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The Effect of Thiol-Disulfide Homeostasis on Prognosis in Patients with Early-Stage Non-Small Cell Lung Cancer

Erken Evre Küçük Hücreli Dışı Akciğer Kanserli Hastalarda Tiyol-Disülfid Homeostazisin Prognoz Üzerine Etkisi

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ABSTRACT

Objective: Lung cancer has a poor prognosis. Thiol groups with high antioxidant capacity are converted to disulfide bonds through biochemical reactions that neutralize different oxidant compounds. The thiol-disulfide (SH-SS) homeostasis has significant effects on cell mechanisms, transcription, and apoptosis. Here we present the prognostic role of dynamic SH-SS homeostasis in patients operated for NSCLC.

Materials and Methods: Patients operating for early-stage NSCLC were prospectively analyzed. SH-SS homeostasis tests were measured using the automated spectrophotometric method.

Results: This study enrolled 138 subjects, including 77 patients and 61 healthy controls. Native thiol and total thiol levels were significantly lower in the patient group. The disulfide-to-native thiol ratio, which is an indicator of oxidative stress in SH-SS homeostasis, also reached a level of statistical significance in the patient group ($p<0.001$). According to the cut-off values (305 and 326.3), the median overall survival rate was significantly shorter in patients with low native thiol and total thiol levels ($p<0.001$).

Conclusions: This study demonstrated decreased native thiol and total thiol levels as well as decreased disulfide levels and SS/SH ratio in early-stage NSCLC. Impaired SH-SS homeostasis may contribute to lung cancer pathogenesis and poor prognosis because of enhanced oxidative stress.

Keywords: Disulfide, early-stage lung cancer, NSCLC, oxidative stress, thiol

ÖZ

Amaç: Akciğer kanseri kötü prognoza sahiptir. Antioksidan kapasitesi yüksek olan tiyol grupları, farklı oksidan bileşikleri nötralize eden biyokimyasal reaksiyonlarla disülfid (SS) gruplarına dönüşürler. Tiyol/Disülfid homeostazı (TDH) hücre mekanizmaları, transkripsiyon ve apoptoz üzerinde önemli etkilere sahiptir. KHDAK nedeniyle ameliyat edilen hastalarda dinamik TDH'nin prognozunun rolünü sunuyoruz.

Materyal ve Metot: KHDAK ile erken evre opere edilen hastalarda prospektif olarak analiz edildi. TDH testleri otomatik spektrofotometrik yöntemle ölçüldü.

Bulgular: Bu çalışmaya 77 hasta, 61 sağlıklı olmak üzere toplam 138 kişi katıldı. Native tiyol (NT) ve total tiyol (TT) düzeyleri hasta grubunda anlamlı olarak düşüktü. TDH'nin oksidatif stres göstergesi olan SS/NT oranı da hastalarda istatistiksel olarak anlamlı bulunmuştur ($p<0,001$). Medyan OS, NT ve TT düzeyi düşük olan hastalarda cut-off değerine göre (305 ve 326,3) anlamlı olarak daha kısaydı ($p<0,001$).

Sonuç: Bu sonuçlar erken evre KHDAK'de; doğal tiyol ve toplam tiyol seviyeleri azaldığını, disülfid seviyeleri ve disülfid/NT oranının da bozulduğunu göstermektedir. Bozulmuş tiyol/disülfid homeostazı, artan oksidatif stresin bir sonucu olarak akciğer kanseri patogenezine ve kötü prognoza katkıda bulunabilir.

Anahtar Kelimeler: Disülfid, erken evre akciğer kanseri, KHDAK, oksidatif stres, tiyol

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INTRODUCTION

Lung cancer is the leading cause of cancer deaths.[1] Non-small-cell lung cancer (NSCLC) accounts for most lung cancer cases. Despite the availability of new genetic technologies and advancements in surgical techniques, the overall 5-year survival rate for lung cancer remains at 15.6%. Genetic and local tissue modifications are also involved in lung carcinogenesis. Exposure to carcinogens modifies normal cells before clinically detectable malignant tumors occur.[2]

There are limited data about clear antioxidant information on the antioxidant levels in lung cancer. Reduction and oxidation stabilities depend on the action between the antioxidant systems. Thiols contribute to the total antioxidants present in the body and play a vital role in the defense against ROS. Total thiols consist of thiols either in the free form or reduced glutathione (GSH).[3]

The serum level of thiols indirectly reflects the antioxidant defense system. Disulfide bonds can be reconverted to thiols, consequently resulting in dynamic thiol-disulfide (SH-SS) homeostasis, which is the reversal of thiol oxidation. Dynamic SH-SS homeostasis plays a critical role in antioxidant protection.[4,5]

Abnormal SH-SS homeostasis status is involved in the pathogenesis of various diseases. Erel and Neselioglu developed a novel automated colorimetric method to be able to separately measure the two elements for the serum value of thiol. [4]

This prospective case-control study evaluated the relationship between dynamic SH-SS homeostasis and early-stage NSCLC.

MATERIALS AND METHODS

Ethical Statement: This study was approved by the Ethics Committee of Yıldırım Beyazıt University Faculty of Medicine (Date: 15.06.2020, decision no: 185). All participants provided informed consent. The study adhered to the principles of the Declaration of Helsinki and was conducted by the international declaration guidelines.

Design, Participants, and Setting: This was a prospective, non-randomized, and case-control study. Patients who were operated on early-stage NSCLC between 2014 and 2019 were analyzed. A total of 138 individuals, including 77 patients and 61 health controls, participated in this study. Seventy-seven patients (54 male and 23 female, with a mean age of 58 years) included in this study were newly diagnosed with and operated on stage I and II NSCLC. Serum samples of patients were collected at the time of diagnosis and before surgery. The American Joint Committee on Cancer staging system was used to classify lung cancer. Positron Emission Tomography (PET) and contrast-enhanced brain Magnetic Resonance Imaging (MRI) were performed for cancer staging. The control group consisted of demographically-matched volunteers who presented for health screening. The patients did not receive any anticancer treatment at diagnosis. The overall survival was calculated from the date of diagnosis to the last follow-up or death from any cause. Subjects with an active infection, chronic inflammatory, or autoimmune disease, both in the patient and healthy control groups, were excluded from the study. The status of SH-SS homeostasis was evaluated in both groups.

Measurement of SH-SS Homeostasis Parameters

Samples: Blood samples were obtained from a phlebotomist. Five ml of blood was collected from each subject into serum-separating tubes to measure the SH-SS homeostasis parameters. To measure the SH-SS homeostasis parameters, serum-separating tubes were allowed to clot for 15 min and then centrifuged. Serum samples were stored at -80°C. All samples were thawed on the same day of measurement, and laboratory analyses were carried out using an automated analyzer (Cobas c501, Roche-Hitachi, Mannheim, Germany).

Determination of SH-SS Homeostasis Parameters: Serum samples were used to determine the SH-SS homeostasis parameters using the method described by Erel and Neselioglu.[5]

This method involved two parallel analyses. Briefly, native SH contents of the sample were first measured with Ellman's reagent [5,5′-dithiobis (2-nitrobenzoic acid), DTNB]. In a parallel analysis, dynamic disulfide bridges were reduced to thiol groups by sodium borohydride. After the reduction procedure, excess unused sodium borohydride was removed with formaldehyde. After this reduction and removal process, the total thiol contents (2 thiols from each disulfide and one thiol from each native thiol of samples) of the sample were measured using DTNB. Disulfide levels were calculated as half the difference between serum levels of total and native thiol. Total thiol (SH+SS), native thiol (SH), and disulfide (SS) were expressed as μmol/L. Moreover, the percentage ratios of disulfide-to-native thiol (SS/SH), disulfide-to-total thiol (SS/SH+SS), and native thiol-to-total thiol (SH/SH+SS) were calculated.

Statistical Analysis: Statistical analysis was conducted using the IBM SPSS Statistics (Version 20) computer program (IBM, Armonk, NY, USA, 2011). Normal assumptions of variables were analyzed with the Kolmogrov-Smirnov test. Descriptive were expressed as median (1^{st}-3^{rd} quartile value) or mean±standart deviation for non-parametrically or parametrically distributed variables, respectively. Comparison of continuous variables was performed with the Mann-Whitney U test or Student's t-test for

non-parametrically or parametrically distributed variables, respectively. Categorical variables were shown as numbers (%) and compared with the Pearson Chi-square test or Fisher's exact test, which was appropriate. Correlation analysis was conducted with the Spearman test. Cut-off points were determined by receiver operating characteristic (ROC) curve analysis based on survival status. Sensitivity and specificity were calculated according to these cut-off points, and the patients were divided into two groups according to this cut-off point. Kaplan-Meier survival analysis was performed to evaluate the effects of the determined cut-off point on survival. A p -value<0.05 was accepted as statistically significant.

RESULTS

This study enrolled 77 patients with NSCLC (mean age of 58.36±9.16 years) and 61 health controls (mean age of 60.56±7.92 years). There was no significant difference between the patient and the control groups regarding age or gender (p>0.05 for both). The native thiol (SH) and total thiol (SH+SS) levels were lower in the patient group. The disulfide level and the percentage ratios of SS/SH, SS/SH+SS, and SH/SH+SS were statistically higher in the patients (p<0.001). All patients were classified by mortality and survival rates. There was no difference in mortality by age, gender, and histopathological types. Native thiol and total thiol levels were lower in those who died during the follow-up period. However, the percentage ratios of SS/SH, SS/SH+SS%, and SH/SH+SS were not correlated with mortality (Table 1).

In this study, the cut-off value for the native thiol level to predict mortality was determined using ROC analysis. The sensitivity of the native thiol level and the specificity of the disulfide level were 91.3% and 50%, respectively (p=0.0002) (Figure 1).

The sensitivity and specificity of the total thiol level were 65.2% and 70.4%, respectively (Figure 2).

Table 1. Thiol/disulfide homeostasis parameters and histopathological types of mortality and survival groups.

	Control Group (n=61)	Patient Group (n=77)	p-value
Age, years	58.36±9.16	60.56±7.92	0.140
Gender (female/male)	9/52	8/69	0.438
Native thiol (SH), µmol/L	430.41±48.46	288.41±62.07	0.001
Total thiol (SH+SS), µmol/L	470.4±49.41	339.95±70.26	0.001
Disulfide (SS), µmol/L	19.99±6.61	25.6±11.68	0.001
SS/SH%	4.71±1.66	9.27±4.78	0.001
SS/SH+SS%	4.27±1.38	9.77±13.41	0.001
SH/SH+SS%	91.47±2.76	84.86±6.10	0.001
Patients	Mortality Group (n=23)	Survival Group (n=54)	p-value
Age, years	59(54-66)	62(55.75-65.25)	0.978
Female/Male	1/22	7/47	0.423
Squamous cell ca/Adenocarcinoma	13/10	22/32	0.203
Survival, months	13(7-19)	34.5 (27-40)	0.001
Native thiol (SH); µmol/L	252.87±58.4	303.55±57.68	0.001
Total thiol (SH+SS); µmol/L	303.36±71.14	355.54±64.41	0.002
Disulfide (SS); µmol/	20.88(15.12-36.4)	25.9 (16.93-34.13)	0.361
SS/SH%	8.55(6.49-12.64)	7.91 (5.88-11.91)	0.701
SS/SH+SS%	7.35(6.03-10.33)	7.2 (5.26-9.64)	0.407
SH/SH+SS%	85.39(79.82-88.5)	86.72 (80.76-89.48)	0.567

Numerical data are shown as the median ± standard deviation.

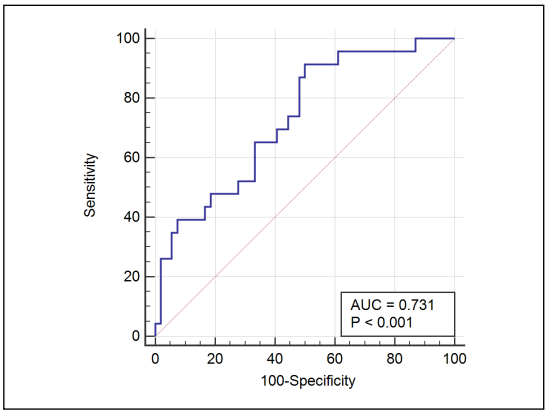

Figure 1. Receiver operator characteristic curve for the native thiol level in the prediction of mortality.

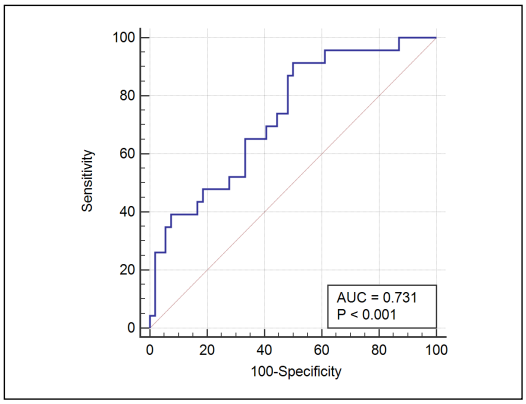

Figure 2. Receiver operator characteristic curve for the total thiol level in the prediction of mortality.

The median follow-up duration of the patient group was 35 months. According to the cut-off value (305 µmol/L and 326.3, respectively), the median overall survival (OS) was significantly shorter in patients with low native thiol (Figure 3) and total thiol levels (Figure 4) (p<0.001).

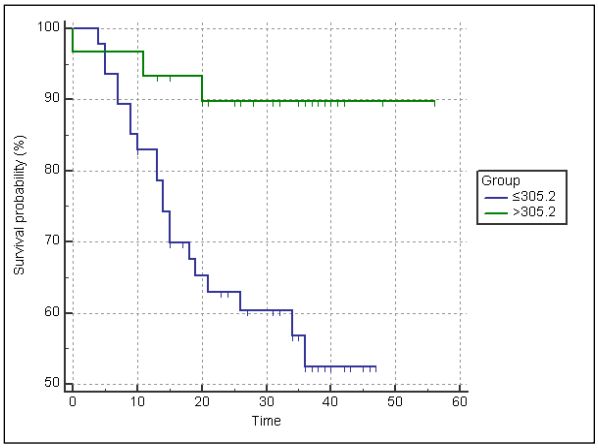

Figure 3. Kaplan-Meier survival analysis for native thiol with a cut-off point of 305.2 (Log-rank test, P=0.0001).

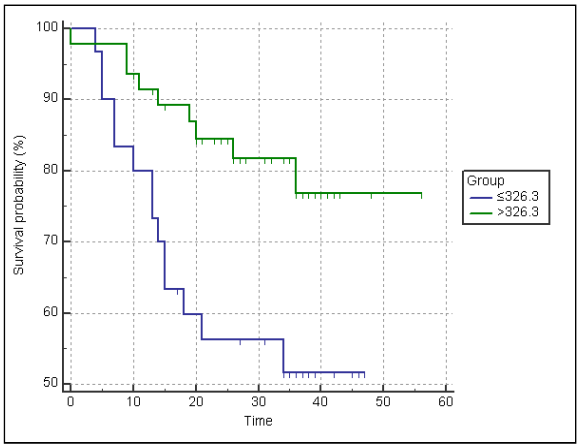

Figure 4. Kaplan-Meier survival analysis for total thiol with a cut-off point of 326.3 (Log-rank test, P=0.0110).

DISCUSSION AND CONCLUSION

This study demonstrated reduced native thiol and total thiol levels in patients compared to the control subjects. The percentage ratios of SS/SH, SS/SH+SS, and NT/SH+SS, which are indicators of oxidative stress, also reached a level of statistical significance in patients. The data of this study suggest that the status of SH-SS homeostasis may be altered in patients with early-stage lung cancer due to ROS-induced oxidation/reduction reactions. These results suggest that low plasma levels of native thiol and total thiol are associated with poor survival.

In a study, NT and TT levels were found to be low in patients with lung cancer. This study included patients with both early-stage and advanced-stage lung cancer. Despite this heterogeneous population, these low levels indicate that oxidant/antioxidant balance is impaired by lung cancer.[1]

While the native thiol and total thiol levels were lower in lung transplant patients compared to the control group, their disulfide levels were similar. Higher disulfide-to-native thiol (SS/SH) and disulfide-to-total thiol (SS/SH+SS) ratios have been reported in lung transplant recipients.[6] The lung is a major target organ for damage caused by exogenous oxidants. Oxidative mechanisms play a role in carcinogenesis initiation, promotion, and progression. The lungs need antioxidants to protect against possible oxidative injury due to direct exposure to cancer agents and very high quantities of oxygen.

The study by Erel showed higher disulfide levels in inflammatory diseases but significantly lower disulfide levels in renal cell carcinoma and colon carcinoma.[4]

The lowest disulfide levels have been observed in aggressively growing tumors; however, in slow-growing tumors like basal cell carcinoma, the decrease has been reported to be at a subnormal level.[7]

In our study, the native thiol and total thiol levels were lower in the patient group. Moreover, the disulfide levels were higher in the patient group. There was no difference between the deceased and surviving patients. This can be explained by the fact that all patients evaluated in this study may have had early-stage lung cancer. There are a limited number of studies investigating the effects of thiol-disulfide hemostasis on cancer patients. In our study, the native and total thiol levels were lower in those who died during the follow-up period (p<0.001).

A study on patients with diabetic ketoacidosis (DKA) found low levels of native, and total thiol. This indicates the presence of oxidative stress in these patients. Disulfide bonds are formed because of thiol metabolism. According to Otal et al.[8] the disulfide level, which does not increase with increased thiol use, indicates the possibility of exposure of advanced proteins to oxidation in DKA patients.

The total thiol levels of our patients were similarly low, but the disulfide levels increased compared with the control group. This result demonstrates the oxidation mechanism in patients with early-stage lung cancer, but no intensive oxidation mechanism as in DKA patients. Thiol-disulfide homeostasis is impaired in many diseases of unknown etiology.[9]

A review assessed thiol-disulfide homeostasis in various skin diseases and found a shift toward the disulfide or thiol side in patients; however, it remained unchanged in some patients. This may vary depending on the stage and severity of the disease, and the level of oxidative stress and antioxidant capacity.[10]

In this study, the cut-off value for the native thiol level to predict mortality was determined using ROC analysis. The sensitivity of the native thiol level and the specificity of the disulfide level were 91.3% and 50%, respectively. The sensitivity and specificity of the total thiol level were 65.2% and 70.4%, respectively. The decreased antioxidant levels in patients with early-stage lung cancer may be associated with poor prognosis.

There is no information on the level of antioxidants, particularly in lung cancer. Studies have reported an increase in antioxidant activity in lung cancer, whereas other studies have shown decreased antioxidant activity.[11]

High oxidative biomarker levels are associated with metastasis and tumor aggressiveness in lung cancer. Therefore, evaluating biomarkers for early-stage cancer is not applicable. However, our results revealed an association between reduced antioxidant activity and mortality in early-stage NSCLC.

In conclusion, these results may demonstrate the roles of oxidative stress in lung cancer and the biomarkers that may be used to predict prognosis.

Ethics Committee Approval: This study was approved by the Ethics Committee of Yıldırım Beyazıt University Faculty of Medicine (Date: 15.06.2020, decision no: 185). All participants provided informed consent. The study adhered to the principles of the Declaration of Helsinki and was conducted by the international declaration guideline.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – MK, CAB; Supervision – CAB, ÖE, MA; Materials – MA, SAB, SAE; Data Collection and/or processing – AÖ, İY, MK, MA; Analysis and/or interpretation – SAB, EEK; Writing – SAE, MA, MK, ÖE.

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