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Effects of smoking on thiol/disulfide homeostasis

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Abstract. – OBJECTIVE: Cigarette smoking is an important risk factor for many diseases. This study aimed to evaluate whether cigarette smoking is associated with changes in the thiol/disulfide homeostasis (TDH), a novel biomarker of systemic oxidative stress.

PATIENTS AND METHODS: Eighty-four smokers and 86 non-smoking healthy volunteers were enrolled. Serum native thiol, disulfide and total thiol levels, disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol ratios were analyzed using a new colorimetric method. Carbon monoxide (CO) levels were measured by a piCO smokerlyzer instrument.

RESULTS: The native, total, and native/total thiol levels of smoking patients were significantly lower (p<0.001 for each), and disulfide, disulfide/native thiol, and disulfide/total thiol levels were significantly higher in smokers than the healthy controls (p<0.001 for each). The CO levels of all study participants were negatively correlated with native thiol (r= -0.627, p<0.001), total thiol (r= -0.569, p<0.001), native thiol/total thiol (r= -0.515, p<0.001), and positively correlated with disulfide (r=0.398, p<0.001), disulfide/native thiol (r=0.515, p<0.001) and disulfide/total thiol (r=0.515, p<0.001) levels.

CONCLUSIONS: To our knowledge, this investigation is the first in the literature that investigated TDH in cigarette smokers. Our results show that cigarette smoking may lead to oxidative stress and TDH shifts through disulfide side compared to the healthy group. Further studies with larger sample size are needed to confirm our results for showing the changes in TDH to contribute to the clinical practice.

Key Words:
Cigarette smoking, Oxidative stress, Thiol/disulfide homeostasis.

Introduction

Cigarette smoke contains more than 4000 chemicals, including tobacco-specific N-nitrosamines, polynuclear aromatic hydrocarbons, and aromatic amines. These chemicals are highly carcinogenic, and also may cause oxidative stress by inducing the formation of free radicals[1,2]. This redox imbalance may result with toxic effects that damage all elements of the cells, particularly proteins, lipids, and nucleic acids. As a consequence, oxidative stress is thought to play a role in the progression of cancer, cardiovascular disease, Parkinson's disease, Alzheimer's disease, and chronic fatigue syndrome[3]. Previous researches[4,5] suggest that acute and chronic effects of smoking may debilitate antioxidant defense systems, which eventually raises long-term pathologies.

The balance between production and elimination of the free radicals is known as oxidative balance. Limiting the damage due to free radicals is only possible by maintaining the oxidative balance. Thiol is an important antioxidant, and plays a key role in the eradication of free oxygen radicals through enzymatic and non-enzymatic pathways[6,7]. Certain protein-thiols with cysteine, homocysteine, glutathione, and albumin constitutes the plasma thiol pool. Thiol/disulfide homeostasis (TDH) is an indicator of oxidative stress, and it is necessary for regulation of detoxification, apoptosis, signaling pathways, and enzymatic reactions[8,9]. Increased production of proinflammatory cytokines is related to the increased mediators of oxidative stress in many diseases[10,11]. Abnormal TDH is related to many inflammatory diseases.

There is no calorimetric method for determining the quantitative TDH. High-performance liquid chromatography, fluorescence capillary electrophoresis, and bioluminescence methods were utilized for determining the TDH in previous studies. But these methods are time-consuming, complex, and not cost-effective[12,13]. Today, thiol and disulfide levels can be measured either separately or simultaneously by the practical and

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full-automatic method that developed by Erel and Neselioglu[14]. Previous works utilized various biomarkers to measure the oxidative stress due to smoking[15]. To the best of our knowledge, TDH levels in smokers have not been studied previously. This investigation aimed to evaluate the TDH in smokers by the novel method of Erel and Neselioglu.

Patients and Methods

This work was conducted in Health Sciences University, Konya Education and Research Hospital. Eighty-four consecutive patients who admitted to Smoking Cessation Clinic and were volunteered to participate the study were included as patients. And, 86 non-smoking individuals were included as control group. Informed consent forms compatible with the Helsinki Declaration of World Medical Association were obtained from each participant before the study. This investigation was approved by the Ethical Committee of Selcuk University.

Exclusion criteria were:

- Patients younger than 18 and older than 65 years of age
- Smokers with a pack/year less than 10
- Patients with a cardiovascular system disease such as acute coronary syndrome, myocarditis, left ventricular dysfunction, and heart failure
- Patients with a chronic inflammatory-autoimmune disease
- Patients using antioxidant drugs or herbal supplements (angiotensin converting enzyme inhibitors, antioxidant beta-blockers or antioxidant vitamins)
- Patients with diabetes mellitus, chronic liver failure, chronic renal failure, cancer, Parkinson disease, and Alzheimer's disease
- Age, height, weight, body mass index (BMI) and carbon monoxide levels of participants were measured.

CO (Carbon monoxide) Measurement

CO levels were measured by a piCO Smokerlyzer instrument (Micro$^+$ Smokerlyzer, Bedfont, England) instrument (0-100 ppm) from the expiratory air flow. Those with a CO level of 5 ppm and less were considered to be non-smokers[16].

Smoking Characteristics

The Turkish version of the questionnaire, which was proposed by Prochaska et al (17) and used in the US for the evaluation, ranking, and classification of the stages of change in the cessation of smoking and its characteristics, was used for the study participants. While, the Fagerstrom Test for Nicotine Dependence (FTND) (18) was used for the scoring and classification of dependence.

Laboratory Analysis

Blood samples were taken from the patients and the controls into serum separating tubes. Sera were obtained by centrifugation at 1500×g for 10 min. The samples were immediately put in the freezer at −80°C. The same process was applied to all the specimens. When the study was completed, the samples were shipped to the Biochemical Laboratory of Ataturk Training and Research Hospital, Ankara, Turkey to measure thiol/disulfide parameters.

Total thiol (-S-S- + -SH) consists of native and reduced thiol. Thiol/disulfide tests were measured using a novel automatic and spectrophotometric method developed by Erel and Neselioglu. The principle of the thiol/disulfide measurement method is the reduction of dynamic disulfide bonds (–S–S–) to functional thiol groups (–SH) by a reductant solution (10 μL), sodium borohydride ($NaBH_4$). The unused $NaBH_4$ remnants are completely removed by formaldehyde. So, this prevents further reduction of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) as well as any disulfide bonds resulting from the reaction with DTNB. Total thiol content in the samples was determined by using modified Ellman reagent after reaction with DTNB. The disulfide parameter is a value which can be calculated automatically as half of the native thiol content and total thiol content. After determination of the main parameters (native thiol, total thiol, and disulfide values) disulfide/total thiol percent ratios, native thiol/total thiol percent ratios and disulfide/native thiol percent ratios were calculated[14].

Statistical Analysis

The descriptive statistics of the study were presented with frequency and percent for categorical variables, and mean and standard deviations for numerical variables. Independent group comparisons were conducted with Student *t*-test for numerical variables, and chi-square test for categorical variables. Multivariate linear regression analysis was used for identifying the independent determinants of thiol/disulfide homeostasis. Comparisons of thiol values between pack/year categories were conducted with Kruskall-Wallis non-parametric

Table I. Baseline characteristics of study groups.

		Smokers		Controls		
		n=84	n(%)	N=86	n(%)	*p*
Gender	Female	17	20.2	68	79.1	
	Male	67	79.8	18	20.9	<0.001
		Mean±SD		Mean±SD		
Age (year)		39.1±9.0		37.3±9.1		0.154
Height (cm)		169.8±6.8		165.5±8.3		<0.001
Body weight (kg)		77.6±15.1		72.2±15.8		0.014
BMI (kg/m^2)		26.9±4.9		26.3±5.2		0.436

variance analysis. In case of a significant result in multiple independent group comparisons, post-hoc tests were performed with Mann-Whitney U test. Spearman test was used for the correlations between CO and other parameters. All analyses were performed as two-sided hypotheses with a 5% of significance level and 95% of confidence interval. SPSS® 21 (IBM Inc, Armonk, NY, USA) software was used for the data analyses.

Results

Basic characteristics of the study groups were presented in Table I. Accordingly, the gender distribution was male dominant (p<0.001), and height (p<0.001) and body weight (p=0.014) were higher in smokers, but the body mass index was similar with the control group (p=0.436).

Mean age of smoking initiation was 15.4±5.0 years. Mean number of cigarette consumption was 26.8±1.7 pack/years. The CO levels of smokers and control group were 6.0±0.3 ppm and 0.3±0.5 ppm, respectively, and the difference was statistically significant (p<0.001).

The native, total, and native/total thiol levels of smoking patients were significantly lower (p<0.001 for each), and disulfide, disulfide/native thiol, and disulfide/total thiol levels were significantly higher than the control group (p<0.001 for each) (Table II).

The multivariate linear regression analysis for determining the independent determinants of TDH in smokers revealed that native thiol levels were affected by age, and total thiol levels were affected by gender and age. Increases in age significantly decreased both parameters, and male gender significantly increased the total thiol levels (Table III).

When the smokers were grouped according to smoking history as <20 pack/years, 21-40 pack/years, and ≥41 pack/years, no significant differences were found between groups for native thiol (p=0.185), total thiol (p=0.150), disulfide (p=0.618), disulfide/native thiol (p=0.963), disulfide/total thiol (p=0.963), and native thiol/total thiol (p=0.963) (Table IV).

The CO levels of all study participants were negatively correlated with native thiol (r= -0.627, p<0.001), total thiol (r= -0.569, p<0.001), native thiol/total thiol (r= -0.515, p<0.001), and positively correlated with disulfide (r=0.398, p<0.001), disulfide/native thiol (r=0.515, p<0.001) and disulfide/total thiol (r=0.515, p<0.001) levels (Table V).

Table II. Plasma thiol/disulfide levels in smokers and control group.

	Smokers Mean±SD	Controls Mean±SD	*p*
Native thiol (µmol/L)	328.8±52.93	407.59±50.2	<0.001
Total thiol (µmol/L)	370.31±52.9	435.7±48.87	<0.001
Disulfide (µmol/L)	20.76±7.29	14.05±6.5	<0.001
Disulfide/native thiol (%)	6.58±2.84	3.55±1.87	<0.001
Disulfide/total thiol (%)	5.71±2.13	3.26±1.57	<0.001
Native thiol/total thiol (%)	88.58±4.27	93.47±3.13	<0.001

Table III. Factors affecting thiol levels.

		B	Standard Error	95% CI	p
Native thiol (μmol/L)	Constant	375.811	25.545	325 - 426.6	<0.001
	Age (year)	-1.201	0.636	-2.5 - 0.1	0.063
Total thiol (μmol/L)	Constant	365.051	37.304	290.8 - 439.3	<0.001
	Gender (reference: female)	27.151	13.969	-0.6 - 54.9	0.055
	Age	-1.113	0.628	-2.4 - 0.1	0.08

Table IV. Comparisons between pack/year groups.

	<= 20 pack/years Mean±SD	21-40 pack/years Mean±SD	>40 pack/years Mean±SD	p
Native thiol (μmol/L)	337.45±51.94	318.32±56.61	342.24±25.58	0.185
Total thiol (μmol/L)	379.8±48.04	358.42±59.44	387.1±19.01	0.150
Disulfide (μmol/L)	21.17±8.61	20.05±6.14	22.43±6.58	0.618
Disulfide/native thiol (%)	6.58±3.17	6.56±2.69	6.67±2.28	0.963
Disulfide/total thiol (%)	5.68±2.43	5.71±1.96	5.82±1.76	0.963
Native thiol/total thiol (%)	88.63±4.85	88.58±3.92	88.36±3.53	0.963

Discussion

Oxidative stress is defined as a condition in which the level of oxidants is higher than the antioxidants, or a condition that causes free radical damage due to the imbalance in oxidant and antioxidant systems[19]. Cigarette smoke contains many compounds, which majority of them are oxidants and pro-oxidants that produce free radicals and causes damage in the organism by oxidative stress[20,21]. As a consequence, cigarette smoke creates a chronic oxidant stress on the respiratory system. Previous studies showed that free radicals in cigarettes cause oxidative stress in active and passive smokers[2]. Various techniques were used in those researches to determine the increased oxidative stress due to smoking[2,22]. TDH is one of the most important systems that ensure optimal redox balance in humans[23]. To the best of our knowledge, this is the first study that evaluates the TDH in smokers. According to our results, we found that TDH changes thru disulfide in these patients.

As a member of the antioxidant cascade, thiol group eliminates reactive oxygen species (ROS), and total thiol levels can be used to evaluate oxidative status[24]. Native thiol capacity was shown to linearly decreased by application of oxidation process[14]. Our work showed that native thiol and total thiol levels were significantly lower in smokers when compared to control group.

Thiol/disulfide balance causes a rapid and dynamic regulation, provides a redox signal, and plays a central role as a target of the oxidative stress. These features make serum thiol/disulfide ratio a useful measure for the clinical evaluation of the oxidative stress[14]. In this study, the disulfide levels, and disulfide/native thiol and disulfide/total thiol levels were found to be significantly higher in smokers when compared with the control group. This shows that TDH shifts to the left and oxidative stress increases in smokers.

Yilmaz et al[25] found in asphalt workers and Bal et al[26] found in individuals who were occupationally exposed to lead that disulfide, disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol levels were significantly higher when compared to control groups, and reported

Table V. Correlations between CO and other parameters.

	CO	
	r	p
Native thiol	-0.627	<0.001
Total thiol	-0.569	<0.001
Disulfide	0.398	<0.001
SSSH	0.515	<0.001
SSTotal thiol	0.515	<0.001
SH Total thiol	-0.515	<0.001

that TDH shifts thru disulfide. In the current study, we found that CO levels of all patients were negatively correlated with native thiol, total thiol, native thiol/total thiol, and positively correlated with disulfide, disulfide/native thiol, and disulfide/total thiol levels. This shows that TDH shifts thru disulfide and oxidative stress increases as the CO level increases.

Ates et al[27] reported that there is a positive correlation between oxidative stress and age, and native thiol levels are decreased with the increasing age. Babaoglu et al[28] also reported that there is a negative correlation between age, and total and native thiol. These data inform us that oxidative stress may increase with increasing age, and thiol/disulfide homeostasis may shift towards disulfide. Similar to the literature data, we found that native thiol and total thiol levels were decreased significantly with the increasing age. There were no statistically significant differences between the ages of smokers and non-smokers. As a consequence, age was not found as a determinant of oxidative stress in our study.

The gender distribution of smokers and non-smokers were significantly different in our work, and there were more males in the smoking group. The male gender was found to cause a significant increase in total thiol levels. High frequency of male patients among smokers might increase the total thiol levels, but total thiol levels were found to be significantly lower than the non-smoker group. If the distribution of the genders were even in both study groups, then the total thiol levels would be lower in smoking group.

Previous studies showed that BMI affects thiol levels[27,29]. In our investigation, there was no statistically significant difference between BMIs of smoking and non-smoking patients. So, we concluded that BMI had no effect on oxidative stress.

Determining the dynamic thiol/disulfide homeostasis may provide valuable information about the physiological and pathologic biochemical processes of diseases related to cigarette smoking. TDH is an indicator of oxidative stress. It is important to determine the dynamic thiol/disulfide status in diseases, which the oxidative stress plays important roles in the pathogenesis[14]. Because, interventions for increasing the thiol levels may prevent the pathological processes due to deteriorations in thiol/disulfide homeostasis[28]. Further studies are needed about the utilization of increased thiol and decreased disulfide levels for the follow-up of antioxidant treatments[30].

Conclusions

Smoking changes thiol/disulfide homeostasis in favor of disulfide. This suggests that oxidant activity significantly increases, and antioxidant activity decreases. As a consequence, smoking causes an increase in oxidative stress. Increased oxidative stress due to smoking may trigger many chronic diseases. As determining the dynamic thiol/disulfide homeostasis might provide valuable information for elucidating the etiopathogenesis of smoking-related diseases, further studies for showing the changes in thiol/disulfide balance are warranted to contribute to the clinical practice.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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