=0.031; 0.65±0.40 arbitrary unit vs. 0.39±0.10 arbitrary unit, p=0.001). On the other hand; T-SH level was decreased in the patient group compared to the control group (respectively, 282.00±183.91 μmol/l vs. 472.11±307.15 μmol/l p=0.013).

Conclusion: Altered levels of T-SH as an antioxidant and also increased levels of TOS and OSI at STEMI patients may give an idea about the role of oxidative stress at the pathogenesis of STEMI , thus; usage of oxidative stress tests cooperative with the existing tests may be approved, but more comprehensive and well-designed studies are demanded.

Key words: Oxidative stress, "ST" Elevation myocardial infarction, TAS, TOS, T-SH

INTRODUCTION

Acute Coronary Syndrome (ACS) is one of the most often causes of attending to emergency departments and it is the most common cause of sudden cardiac death. Wide range of clinical spectrum includes unstable angina pectoris, non-STEMI and STEMI. STEMI usually occurs when a fibrin-rich thrombus completely occludes an epicardial coronary artery. [1,2]

The diagnosis of acute ST elevation acute myocardial ischemia (STEMI) is based on clinical characteristics and persistent ST-segment elevation as demonstrated by 12-lead electrocardiography. But, it is somewhat difficult to find out abnormal findings of ECG when it is taken in early phases of ACS. Therefore, it is necessary to record ECG several times and to follow up even if there is no ECG abnormality at the first recording. In recent years, the diagnosis and management of patients with ACS have evolved dramatically. The most sensitive and specific markers of myocardial injury are cardiac troponin and creatine kinase. Recent studies have revealed several novel biomarkers. Elevated levels of C-reactive protein and interleukin-6 are strong independent markers of increased mortality among patients with ACS.[3]

Oxidative stress may have an important role in the pathogenesis of acute coronary diseases as it is involved in the pathophysiology of several diseases.[4] Disturbance in the equilibrium status of prooxidant/ antioxidant systems in intact cells is termed as oxidative stress. Most of the potentially deleterious effects of oxygen are because of the formation and activation of a number of chemical compounds, defined as reactive oxygen species, which have a high tendency to donate oxygen to other substances. Many such reactive species are actually free radicals. Free radicals are some kind of molecules that have one or more unpaired electrons and thus unstable and highly reactive. The balance between the production of free radicals and antioxidant defenses in the body has important health implications. If there are too many free radicals or too few antioxidants for protection, a condition of oxidative stress develops, which may cause chronic and permanent damage.[5]

Particularly for acute STEMI, the effective interventions for patients are extremely time-limited. It is imperative that we evaluate efficient risk stratification and effective treatment of patients with non-STE ACS, as soon as possible.[2,6,7] Patients with acute STEMI should be assessed rapidly for reperfusion therapy and a reperfusion strategy should be implemented immediately after the patient's contact with health care system. Two methods are currently available for establishing timely coronary reperfusion: primary percutaneous coronary intervention and fibrinolytic therapy. Percutaneous coronary intervention is the preferred method, but is not always available. Antiplatelet agents and anticoagulants are critical adjuncts to reperfusion.[8]

The changes in the levels of T-SH,TOS and OSI at STEMI patients, which were included in this study, let us think that oxidative stress has a role at the patogenesis. Thus these tests can be runned as cooperative with excisting tests. Anyway much more comprehensive and well designed studies are still demanded.

MATERIALS AND METHODS

Study population and protocol

A total of 31 consecutive patients with electrocardiography and cardiac enzyme results confirmed as ST-elevation myocardial infarction were retrospectively included in the study. Thirty five volunteers were included as the control group. On admission to the emergency department, peripheral venous blood sample was taken for measuring oxidative stress parameters. The study was performed between August 2010 and December 2010. It was approved by the institution's ethic committee and informed consents were obtained for this study.

On admission, the data related to the demographic characteristics were recorded and patients were included deterministically in the study if diagnosis of acute STEMI was confirmed by electrocardiography (ECG) and cardiac enzyme results including CK, CK-MB and troponin evaluation carried out immediately. Data were collected for confirmed cases of acute STEMI. The cases with ECG signs and cardiac enzyme results incompatible with acute STEMI were excluded. Additionally, patients with renal, hepatic or malignant diseases were also excluded. There was no condition which might affect oxidative stress in the control group. During the study, patients with renal, hepatic or malignant diseases, which can affect oxidative stress levels, were also excluded.

On the other hand; on admission to the emergency department, venous blood was drawn into blood tubes from acute STEMI patients, confirmed later by ECG and cardiac enzymes, and serum was separated from the cells by centrifugation at 1500 g for 10 min, and the serum samples were stored at –80 °C until the analyses. Laboratory staffs haven't been informed. Time frame of blood taking was exactly at the time of diagnosis, before all treatment medications. Serum antioxidative status was evaluated by measuring TAS and T-SH levels in patients with acute STEMI and in the control group. Serum oxidative status was evaluated by measuring TOS. The ratio of TOS level to TAS level was accepted as OSI. Similar studies were also performed in the control group.

Determination of Serum Total Oxidant Status (TOS) Levels

TOS levels were measured using commercially available kits (Rel assay, Turkey). In the new method, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/l).[9]

Determination of Serum Total Antioxidant Status (TAS) Levels

TAS levels were measured using commercially available kits (Rel assay, Turkey). The novel automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as μmol Trolox equivalent/l.[10]

Calculation of oxidative stress index (OSI)

The TOS: TAS ratio was used as the OSI, which was calculated as follows: should be

OSI (arbitrary unit) = TOS (μmol H_2O_2 equivalent/l) / TAS (μmol Trolox equivalent/l).[11-13]

Determination of Serum Total Thiol (T-SH) Levels

Serum thiol (total – SH group) content was measured by using dithionitrobenzoic acid (DTNB).[14]

Statistics

For statistical evaluation, we used the software package SPSS 15.0 and probability value of less than 0.05 was accepted as statistically significant. As the data were normally distributed (Kolmogorov-Smirnov test) and independent, statistical analysis was performed using Student's t-test when comparing groups. The results are given as the mean ± standard deviation (SD).

RESULTS

There were 29 (93.5%) male and 2 (6.5%) female patients with STEMI. Mean age was 54.90±12.23

years. In control group, there were 32 (91.4%) males and 3 (8.6%) females. Mean age of control group was 36.50±4.50. Average time between the beginning of symptoms and patients admission to the emergency department was 35.00±7.07 minutes. On admission to the emergency department, the ratio of the presentation symptoms were respectively; 66,7% dyspnea, 100% chest pain and 5.3% diaphoresis. The ratio of patients who have had tacyhpnea was 4,8% (>20/min) and tachycardia was 9,5% (>100/min). According to their ECG's, 71.4% of them were diagnosed as inferior MI, 23.8% as anterior MI, 9.5 % as diffuse MI and 19% as posterior MI.

The levels of TOS, TAS, OSI and T-SH on arrival are shown on Table 1. TOS, TAS and OSI levels increased in the patient group compared to the control group (respectively, 15.02±10.82 µmol H_2O_2 equivalent/l vs. 8.13±2.71µmol H_2O_2 equivalent/l, *p=0.001*; 2310.95±549.80 µmol Trolox equivalent/l vs. 2065.71±280.98 µmol Trolox equivalent/l *p=0.031*; 0.65±0.40 arbitrary unit vs. 0.39±0.10 arbitrary unit, *p=0.001*) (Figure 1, 2, 3). On the other hand T-SH level was decreased in the patient group compared to the control group (respectively, 282.00±183.91 µmol/l vs. 472.11±307.15 µmol/l *p=0.013*) (Figure 4). There was no significant difference between high or normal serum troponin level groups in terms of levels of TOS, TAS and OSI. On the other hand<, T-SH levels were 390.62±220.98 in the high level troponin observed patients vs. 175.45±82.44 in the normal level troponin observed ones (*p=0.008*)

DISCUSSION

In this study, we demonstrate that oxidative stress is elevated in acute STEMI patients compared with healthy controls.

In organism, activation of certain systems such as sympathetic nervous system, renin-angiotensin system and neutrophils may increase the formation of oxyradicals and oxidants, which in turn increase the oxidative stress and thereby result in ventricular dysfunction.[15] Oxidative stress can be defined as an increase in oxidants and/or a decrease in antioxidant capacity.[16] Oxidative stress plays a pivotal role in the inflammatory process leading to atherosclerotic plaque formation. In clinical studies, several novel markers of oxidative stress have been found to be associated with ACS and valuable to predict future cardiovascular events, independent of traditional cardiovascular risk factors.[17,18]

Oxidative stress impairs endothelial function and may play an important role in the pathogenesis of acute cardiovascular diseases.[19] These results indicate that the mentioned markers may be useful both in understanding plaque destabilization and in determination of risk stratification of patients. Also, measurement of these markers may provide a non-invasive perspective to study atherosclerotic lesions. Oxidative stress is associated with the origination of free oxygen radicals that may impair cells and tissues. Free oxygen radicals are able to bind to lipoproteins, proteins, nucleic acids and enzymes. Lipid peroxidation is reported to be the significant factor in the cardiovascular diseases. Increases in these oxidant markers may provide an earlier assessment of overall patient risk and aid in identifying patients with higher risk of having an adverse event. The damaging free radicals may cause either direct arterial wall injury or initiate secondary processes including depletion of antioxidants such as vitamin C or vitamin E. These oxidant and antioxidant markers may have potential clinical utility to identify high risk patients, who may require early treatment.[20]

In previous studies, oxidative stress parameters in coronary artery diseases were examined individually such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and malondialdehyde,but, oxidants and antioxidants have additive effects. Although the concentration of oxidant and antioxidant components can be measured individually, these measurements are time and cost consuming and require sophisticated systems. In addition, it may not accurately reflect the TAS and TOS.[21]

T–SH which is a member of the antioxidant family is required for reducing the oxidative stress in biological materials. [22-25]

Antioxidant defense system which consists of free radical scavengers including superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase and non enzymatic antioxidants such as

T-SH and various other substances taken along with food such as vitamin E, vitamin C, β-carotene and flavanoids, is able to cope with harmfull effects of ROS (reactive oxygene species) in normal conditions. Antioxidant defense systems work cooperatively to alleviate the oxidative stress caused by enhanced production of the free radicals. Imbalance between increased oxidative stress and impaired antioxidant defense may contribute to the pathogenesis of cardiovascular events.[26]

There is strong proof about the vital role of oxidative stress which has been caused by the deterioration of the balance between antioxidant defense and reactive oxygen species production, at the pathogenesis of coroner atherosclerosis and endothelial disfunction. The relationship between oxidative stress and atherosclerosis has been examined by various research groups on both human and animal experiments. [27]

In our study, the increase of oxidative stress in STEMI patients compared to healthy control individuals are compatible with the prediction that oxidative stress plays an important role in the pathophysiology of STEMI. The elevation of TAS levels in the patient group indicates that the antioxidant activity is increased by the organism for the compensation of harmful effects of oxidative stress. Similarly, the increased T-SH amount in high level troponin observed patients are considered that the organism counteracts against destructive impacts through antioxidant activity. To the best of our knowledge, this is the first study to demonstrate an association of a novel marker for oxidative stress with acute myocardial infarction. Despite the small sample size of this study, our prospective data do indicate that oxidative stress parameters may potentially provide an easily applicable tool to identify patients with STEMI, however; since this was a relatively small study, these observations should be confirmed in a larger follow-up studies in patients with STEMI to establish the usefulness of oxidative stress parameters in this clinical setting.

Limitations

At the beginning of the study, we planned to collect only STEMI patients but non- STEMI and USAP (unstable angina pectoris) patients could be worked in the future studies. This is a prestudy for future studies, of course increased number of patients would be more appropriate for following studies. We didn't exclude the smokers in the study, because we already had a small number of patients in the study group and if we have excluded the smokers, the number would be much less, because smoking is already a risk factor for Acute Coronary Syndrome. All the data were obtained in the emergency service. During this study we didn't colloborate with the angiogram unit, so we didn't get any angiogram results.

CONCLUSION

In this study, altered levels of T-SH as an antioxidant and also increased levels of TOS and OSI at STEMI patients may give an idea about the role of oxidative stress at the pathogenesis of STEMI , thus; the usage of oxidative stress tests cooperative with the excisting tests may be approved, but more comprehensive and well-designed studies are demanded.

Acknowledgements: No company foundation was used during the study. We applied to our ethical committee for acceptation after the approval, all the expenses were provided by hospital sources.

Table 1. TOS, TAS, OSI and T-SH levels between patient and control groups (mean± SD)				
Parameters	**Group**	**mean**	**SD**	***p***
TOS (μmol H_2O_2 equivalent/l)	patient	15.02	10.82	*p=0.001*
	control	8.13	2.71	
TAS (μmol Trolox equivalent/l)	patient	2310.95	549.80	*p=0.031*
	control	2065.71	280.98	
OSI (arbitrary unit)	patient	0.65	0.40	*p=0.001*
	control	0.39	0.10	
T-SH (μmol/l)	patient	282.00	183.91	*p=0.013*
	control	472.11	307.15	

(TOS=total oxidant status, TAS=total antioxidant status, OSI=oxidative stress index, T-SH=serum total thiol)

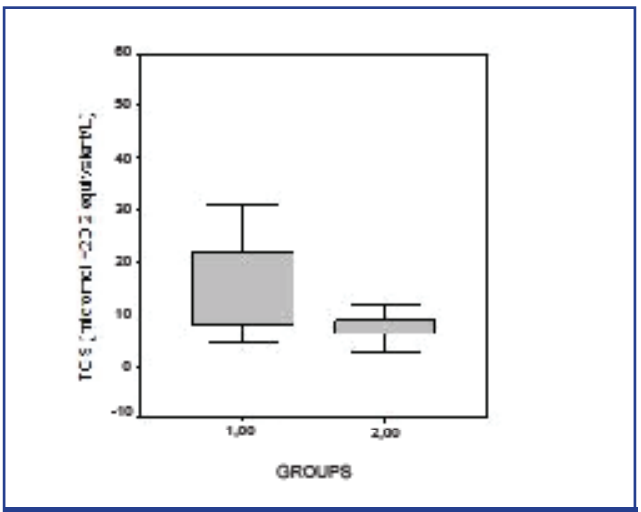

Fig 1. Differences in TOS (total oxidant status) levels between STEMI patients (Group 1) and control group (Group 2).

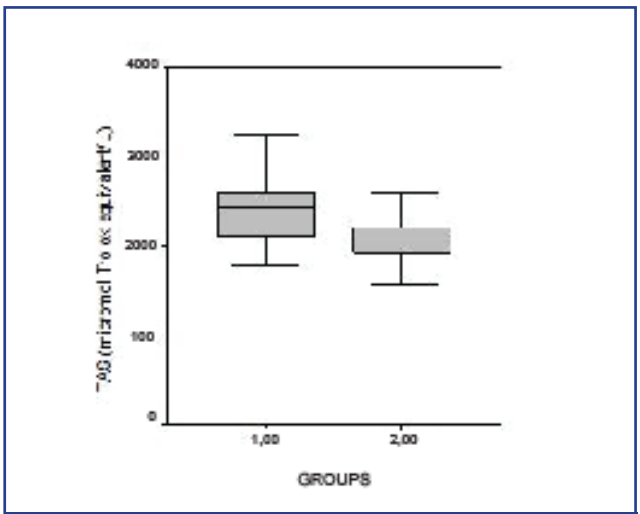

Fig 2. Differences in TAS (total antioxidant status) levels between STEMI patients (Group 1) and control group (Group 2)

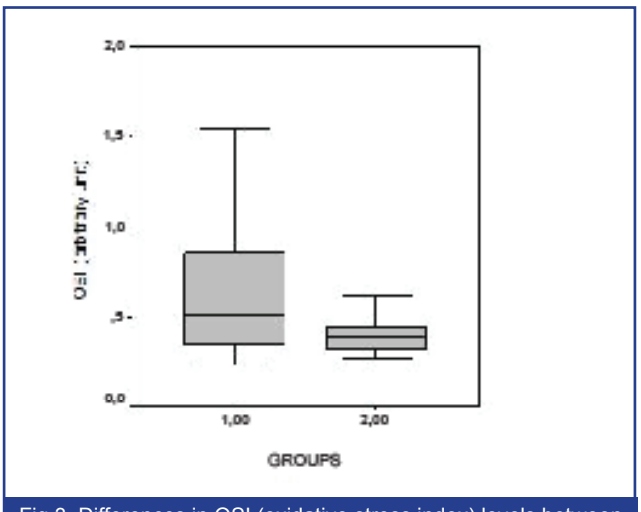

Fig 3. Differences in OSI (oxidative stress index) levels between STEMI patients (Group 1) and control group (Group 2)

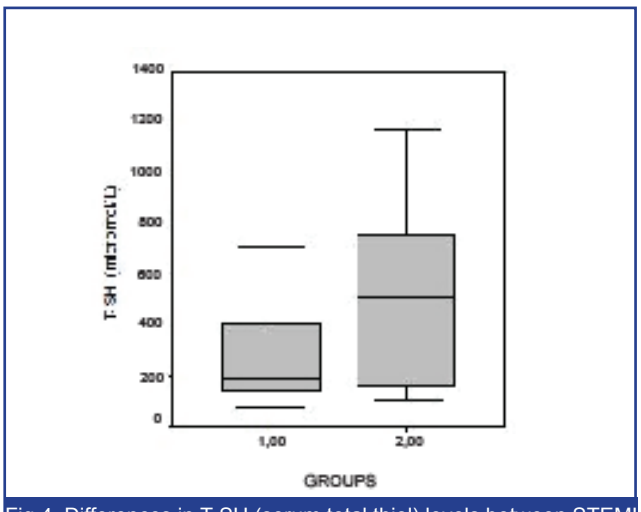

Fig 4. Differences in T-SH (serum total thiol) levels between STEMI patients (Group 1) and control group (Group 2)

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