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Original Article

Evaluation of Thiol/Disulfide Homeostasis in Lung Cancer

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Abstract

OBJECTIVES: Lung cancer is one of the most common causes of mortality all around the world. The increased production of reactive oxygen species occurs with cell damage, and cysteine is an important factor in preventing oxidative damage by its functional thiol group. The objective of this study was to evaluate the relationship between thiol/disulfide homeostasis (TDH) and the risk factors, disease severity, and physical condition of patients with lung cancer.

MATERIALS AND METHODS: This is a prospective, controlled, nonblinded study, which included healthy volunteers and patients diagnosed with lung cancer who had not yet started any treatment.

RESULTS: There were 45 male (90%) and five female (5%) patients (mean age 64±9 years), and 41 male (82%) and nine female (18%) healthy volunteers (mean age 65±17 years) were included in this research. Overall, the thiol levels were lower in patients than the control group ($p<0.001$). The native thiol level means were 275±72 µmol/l in the patient group and 414±80 µmol/l in the control group, and the total thiol level means were 309±74 and 451±79 µmol/l, respectively. However, the disulfide parameter was not statistically significantly different between the two groups. There were no correlations between the tumor size and overall survival and the total thiol, native thiol, and disulfide levels.

CONCLUSION: This study showed that there is a significant relationship between lung cancer and TDH, but there were no correlations with the disease stage and the clinical performance status.

KEYWORDS: Lung cancer, oxidative stress, thiol/disulfide homeostasis, mortality

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INTRODUCTION

Lung cancer is a common cancer type, and it is an important cause of mortality worldwide. Oxidative stress describes a deterioration in the balance between the oxidation mechanisms and free oxygen radicals and the protective role of antioxidants. This causes damage to important cellular components, such as proteins, lipids, and DNA, which can result in mutagenesis [1]. When the amount of reactive oxygen species (ROS) exceeds the physiological levels, the direct oxidative effect on DNA causes an increase in lipid peroxidation or structural changes in DNA, causing cellular structure deterioration [2].

Thiol is an organic compound containing a sulfhydryl group that plays a critical role in preventing cellular oxidative stress. Cysteine, which is one of the defensive protein components of the body, plays an important role in preventing oxidative damage via its functional thiol group [3]. The thiol groups of the sulfur-containing amino acids (cysteine, methionine) in proteins are the primary target of ROS [3]. In the presence of ROS, the thiol groups of the proteins, low molecular weight compounds, and cysteine residues, as well as other thiol groups, are oxidized in the cellular environment, and they are converted into structures with reversible disulfide bonds. These disulfide bond–containing structures can be reduced back to thiol groups, which is the mechanism by which thiol/disulfide homeostasis (TDH) works [3]. Oxidative stress increases the redox imbalance in cancer cells, when compared with normal cells, which suggests that a redox imbalance may be associated with oncogenic stimulation [4]. Dynamic TDH plays critical roles in antioxidant defense mechanisms, detoxification apoptosis, enzyme activity regulation, transcription, and cellular signal transduction mechanisms [3]. Some of the antioxidant system components that work to eliminate free oxygen radicals are catalase, superoxide dismutase, glutathione (GSH) redox system parameters, GSH disulfide, GSH peroxidase, reduced GSH , thioredoxin system components, vitamin E, and vitamin C [2].

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Lung cancer cells develop in a broad oxidative environment, which is believed to increase tumor progression and metastasis development. Moreover, high oxidative biomarker levels are associated with tumor aggressiveness [5]. The objective of this study was to evaluate the relationships between the oxidative status and lung cancer.

MATERIALS AND METHODS

This was a controlled, nonblinded prospective study. An ethics committee approval was obtained from the local ethics committee. The study included 50 patients with lung cancer who were newly diagnosed and had not yet undergone any treatment and 50 healthy volunteers. All of the study participants were over 18 years of age, and informed consent was obtained from all of them. Blood samples were collected from each participant once during admission. The parameters that were used to evaluate the TDH were measured with an automatic analyzer via the Erel and Neşelioğlu spectrophotometric method. The disulfide (D), native thiol (NT), and total thiol (TT) levels were studied using this method. In addition, indexes 1-3 (index 1=D/NT, index 2=D/TT, and index 3=NT/TT) were calculated with these parameters.

For the lung cancer staging, the 7th edition of the International Association for the Study of Lung Cancer Tumor/Node/Metastasis Classification of Malignant Tumors was used (6). After the blood samples were taken from the patients, treatment was provided according to the National Comprehensive Cancer Network guidelines. The period ranging from the diagnosis to the time of death for any reason was defined as the overall survival, and this was recorded for each patient. The Eastern Cooperative Oncology Group (ECOG) Performance Status scale was used to evaluate the performance status of each of the patients.

Statistical Analysis

The Statistical Package for the Social Sciences for Windows 16.0 (SPSS Inc.: Chicago, IL, USA) was used for the statistical analysis. The normality of data was analyzed with the Kolmogorov-Smirnov test, and the mean±standard deviation (SD) was used for the normally distributed data, and the median and minimum-maximum (min-max) were used for the data that were not normally distributed. A Mann-Whitney U test was used for the parameters that were not normally distributed, and an independent samples *t* test was used for the parameters that were normally distributed. For the independent multiple groups, a one-way analysis of variance was used for the data that were normally distributed, and a Kruskal-Wallis test was used for the data that were not normally distributed. The Bonferroni correction was used for the subgroup analysis. For the correlation analysis, a Pearson test and Spearman test were used according to normality. A p value of <0.05 was used for statistical significance.

RESULTS

This study included 45 males (90%) and five females (10%) in the patient group and 41 males (82%) and nine females (18%) in the control group (healthy volunteers). The gender distributions were homogeneous between the patient and control groups (p=0.249). The mean ages were 64 ± 9 years for the patient group and 65±17 years for the control group (p=0.063).

The lung cancer types are shown in Table 1. a, b, with adenocarcinoma (n=21, 42%) being the most common type in this study. The lung cancer stages and performance statuses are also shown in Table 1. a, b. The NT, TT, and index 3 values were statistically significantly lower, whereas values for indexes 1 and 2 were statistically significantly higher in the lung cancer group (p<0.001). The D levels were not signifi-

Table 1a. Demographic features and performance status

Gender, n (%)	Patient (n=50)	45 (90%) Male
	Control (n=50)	41 (82%) Male
Age (Mean±SD)	Patient	64±9
	Control	65±17
ECOG		
0≤2	41 (82)	
0>2	9 (18)	

ECOG: The Eastern Cooperative Oncology Group

Table 1b. Cancer characteristics

Type and Stage	Values, n (%)
Type	
Histologic type	
Adenocarcinoma	21 (42)
Squamous	15 (30)
Small	11 (22)
Others	3 (6)
Stage	
1B	1 (2)
3A	6 (12)
3B	7 (14)
4	36 (72)
Metastasis	
No	12 (24)
Yes	38 (76)

MAIN POINTS

- Lung cancer cells develop in a broad oxidative environment, which is believed to increase tumor progression and metastasis development.
- Oxidative stress increases the redox imbalance in cancer cells which suggests that a redox imbalance may be associated with oncogenic stimulation.
- Dynamic thiol/disulfide homeostasis plays critical roles in antioxidant defense mechanisms, detoxification apoptosis, enzyme activity regulation, transcription, and cellular signal transduction mechanisms
- This study showed that low thiol levels were related to an oxidant/antioxidant balance deterioration in the lung cancer.

Table 2. Age and thiol/disulfide homeostasis parameters in patient and control groups

	Control			Patient			
Parameters	**Mean±SD**	**Med**	**Min-Max**	**Mean±SD**	**Med**	**Min-Max**	**p**
Age*	65±17	72	21-84	64±9	66	37-85	0.063
NT**	414.4±80.2	421.7	186-578.3	275.2±73	276.5	132.2-484.5	<0.001
TT**	450.9±79.1	461.6	215-607.3	309±73.2	313.2	153.4-513.8	<0.001
D**	18.3±6.9	18.1	5.1-35.7	16.9±5.9	16.7	4.5-33.5	0.28
Index 1*	0.046±0.02	0.046	0.009-0.103	0.067±0.038	0.058	0.017-0.253	0.001
Index 2*	0.042±0.017	0.043	0.009-0.085	0.057±0.026	0.052	0.017-0.168	0.001
Index 3*	0.916±0.034	0.915	0.829-0.983	0.886±0.052	0.896	0.664-0.967	0.001

*Mann-Whitney U test **Independent samples t test
NT: native tiyol; TT: total tiyol; D: disulfide; Index-1: D/NT, Index-2: D/TT; Index-3: NT/TT

Table 3. Relationship between tumor size and overall survival with NT, TT, and D

			NT	TT	D
Pearson correlation	Tumor size	Pearson correlation	-0.08	-0.099	-0.122
		p	0.583	0.494	0.4
		n	50	50	50
Spearman rho	Overall survival	Correlation coefficient	0.064	0.045	-0.07
		p	0.658	0.756	0.63
		n	50	50	50

NT: native thiol; TT: total thiol; D: disulfide

Table 4. The relationship between the presence of metastasis and TDH (independent samples t test)

	Metastasis						
	No (n=12)			Yes (n=38)			
	Mean±SD	**Med**	**Min-Max**	**Mean±SD**	**Med**	**Min-Max**	**p**
NT	271.4±61.2	271.9	177.2-371.3	276.7±77.8	279.2	132.2-484.5	0.821
TT	302.0±63.8	304.7	202.4-405.9	311.7±77.2	315.5	153.4-513.8	0.677
DD	15.3±5.3	14.2	10.2-27.1	17.5±6.1	17.3	4.5-33.5	0.236

NT: native thiol; TT: total tiyol; D: disulfide

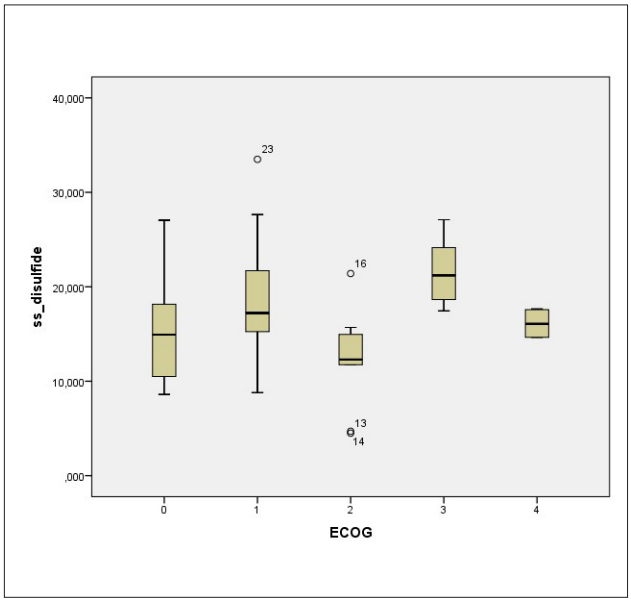

Figure 1. ECOG performance status-disulfide levels

cantly different (p=0.280). The NT value means were 275±72 µmol/l in the patient group and 414±80 µmol/l in the control group, and the TT value means were 309±74 and 451±79 µmol/l, respectively (Table 2). The tumor size median was 55 mm (min-max: 15-100 mm). In 12 (24%) of the total patients, metastases were not detected during diagnosis, but 38 (76%) patients had metastases. There was no correlation between the tumor size and the overall survival and the NT, TT, and D values (Table 3). In addition, there were no statistically significant differences in the NT, TT, and D values between the groups in terms of the metastasis presence (Table 4).

The patients were grouped according to their ECOG values (0-4), and the differences in the NT, TT, and D values and indexes 1-3 were analyzed. On the basis of the results of the Kruskal-Wallis test, a difference was only detected in the D parameter (p=0.020). A *post hoc* analysis was performed for the D parameter, and the difference was due to the differences between ECOG groups 2 and 3 (adjusted p=0.022) (Figure 1).

Table 5. NT, TT, and DD in overall survival time

Statistics		n	Mean±SD	Standard error	95% CI	Min-max	p*
NT	0-6 months	21	261.4±70.1	15.3	229.5-293.4	144-441.2	0.138
	6-12 months	12	312.6±74.7	21.6	265.1-360	207.7-484.5	
	12-18 months	7	241.7±72.7	27.5	174.5-309	132.2-351.8	
	Over 18 months	10	282.8±67.1	21.2	234.8-330.7	161.3-377.3	
	Total	50	275.2±73	10.3	254.5-296	132.2-484.5	
TT	0-6 months	21	295.5±75.5	16.5	261.1-329.9	153.4-474.4	0.186
	6-12 months	12	344.9±72.1	20.8	299.1-390.7	241.9-513.8	
	12-18 months	7	279.2±72.4	27.3	212.3-346.2	199.2-405.9	
	Over 18 months	10	315±61.4	19.4	271.1-358.9	208.9-401	
	Total	50	309±73.2	10.3	288.2-329.8	153.4-513.8	
D	0-6 months	21	17±6.4	1.4	14.1-19.9	4.5-27.7	0.797
	6-12 months	12	16.2±4.7	1.3	13.2-19.1	8.6-24.2	
	12-18 months	7	18.8±8.3	3.1	11.1-26.4	11.9-33.5	
	Over 18 months	10	16.1±5	1.6	12.6-19.7	10.3-24.6	
	Total	50	16.9±5.9	0.8	15.2-18.6	4.5-33.5	

*One-way ANOVA

The mean overall survival time was 10.6±9 months (min-max: 0.1-29.0 months). There were no correlations between the NT, TT, and D values and indexes 1-3 and the overall survival (p>0.05) (Table 5). Moreover, we did not detect any significant differences in the NT, TT, and D parameters when the overall survival time was grouped by 6-month intervals (Table 5).

DISCUSSION

ROS play important roles in the pathogenesis of many lung diseases, such as pneumonia, bronchial asthma, bronchopneumonic dysplasia, pneumoconiosis, emphysema, acute respiratory distress syndrome, hyperoxia, cystic fibrosis, and bleomycin toxicity [7]. One study revealed that the total antioxidant status serum values were significantly lower in patients with severe asthma attacks and community-acquired pneumonia than in healthy subjects. It has also been reported that these results indicate an oxidant/antioxidant balance disorder, probably related to an excessive oxidative load [7].

Oxidant/antioxidant balance degradation is involved in the etiology of lung cancer, and in many previous studies, high oxidant levels and insufficient antioxidant effects in the lung peripheral blood, exhaled respiratory condensate, epithelial fluid, and tumor biopsies were found to be related with lung cancer development [8, 9]. In particular, the nonenzymatic antioxidant effects (especially the TT and nonprotein thiol) are very important because these thiol groups maintain normal cellular functions and the main redox homeostasis [10].

In our study, the NT and TT values were statistically significantly lower in the lung cancer group. The fact that the thiol level, a nonenzymatic antioxidant, was low in the lung cancer group also supports the results of previous studies. In the Gupta et al. [11] study, the GSH and superoxide dismutase levels were lower in the non-small-cell lung cancer group than the control group. On the basis of these results, one can interpret that the inadequacy of the antioxidant system in the detoxification of superoxide radicals is important in the development of cancer.

In our study, no remarkable relationship was detected between the tumor size and the presence of metastasis and the TT, NT, and D values. Lung cancer develops in a broad oxidative environment, and high oxidative biomarker levels are believed to be associated with metastasis and tumor aggressiveness. Therefore, it is believed that the evaluation of biomarkers in early stage cancer is not useful [12]. In our study, a comparative analysis with advanced stage cancer could not be performed because the number of cases with early stage lung cancer was low. However, no difference was found between stage 3 and stage 4 lung cancer. Different results may be obtained in studies with early stage lung cancer cases. In one breast cancer study, the total oxidant status and oxidative stress index (OSI) were significantly increased in the disease group, and these values were closely related to the disease stage [13]. Many studies have been conducted on the roles of oxidative stress in metastasis and tumor aggression, and the ROS levels have been associated with increased tumor invasion and a poor prognosis [14-16]. In some previous studies, isothiocyanates, which are natural antioxidants found in cruciferous vegetables, were found to increase apoptosis and arrest in the cell cycle and to decrease the development of metastases through different enzymatic pathways [17, 18].

We did not detect any significant difference in the NT and TT values between the ECOG performance status groups; however, there was a significant difference in the D levels between ECOG groups 2 and 3. As the D value is also affected by different pathways, it may not be possible to discuss D alone [19]. In a similar study, the NT and TT levels were negatively correlated with the Karnofsky performance status [5].

In our study, there were no relationships between the overall survival time and the NT, TT, and D levels. In the Dirican et al. [5] study, a relationship was found between TDH and overall survival (OS), and low plasma NT and D levels were found to be associated with a short OS. In one study, there were no differences between the oxidative stress status and the OS and performance statuses of terminal cancer patients, but the performance status of the patient group in this study consisted of only ECOG groups 3 and 4 [20]. However, in a study in which patients with nonmetastatic breast cancer were included, a significant relationship was found between the antioxidant and oxidant biomarker levels and OS [21]; in another similar study [22], only oxidant stress level was found to be correlated with survival in patients with metastatic breast cancer. It has been found that the GSH level and GSH-related enzyme activity were associated with chemotherapy resistance in the treatment of glioblastoma multiforme [23]. In another study, low GSH reductase activity was associated with cisplatin resistance in epithelial ovarian cancer cases [24]. On the basis of these results, one can argue that oxidative stress can lead to a poor prognosis and treatment resistance. In a study of patients with stage 3 gastric cancer by Du et al. [25], it was emphasized that the OSI level was an independent biomarker predictor in the overall survival evaluation. Additionally, percentages of mortality were higher in the patients with higher OSI levels, regardless of lymph node involvement and the histological subtype [25]. To understand the value of TDH in the prognosis determination, additional studies are needed with larger patient groups in which the cancer subtypes are homogenized.

Our study showed that the NT and TT levels were low in the lung cancer cases, and these values were related to an oxidant/antioxidant balance deterioration. These results express the roles of the oxidative stress factors in lung cancer cases. Some of the important limitations of this study were the nonhomogeneous distribution of the disease stages and the low number of cases. There is a need to conduct studies with higher patient numbers to evaluate the roles of TDH in the clinical stages and in estimating the prognosis. Future studies should be planned to evaluate TDH with regard to the treatment response and drug resistance, and these studies should be performed before and after the treatment. Finally, we believe that this easily accessible biomarker method can be used in the follow-up of the treatment of patients with lung cancer in the future.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethical Committee of Keçiören Training and Research Hospital in September 28, 2016 (no: 15/1203).

Informed Consent: Written informed consent was obtained from the patients participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.U.Ş.; Design - M.U.Ş., Ö.S.; Supervision - İ.A.K., Y.E.; Resources - M.U.Ş., Ö.E.; Materials - S.B., Ö.S.; Data Collection and/or Processing - M.U.Ş, Ö.S.; Analysis and/or Interpretation - M.U.Ş, M.A.; Literature Search - M.U.Ş, Ö.S., Y.E.; Writing Manuscript - M.U.Ş.; Critical Review - Y.E., Ö.E.

Conflict of Interest: The authors have no conflicts of interest to declare.

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