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Evaluation of Dynamic Thiol/Disulfide Homeostasis in Patients with Non-Muscle Invasive Bladder Tumor

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What's known on the subject? and What does the study add?

Reactive oxygen species (ROS) can cause oncogenic transformation by damaging the normal functions of DNA and cellular structures. In transformed cells, intracellular ROS levels are maintained at a higher level than in normal cells due to abnormal metabolism. This may contribute to gene mutations involved in cancer initiation. Non-enzymatic antioxidants such as total thiol and non-protein thiol groups play a critical role in maintaining the intracellular structure and ensuring the function of normal cells. Sulfhydryl groups mediate the maintenance of redox homeostasis and elimination of free radicals. This study was determined that disruption in thiol/disulfide homeostasis may be effective in the development of superficial bladder tumors in cases where oxidant stress predominates over antioxidant mechanisms. The total thiol/disulfide balance plays a role in the etiology of bladder tumors and in many tumors and inflammations, and based on this, antioxidants may be beneficial in the prevention and treatment of bladder tumors. However, further more comprehensive studies should be conducted in order to obtain clearer and more definitive results on this subject.

Abstract

Objective: To evaluate the thiol-disulfide homeostasis in patients with a diagnosis of non-muscle invasive bladder tumor (NMIBC), which is a new oxidative stress marker, and to investigate the relationship between the development of NMIBC and native thiol, total thiol, and dynamic disulfide values.

Materials and Methods: Fifty-three patients who were operated for bladder tumor in Karabük University Karabük Training and Research Hospital, Clinic of Urology between February and November 2020 and diagnosed with NMIBC in the pathological examination and 60 healthy volunteers were included in the study. Plasma native thiol, total thiol and disulfide levels of these two groups were measured and compared.

Results: There was a statistically significant difference between the two groups in terms of native thiol, total thiol and disulfide values. In the subgroup analysis in those diagnosed with NMIBC, native thiol values were found to be 255,870 µmol/L in the low grade patient group and 169,420 µmol/L in the high grade patient group. This difference was statistically significant.

Conclusion: The thiol disulfide homeostasis shifted to the disulfide side in the NMIBC group. It was determined that an increase in serum disulfide level and a decrease in native thiol level may have diagnostic value in predicting NMIBC. In addition, in the group diagnosed with NMIBC, there was a significant decrease in native thiol values as the pathological grade increased. This was interpreted as a shift of the equilibrium towards the oxidant side as the tumor showed an aggressive course.

Keywords: Bladder cancer, thiol, disulfide

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Introduction

Bladder cancer, which is the second most common malignancy of the urinary system, is an important public health problem due to its aggressive course and poor prognosis (1,2). 70-80% of patients have a non-muscle invasive bladder tumor (NMIBC) at the time of diagnosis (3).

Oxidative stress plays an active role in the emergence and progression of various diseases, such as diabetes, hypertension and cancer (4). Reactive oxygen species (ROS) are physiologically released from aerobic cells and the release amount into the circulation increases in cases of cell damage (5).

ROS can cause oncogenic transformation by damaging the normal functions of DNA and cellular structures. In transformed cells, intracellular ROS levels are maintained at a higher level than in normal cells due to abnormal metabolism. This may contribute to gene mutations involved in cancer initiation (6). Chronic inflammation causes tissue damage by damaging nucleic acids, proteins and lipids because of ROS production. Tissue damage causes stem cell activation for tissue regeneration. Stem cells are damaged by ROS, and the resulting mutations can accumulate and lead to the development of carcinoma (7,8).

Non-enzymatic antioxidants such as total thiol and non-protein thiol groups play a critical role in maintaining the intracellular structure and ensuring the function of normal cells. Sulfhydryl (SH) groups mediate the maintenance of redox homeostasis and elimination of free radicals (9). In this study, we evaluated the relationship between the presence of oxidative stress and tumor formation and tumor characteristics by comparing the serum dynamic thiol/disulfide levels of patients with histologically diagnosed bladder tumors and healthy individuals. This is the first study on this subject in the English literature.

Materials and Methods

Study Groups

This study was planned as a prospective, non-randomized case-control study. Accordingly, patients who applied to Karabük University Training and Research Hospital, Clinic of Urology, between February 2020 and November 2020 were evaluated. Patients were divided into two groups as patients with NMIBC and healthy individuals. Fifty-three patients who underwent transurethral resection of bladder tumor (TUR-M) and were found to have NMIBC by histopathological analysis were included in the patient group. The control group was formed of 60 demographically matched volunteer participants among healthy individuals without any oncological diagnosis who applied to our clinic for general health screening.

In the control group, 2 patients with uncontrolled diabetes, 3 patients receiving anti-inflammatory therapy, 1 patient with hyperthyroidism, 2 patients with renal dysfunction, 1 patient with symptomatic heart failure, and 1 patient-receiving lymphoma treatment were excluded from the study. In the NMIBC group, 2 patients receiving anti-inflammatory therapy, 1 patient with uncontrolled diabetes, 1 patient with symptomatic heart failure, 4 patients with muscle-invasive bladder tumors in the pathology report, and 1 patient with colon carcinoma invasion into the bladder were excluded from the study.

Plasma native thiol, total thiol and disulfide levels, which indicate dynamic thiol/disulfide homeostasis (TDH), were measured in patients and healthy subjects who were planned to be included in the study after the initial evaluation (9). In the patient group, surgical and pathological data after TUR-B were examined and recorded.

Blood Sampling and Measurement of Dynamic Thiol/Disulfide Homeostasis

In patients diagnosed with NMIBC and healthy volunteers, blood samples were collected in empty and dry biochemistry tubes after 8 h of fasting to determine thiol/disulfite blood levels. After centrifuging at 1.500 rpm for 10 min, serum samples were stored at -80 °C. In this method, dynamic and reducible disulfide bonds in the samples were reduced to free functional thiol groups using sodium borohydride. $NaBH_4$ was removed with formaldehyde to prevent a reduction of unused reduced sodium borohydride to dithionite-2 nitrobenzoic compound (DTNB). Native thiol and total thiol levels were determined after reaction with DTNB and finally their levels were measured. Half of the difference between the amount of total thiol content and native thiol indicated the disulfide level.

At the end of the study, it was investigated whether there was a statistically significant difference between the two groups in terms of demographic data, native thiol, total thiol and disulfide levels. In the patient group diagnosed with NMIBC, a subgroup analysis was conducted to evaluate the relationship between tumor size and pathological data and native thiol, total thiol and disulfide levels.

Statistical Analysis

Statistical analysis was carried out using the SPSS 17.0 statistical package program. The compliance of numerical data with a normal distribution was checked with Kolmogorov-Smirnov test. Categorical variables are presented as frequency and percentage. Numerical variables were presented as mean and standard deviations or median and minimum-maximum values. Two independent means were compared with the Student's t-test and two independent medians were compared with the Mann-Whitney U test. The relationship between two independent categorical variables was examined with the chi-square (Fisher's Exact/Exact) test. All analyses were performed

at 95% confidence level (p<0.05 was accepted as statistically significant).

The G* Power (G* Power Ver. 3.0.10, Franz Faul, University Kiel, Germany, htpp://www.pyscho.uniduesseldorf.de/app/project/gpower) package program was used for determining sample size. The sample size was calculated as at least 31 individuals in each of the two groups with 62 individuals for a study with 95% power, Type I error (alpha) 0.05 and effect size 0.93.

This study was designed in accordance with the Declaration of Helsinki, with the approval of the local ethics committee (Karabük University Ethics Commitee) (2020/154). Informed signed consent was obtained from all participants.

Results

The data of 132 people (70 in the control group and 62 in the NMIBC group) were evaluated. After patients excluded based on exclusion criteria, a total of 113 patients, 101 males (89.4%) and 12 (10.6%) female, were included in the study. The mean age of the people included in the study was 69.9±10.26. Mean age was 72.19±10.24 in the patient group and 67.4±9.83 in the control group (p=0.13). The control group included 55 males (91.7%) and 5 females (8.3%), and the NMIBC group included 46 male (86.8%) and 7 female (13.2%) patients (p=0.401).

The control group and the patient group diagnosed with superficial bladder cancer were evaluated in terms of smoking and concomitant diseases. There were no significant differences between the two groups (Table 1).

When the pathology specimens of patients with NMIBC were evaluated in terms of tumor invasion, pTa tumors were detected in 35.8% (19 patients), pT1 high-grade tumors were detected in 50.9% (27 patients) and pT1 low-grade tumors were detected in 13.2% (7 patients) of the specimens. Additionally, when the patients were examined according to tumor size, tumor size was <3 cm in 41.5% (22 patients) and more than 3 cm in 58.6% (31 patients) of the patient group. In terms of tumor localization, it was observed that 67.9% were located on the right and left side wall, 24.5% on the bladder floor, and 28.3% were located on the bladder dome and posterior wall.

A single dose of intracavitary EpirubicinC 50 mg was given to 37.7% (20 patients) of the patients who did not develop complications in the early postoperative period. Patients with pathology results of T1 and high grade were resectioned approximately 4 weeks after the first operation. Intravesical Bacillus Calmette-Guérin treatment was administered to 34 patients (64.1%) according to the pathology results. Tumor recurrence was observed in 13 patients (24.5%) at a mean follow-up of 20 months.

When the control group and the NMIBC group were evaluated in terms of native thiol values, it was observed that the native thiol values were significantly higher in the NMIBC group (p<0.001) (Table 2).

Total thiol and disulfide values were found to be higher in the NMIBC group compared to the control group. Mean total thiol level was found to be 551.01±223.00 µmol/L in the NMIBC group and 412.21±132.15 µmol/L in the control group. Mean disulfide level was 162.49±105.31 µmol/L in the NMIBC group and 12.62±6.88 µmol/L in the control group (p<0.05).

When the NMIBC group was divided into two subgroups in terms of tumor invasion and grade, Ta and T1 low grade 26 (49.1%) patients and T1 high grade 27 (50.9%) patients were found to have statistically similar total thiol and disulfide levels. In the patient group with low-grade tumor, native thiol values were found to be higher than those in the patient group with high-grade tumors (p<0.05) (Table 3).

Table 1. Smoking and concomitant diseases in the control group and patients with bladder tumor

Variable n/(%)	Control Group	Bladder Tm Group	p-value
Smoking			
Yes	45/75	38/71.7	0.692
No	15/25	15/28.3	
Concomitant disease			
Yes	31/51.7	35/66	0.122
No	29/48.3	18/34	

Table 2. Native thiol disulfide and total thiol values in superficial bladder cancer and control group

	NMIBC	Control	p-value
Native thiol (SH) µmol/L	226.02±143.83	386.96±135.00	<0.001
Total thiol (TT) µmol/L	551.01±223.00	412.21±132.15	
Disulfide (SS) µmol/L	162.49±105.31	12.62±6.88	

NMIBC: Non-muscle invasive bladder tumor

Table 3. Comparison of total thiol, native thiol and disulfide levels between Ta/T1 low grade patient group and T1 high grade patient group

	Ta/T1 Low Grade	T1 High Grade	p-value
Native thiol	255.87	169.42	<0.05
Total thiol	506.55	486.60	>0.05
Disulfide	153.73	166.90	>0.05

When 22 (41.5%) patients with a tumor size of <3 cm and 31 (58.5%) patients with a tumor size of more than 3 cm were compared in terms of total thiol, native thiol and disulfide levels, no significant difference was observed between the two groups (p>0.05). Similarly, no significant difference was found between the total thiol, disulfide and native thiol levels of 13 (24.5%) patients with tumor recurrence in their follow-ups and 40 (75.5%) patients without tumor recurrence (p>0.05).

Discussion

In many epidemiological, experimental and clinical studies, oxidative stress markers have been shown to be associated with cancer development and progression. Higher lipid, protein and DNA oxidation markers detected in bladder cancer tissues confirm a potential role of oxidative stress in the molecular mechanism of the disease. Literature data support the overexpression of NO and a deficiency in antioxidant systems (SOD, CAT and GTPx) in bladder tissue, serum and plasma of patients diagnosed with bladder cancer. It is generally thought that the development of bladder cancer occurs because of a disruption in the antioxidant/pro-oxidant balance (10,11). In this regard, our study has the distinction of being the first study in the English literature.

There are studies investigating the changes in TDH in many diseases where oxidative stress is thought to play a role in the development of the disease (9). Hanikoglu et al. (12) examined patients with prostate cancer and found that native thiol, total thiol, and total oxidant status (TAS) levels in the sixth month follow-ups of patients after radical prostatechromia (RP) increased compared to the levels before RP. In the same study, when prostate specific antigen and thiol levels were compared, it was seen that there was a negative correlation between these two values. It was shown that decreased thiol and TAS levels weaken the antioxidant defense mechanism in patients with prostate cancer, and as a result, the balance shifts to the oxidative side. Similar results were obtained in this study, and it was found that there was a decrease in antioxidant levels in patients with bladder cancer compared to healthy individuals, and there was a shift in balance toward the oxidative side.

In this study, native thiol levelswere significantly higher in the healthy group compared to the NMIBC group. Disulfide levelswere found to be higher in the NMIBC group compared in the healthy group. In the subgroup analysis conducted in the NMIBC group, native thiol levels were found to be significantly higher in patients with low-grade tumors compared with patients with high-grade tumors. These results prove that there is a direct relationship between superficial bladder cancer and oxidative stress.

In another study, Senel et al. (13) reported that the mean plasma levels of native thiol and total thiol were lower in patients with prostate cancer compared in the healthy control group. In the same study, in the prostate cancer group, it was determined that patients with a Gleason score of ≥7 had lower plasma native thiol levels than patients with a Gleason score of <7, and there was no significant difference between the two groups in terms of total thiol and disulfide levels. These findings are similar to the results obtained in this study and are consistent with the data obtained in our subgroup analysis and support the decrease in native thiol levels with increasing tumor grade.

Sönmez et al. (14) performed a subgroup analysis in the prostate cancer group and the control group and found that the native and total thiol/disulfide levels in the prostate cancer group were lower than those in the control group. We concluded that oxidative stress, which is involved in the etiopathogenesis of cancer, also plays a role in the etiology of prostate cancer and that there is a decrease in the level of native thiol due to increased oxidative stress. In this study, native thiol levels were also found to be significantly lower in the cancer group.

In a study by Solak et al. (15) in 2018 between smokers and non-smokers, it was found that native, total and native/total thiol levels were lower in smokers. Furthermore, disulfide, disulfide/native thiol and disulfide/total thiol levels were found to be significantly higher in smokers than in non-smokers. As a result, it was determined that smoking increases oxidative stress and causes a shift in TDH to the disulfide side compared to the healthy group. In this study, no significant difference was observed between thecontrol and NMIBC groups smoking. Therefore, a separate subgroup analysis could not be performed for the relationship between smoking and thiol levels.

When we performed a subgroup analysis in the NMIBC group, no significant correlation was found between the dimensions of the bladder tumor and the native thiol, total thiol and disulfide levels. Similarly, in the NMIBC group, there was no significant difference in total thiol and disulfide levels between patients with pathological stages of and T1 low-grade tumors and patients with T1 high-grade tumors, while native thiol values were significantly higher in the low-grade tumor group. This result shows us that there may be a relationship between tumor aggressiveness and native thiol levels. Although the idea that there may be a relationship between the pathological stage of superficial bladder cancer and the TDH has emerged in this

study, it is necessary to conduct more comprehensive studies involving many patient groups to obtain clearer and more definitive results.

The literature data shows that because of the increase in oxidative stress, there is a decrease in the native thiol levels and the balance shifts to the oxidant side. Similarly, in this study, total thiol and disulfide levels were found to be higher in patients with superficial bladder tumors than in the healthy group. This finding is important as this is the first study in the literature to show dynamic TDH in superficial bladder cancer. The increase in the oxidant level seen because of the changes in TDH in patients with superficial bladder cancer also increases serum total thiol and disulfide levels, confirming the role of oxidative stress in the etiopathogenesis of bladder cancer. This supports the relationship between the formation of bladder cancer and oxidative stress. We believe that important complementary data regarding the etiopathogenesis of bladder cancer and data that help evaluate the severity of the disease can be obtained based on the results of this study. Early detection of the increase in serum total thiol and disulfide levels may be a guide for early cystoscopy or interventional procedures in patients with bladder cancer. Since there is no specific antigen or laboratory test in routine use in the follow-up of bladder cancer, we believe that it will guide patients and physicians in their follow-up since it is inexpensive and accessible if it is routinely used.

Study Limitations

The limited number of patients in our study and the exclusion of muscle-invasive bladder tumors caused the subgroup analyses were rather limited. Additionally, the possibility of disruptions in dynamic thiol-disulfide hemostasis in different diseases in which oxidative stress increases is among the limitations of our study.

Conclusion

In this study, it was determined that disruption in TDH may be effective in the development of superficial bladder tumors in cases where oxidant stress predominates over antioxidant mechanisms. Thus, we believe that the total thiol/disulfide balance plays a role in the etiology of bladder tumors and in many tumors and inflammations, and based on this, antioxidants may be beneficial in the prevention and treatment of bladder tumors. However, further more comprehensive studies should be conducted in order to obtain clearer and more definitive results on this subject.

Ethics

Ethics Committee Approval: This study was designed in accordance with the Declaration of Helsinki, with the approval of the local ethics committee (Karabük University Ethics Commitee) (approval number: 2020/154, date: 27.02.2020).

Informed Consent: Informed signed consent was obtained from all participants.

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Authorship Contributions

Surgical and Medical Practices: S.B., A.A., Concept: S.B., A.A., Design: Ö.B., Data Collection or Processing: Ö.B., M.M.S., Analysis or Interpretation: S.B., M.M.S., Literature Search: S.B., A.A., Writing: S.B.

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