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# Evaluation of serum thiol levels in patients with non ST elevation acute coronary syndrome

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**ABSTRACT**

Imbalance between antioxidant and oxidant systems in the body play an important role in the pathogenesis of coronary atherosclerosis and its complications. The aim of this clinical study was to investigate total serum thiol (TS-H) levels in patients with non ST Elevation Acute Coronary Syndrome (NSTE-ACS) patients and relationships between these results and cardiac markers. This study included 55 patients and a control group consisted of 50 healthy people. Samples for serum total TS-H levels were obtained from the patients considered with NSTE-ACS at the time of admission to emergency department. The patient group was divided into two subgroups according to troponin levels; that is patients with positive troponin levels in the non-ST-elevation myocardial infarction (NSTEMI) group and, and patients with negative troponin levels in the unstable angina pectoris (USAP) group. SPSS for Windows Ver. 15.0 software packages were used for statistical analysis of data in patient and control groups. The patients group included a total of 55 patients with 40 men (72.7%) and 15 women (27.3%) and the control group included 50 healthy individuals people with 28 men (56%) and 22 women (44%). Total serum TS-H values were significantly lower in patients group when compared with the control group. Serum total TS-H values were significantly lower in USAP subgroup when compared with patients with NSTEMI. In addition, negative correlations between total serum TS-H values and troponin I and CK-MB were found. According to this study, total serum levels of thiol which is antioxidant marker TS-H may be useful as a confirming test in diagnosis of patients with NSTE-ACS and in differentiating patients with NSTEMI and USAP.

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## 1. Introduction

Coronary artery disease (CAD) presents the summary of all complications (ischemia, necrosis, etc.) caused by an atherosclerotic occlusive lesion, narrowing the wall of one or more coronary artery and causing coronary blood flow not to meet the increased demand for oxygen in the myocardium (Stary, 1994; Stary et al., 1995). Atherosclerosis makes approximately up to 99% of the pathogenesis of CAD. Atherosclerosis is a chronic, multifactorial disease and usually affects the entire arterial system. The initial step of atherosclerosis is a vascular wall damage, and resulting endothelial cell dysfunction. Following important processes include inflammatory responses in vascular bed, cell proliferation, atherosclerotic plaque formation and revascularization. Acute coronary syndrome (ACS) is a complication of CAD and one of the major life-threatening emergencies. Rapid diagnosis and appropriate treatment is life-saving. Classification of ACS is based on ECG. Patients with acute chest pain and persistent (>20 minutes) elevation in ST-segment are classified as '' ST-elevation acute coronary syndrome'' (STE-ACS), and patients with acute chest pain without persistent ST-segment elevation are named as, ''non-

ST-elevation acute coronary syndrome (NSTE-ACS). In addition NSTE-ACS with troponin elevation is classified as NSTEMI and with normal troponin levels as USAP (Hamm et al., 2011).

Oxidative stress is defined as the loss of balance between oxidants and antioxidants in favor oxidants. Oxidative stress is involved in the pathogenesis of various diseases, such as coronary atherosclerosis and complications, cancer, neurodegeneration, and immunological disorders. (Dogru-Abbasoğlu et al., 1999; Tamer et al., 2002; Surekha et al., 2007). Antioxidants prevent the effects of increasing free oxygen radicals. Many antioxidants such as enzymes, proteins, minerals, vitamins, glutathione and thiol (TS-H) were described (Brent et al., 1991). TS-H are components of sulfhydryl groups associated with carbon atoms (Chung et al., 2005). Thiol is an endogenous molecule TS-H which protects aerobic cells by reacting as a reducer despite the oxidizing environment. TS-H provides a protective effect against damage induced by free radicals in the cells with its exceptional antioxidant effects (Pogocki and Schöneıch, 2001).

In this study, serum total TS-H levels in patients with NSTE-ACS at diagnosis, and prognosis of these levels of cardiac markers contributions aimed to investigate whether the relationship.

## 2. Materials and methods

The study included the patients group with 55 individuals and the control group with 50 individuals. Patient group consisted of the patients admitted to the emergency department with chest pain over the age of 18, and who were suspected to have ACS depending on the story, the clinical presentation, ECG findings, and cardiac markers. Informed consents were obtained from the patients enrolled in the study. Exclusion criteria included being under 18 years of age, creatinine>2 mg/dL, the previously known coronary artery disease, a history of stroke within the last 3 months, history of diabetes mellitus, suspected aortic dissection, malignancy, sepsis, cardiac trauma, a history of injection in recent 2-3 days, crush injury, history of autoimmune disease, history of pulmonary embolism in the last 3 months, and diagnosis of pericarditis / myocarditis.

Venous blood samples were obtained from antecubital region of the patients on admission. For each patient complete blood count, glucose, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum electrolytes, sodium (Na), potassium (K), calcium (Ca), and in terms of cardiac involvement, creatine kinase (CK), creatine kinase-MB (CK-MB) and troponin levels were studied from the first blood samples. For the measurement of total serum TS-H levels, blood samples were kept at room temperature for 30 minutes and centrifuged at 3.500 rpm for 5 minutes. The samples were stored in eppendorf tubes after centrifugation and stored at -80 °C in temperature in the refrigerator until the study was done. Total serum TS-H or sulfhydryl group concentrations were measured using the procedure described by Elmman and modified by Hu. 5,5 '-dithiobis (2 nitrobenzoic acid) (DTNB) interacts with TS-Hand forms a highly colored anion at a maximum peak of 412 Km. In order to obtain total serum TS-H levels this method was adapted to biochemistry analyzer (SIEMENS, ADVIA 2400, Japan) in the biochemistry laboratory of our hospital.

Shapiro-Wilk test was used for analyzing coherence of measured values with normal distribution. Demographic characteristics of the study population, the frequency of clinical symptoms, physical examination findings, and laboratory results and averages were analyzed. Chi-square or chi-square Fisher's exact test were used for analyzing the changes in categorical variables. Independent samples t test was used to examine coherence of comparison of study and control groups with normal distribution in terms of the chosen parameters. Spearman's rank correlation coefficients (Rho -r) were determined to observe the relationships between variables. SPSS for Win. Ver. 15.0 (SPSS Inc. Chicago, IL. USA) software packages were used in all statistical analyzes and calculations. A level of $p<0.05$ was considered statistically significant.

## 3. Results

The patient group included a total of 55 patients with 40 men (72.7%) and 15 women (27.3%) and the control group included 50 healthy individuals people with 28 men (56%) and 22 women (44%). There was no statistically significant difference between groups in terms of gender ($c2=0.073$, $p>0.05$). The mean age of the patient group was 60.42±41.82 years and ranged between 23 and 95 years. The mean age of the control group was 55.42±12.75 years and ranged between 24 and 78 years. The mean ages of the patient and the control groups were statistically similar ($t=1.798$, $p=0.075$). Regarding a relationship between the TS-H levels of the patients and admission method, the average TS-H level of the patients brought by ambulances were lower than the level of the patients who admitted themselves.

Regarding a relationship between the TS-H levels of the patients and admission method, the average TS-H level of the patients brought by ambulances were lower than the level of the patients who admitted themselves. The average level of TS-H of the patients brought by ambulances was 360.40±124.67 $\mu$mol/l, whereas this level was 411.76±102.57$\mu$mol / l in the patients who admitted themselves. Although the difference was not statistically significant, it may suggest that the patients brought by ambulance may be more serious. Patients' vital signs and the final status information are shown in Table 1.

The most common cause of admission to emergency department was chest pain (96.4%). It is followed by perspiration (27.3%), dyspnea (9.1%), nausea and vomiting (5.5%), palpitations (4%), and syncope (3.6%), respectively. Regarding percentages of risk factors, age risk factor in 78.2%, male gender in 72.7%, low HDL in 67.3%,

**Table 1.** Patients' vital signs at admission and final status information

| | |
|---|---|
| **Vital signs** | |
| Systolic blood pressure (mmHg) | 123.70±21.85 |
| Diastolic blood pressure (mmHg) | 71.87±12.85 |
| Pulse | 77.87±19.71 |
| Respiration | 12.9±1 |
| **Final status** | |
| Medical treatment | 21 (%38.2) |
| Stent placed | 25 (%45.5) |
| CABP planned | 3 (%5.5) |
| Angiography wasn't performed | 6 (%10.9) |

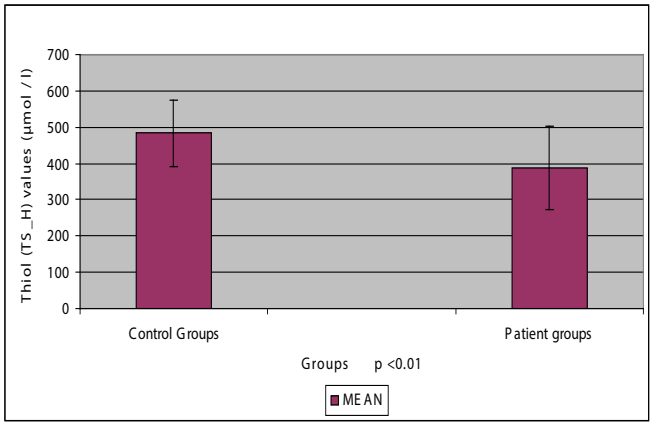


**Fig. 1.** Comparison of TS-H Values of patients and control groups

smoking in 65.5%, hypertension in 54.5%, hyperlipidemia in 40%, family history in 16.4% and obesity in 7.3% of the patients were noted. Diabetic patients were excluded due to exclusion criteria. Mean level of TS-H in the patient group was calculated as 388.41±115 ($\mu$mol/l) and in the control group as 482.94±92.18 ($\mu$mol/l), respectively. A statistically significant difference was found between the mean levels of TS-H of the patient and control groups according to the analysis performed by using independent samples t-test ($p<0.01$). TS-H values of the patient and control groups are shown in Figure 1. Average TS-H level of the patient group was significantly lower than the level of the control group.

TS-H values of NSTEMI and USAP subgroups were compared. The mean value of TS-H measured in NSTEMI patients was 339.66±115.20 ($\mu$mol/l) and in USAP group 435.42 ± 94.87 ($\mu$mol/l), respectively. There was a statistically significant difference between the mean TS-H levels of NSTEMI and USAP patients ($p<0.01$). The mean level of TS-H was significantly lower in patients with NSTEMI than patients with USAP. TS-H levels of NSTEMI and USAP patients are shown in Figure 2.

Regarding a relationship between the TS-H levels of the patients and admission method, the mean TS-H level of the patients brought by 112 ambulances were lower than the level of the patients who admitted themselves. The mean level of TS-H of the patients brought by 112 ambulances was 360.40±124.67 ($\mu$mol/l), whereas this level was 411.76±102.57 ($\mu$mol/l) in the patients who admitted themselves. Although the difference was not statistically significant, it may suggest that the patients brought by ambulance may be more serious.

Regarding a relationship between the TS-H levels and the patients' symptoms, thiol levels of patients with complaints of chest pain, palpitations, and shortness of breath were lower than TS-H levels of the patients who admitted with other complaints. However, this difference was not statistically significant.

There was negative and statistically significant moderate correlation between TS-H and troponin I values of the patients in the study group ($p<0.001$, $r=0.592$). Weak negative correlation was found between TS-H and CK-MB values and ıt was statistically significant. ($p<0.001$, $r=0.487$).

## 4. Discussion

The results of this study revealed that TS-H level of patient group was significantly lower when compared with the control group. In addition, mean TS-H level of non-STEMI patients was lower than mean level of USAP patients. There is evidence that oxidative stress which is a result of deterioration of the balance between the production of reactive oxygen components and antioxidant defense plays a vital role in the pathogenesis of atherosclerosis and coronary endothelial dysfunction at the initial stage (Hamm et al., 2011). Free radicals leading to oxidative stress are produced during the cell metabolism, or they are exogenous molecules with unpaired electrons (Freeman and Crapo, 1982; Lunec and Blake, 1990). Free radicals have been associated with occurrence, progression, and complications of many local and systemic diseases in the organism (Roehm et al., 1971). Their effects on myocardial infarction due to atherosclerosis, diabetes, cancer, cataracts, rheumatoid arthritis, infertility, respiratory diseases, diseases of the central nervous system and genitourinary system, and aging process have been reported by many researchers (Ak et al., 1994). Free radicals affect all the major compounds of cells such as lipids, proteins, DNA, and carbohydrates and cause deterioration of their structures.

The relationship between oxidative stress and atherosclerosis in both humans and experimental animals was investigated by several research groups. Atherosclerosis is created by giving cholesterol in experimental animals and it was shown that pro-oxidant antioxidant balance is affected, and there is a relationship between these changes and the degree of atherosclerotic plaques (De la Cruz et al., 2000).

There are many antioxidants in our body. Antioxidants include enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase (GSH-Px), glutathione reductase, glucose-6-phosphate dehydrogenase), and non-enzymatic antioxidants which serve as collectors of free radicals in food (vitamin E, ascorbic acid, carotenoids and flavonoids). In addition, there are endogenous antioxidants which act as free radical scavengers such as glutathione (GSH), TS-H, uric acid, melatonin, and coenzyme Q.

TS-H groups have highest concentration among the antioxidants. Major sources of TS-H include cysteine, homocysteine, methionine, amino acids and GSH. One can reach an opinion about antioxidant capacity by measuring total serum TS-H levels (Cao et al., 1993; Yardım-Akaydın et al., 2003).

GSH and GSH-Px are well-known natural antioxidants. Morrison et al. control compared total serum levels of GSH

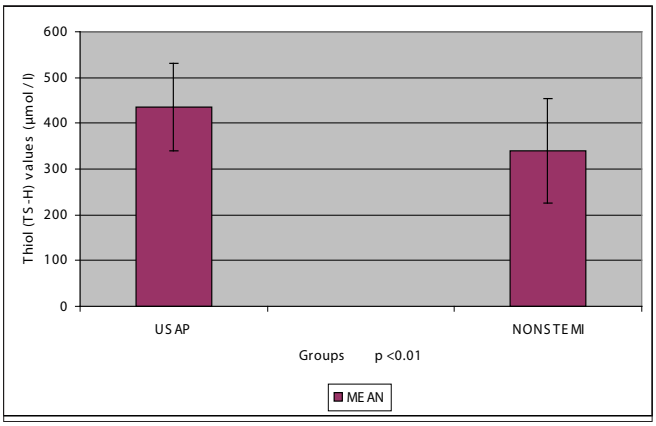


**Fig. 2.** TS-H values of USAP and NSTEMI Patients

between control group (n=78) and CAD group (n=81) in their study. They found that total serum GSH levels of CAD group (20.3±2.2) was significantly lower when compared with the control group (4.35±2.1) ($p<0.001$) (Morrison et al., 1999).

Weinbrenner et al. (2003) in their case-control study that included a total of 64 male participants, compared the complete blood GSH-Px activity in patients group (n=32) and, control group (n=32) and they found that GSH-Px activity of patients with CAD was significantly lower than that of healthy control group ($p<0.001$).

Bridge et al. (1992) compared plasma malondialdehyde (MDA) and GSH levels of patients group (n=58) who underwent coronary angiography and divided into six subgroups, with a control group of healthy subjects (n=50). Plasma levels of GSH, which is an antioxidant, were lower in patients group and levels MDA, which is an oxidant, were higher in all of the six subgroups than the control group. The differences were statistically significant.

In our study, the total plasma TS-H was measured as an antioxidant. TS-H levels in patients with acute coronary syndromes without persistent ST-segment elevation were significantly lower when compared to the control group. In addition TS-H levels were lower in the subgroup of NSTEMI patients when compared with USAP subgroup. Our results are compatible with the above mentioned studies and other literature.

Nojiri and his colleagues (2001) in their study compared total antioxidant status (TAS) levels of patients with CAD with the control group, and found that TAS levels were significantly lower in the patients group. In addition they demonstrated that TAS levels were associated with the number of involved coronary arteries. Likewise, in another study by Surekha et al. (2007) TAS levels of patients with myocardial infarction were significantly lower than the control group. The results of this study were also coherent with the results of our study.

Barsotti et al. in their study published in 2011 investigated the role of Advanced Oxidation Protein Products (AOPP) and TS-H in patients with ACS. They enrolled 34 patients with ACS and 16 healthy control subjects with one or more major variable cardiac risk factors. The measurements were repeated in the first and sixth. months in order to asses changes in pro-oxidants. AOPP levels were significantly higher in the patients with AMI and USAP, in the first month. AOPP levels approximated to normal values four weeks later, whereas there was no significant change in the sixth month. There was no significant change in patients with USAP (Barsotti et al., 2011). TS-H levels were significantly lower in AMI and USAP groups than the levels of the control group in the first measurement. These results are in line with the results of our study.

As a result, TS-H levels at admission can be a supportive diagnostic test for patients with NSTE-ACS in the emergency service. In addition, it may be helpful in the differential diagnosis of NSTEMI and USAP.

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