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The relationship between thiol-disulfide balance and prostate cancer

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Abstract

This study aimed to investigatethe possible association of thiol/disulfidehomeostasiswith prostate-specific antigen levelsin prostate cancer patients and to compare the results with a normal healthy population ofa similar age group for the first time in literature. Forty-twopatients (20 patientswith prostate cancer and 22 volunteers) were included in the study. Thiol/disulfide homeostasis tests were measured with an automated method. Albumin, carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), ischemia modified albumin (IMA), total thiol (TT), native thiol (–SH), and disulfide (–S–S–) levelsand, thiol-disulfide ratios (disulfide / native thiol, disulfide/total thiol and native thiol/total thiol) were assessed to detect any differences between prostate cancer group and control group. The mean PSA value of the prostate cancer group was 2.56 ng/mL, the mean PSA value of the control group was 1.21 ng/mL, and the mean age of the prostate cancer group was 67.32 years, the mean age of control group was 60.09. Although native thiol and total thiol levels were significantly lower in patients with prostate cancer ($p<0.05$), there was no difference between the other values (PSA, IMA, Albumin, CEA,–S–S– and thiol-disulfide ratios) in the prostate cancer group compared to the control group ($p> 0.05$). In our study, we could not show thiol-disulfide values as a biochemical prognostic factor in patients with prostate cancer. We believe that serum TT, –SH, –S–S– concentrations cannot serve as a noninvasive biomarker for prostate cancer. To verify the biochemical role of thiol/disulfide balance, studies need to be done by increasing the number of patients with prostate cancer.

Keywords: Prostate cancer, disulfide, thiol, biomarker

Introduction

Cancer is among themost common causes of death worldwide [1,2,3]. Although prostatitis (chronic inflammation), benign prostatic hyperplasia (BPH), and prostate cancer are common diseases of the prostate, Prostate cancer is one of the most common and common malignancies worldwide [4]. Although it is the leading cause of cancer-related deaths among men in advanced ages (75% over 65 years of age),the mortality rate from prostate cancer is around 3% and it is often confined to the prostate gland [4,5].

Prostate cancer is the most frequentlydiagnosed type of urological cancer among men.Although its incidence varies around the world, it is predicted that factors such as nutritional habits, chronic inflammation of the prostate, sexual disorders, and low exposure to ultraviolet radiation can cause prostate cancer.[4-6].

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Prostate cancer is still being followed up by a glycoprotein called prostate-specific antigen also known as human Kallikrein 3 (hK3) [6].

PSA level is a noninvasive biomarker used in prostate cancer along with tissue biopsy, which may vary according to age, density rate, rate of increase (velocity), and doubling time [4,7,8].

PSA reference range is considered to be 0-4 ng / mL. However, PSA values of prostate cancer have been reported below these values, even at 0.50 ng/ml. It is stated that the 4ng / ml limit of PSA can be significantly overlooked for prostate cancer [7].

To increase the value of PSA in the diagnosis, the need to use PSA with different values such as PSA growth rate, age-specific PSA values, and PSA density has been demonstrated in many studies. It is also known to confirm the diagnosis with biopsy [4,7,8]. Oxidative stress is known to be important in tumor formation. Oxidative stress is thought to accelerate the cancer formation process by disrupting the redox balance [9].

Thiols are not a simple class of antioxidants but play an important role in preventing oxidative stress formation in cells.Thiols are

organic compounds containing a sulfhydryl group (-SH). They can form disulfide bonds by oxidation reactions[3,10]. Thiols in plasma form more protein thiols.However, they are found in low molecular weight thiols (glutathione,homocysteine, cysteine (Cys),etc.).Due to the prevalence of prostate cancer, accurate management of the diagnosis, treatment,and active follow-up process is important for the patient as well as for public health.

In recent studies, it has been reported that no biomarker alone is sufficient in diagnosis and it is necessary to be multivariate in the diagnostic approach [11]. We aimed to analyzethe possible relationship between thiol/disulfideequilibrium and the levels of PSA in prostate cancer patients and to compare the results with the normal healthy population.

Materials and Methods

Study population, assay protocol and ethics approval

This study was conducted with 42 participants from the Department of Oncology and Urology in Turkey.The study was performed according to the Helsinki Declaration (Declaration of Helsinki 2013, Brazil version). It was performed after approval from the Research Ethics Committee of Dumlupınar University in Turkey (2015-KAEK-86/08-158).

The population of our study consisted of voluntary prostate cancer patients and healthy individuals. All patients and volunteers were over 18 years of age. The prostate cancer group consisted of 20 patients and the control group consisted of 22 healthy volunteers. The healthy control group consisted of participants who applied for a check-up to the outpatient clinic. The control group consisted of healthy volunteers who were free of any chronic disease (liver diseases, cardiovascular diseases,pathology of the salivary gland,diabetes mellitus, or cancer).The patient group consisted of participants with prostate cancer. In the control group, participants using chronic drugs and patients with any chronic disease were excluded from the study.

Serum samples were collected in two periods (first period: February-June 2017/ second period: March-September 2018). Sampleswere recollected as it was desired to increase the samples in Congress of Biochemistry (National Congress of Biochemistry, 28th National Biochemistry Congress, September 19-23, 2017). Blood samples (5 ml) were taken from the participants with prostate cancer and the control group into the ethylenediaminetetraacetic acid (EDTA) tubes. Samples collected from the prostate cancergroup (n = 20) and the control group (n = 22)were separated by centrifugation (1500 rpm for 10 min). Serums of blood samples were stored in -80 Celsius in the deep freezer until the analysis. Serum samples were sent to YıldırımBeyazıt University in dry ice and then analyzed. Native thiol, total thiol, and disulfide levels were measured using an automatic spectrophotometric method (19).

This method was performed according to the specific protocol.

The specific protocol was comprised of the following steps:

1.Di-sulfide bonds were reduced to free thiol groups by sodium borohydride (NaBH4).

2.NaBH4 residues were removed by CH2O (formaldehyde).

The total thiol was measured using reagent (modified Ellman). A spectrophotometric method byErel and Neselioğlu was used to measure the concentrations of TT,–SH, and –S–S, the ratios of SS/(SH + SS), and SS/SH, and the ratios of SH/(SH + SS).

Serum PSA and CEA were determined using an automated clinical biochemistry analyzer with original reagents. Total IMA and albumin levels were measured ing/dl in an automatic analyzer with commercially available assay kits. The assay was performed according to the manufacturer's kit protocol. Serum PSA was measured in ng /ml in the automatic analyzer with commercially available assay kits.

Statistical Analysis

Results were evaluated by using SPSS 17 program. Values (mean and median) and spread (standard deviation and coefficient of variation) of continuous data were calculated. In comparing the parametric data between the groups, the Mann-Whitney-U test will be used to compare non-parametric and normally non-dispersible values between the groups.The relationship between the variables was examined by Pearson Correlation Test.

$P < 0.005$ is considered to be significant.

Results

Demographic and thiol-disulfide equilibrium parameters (Native thiol/ disulfide, native thiol, total thiol, and disulfide levels) of all study subjects are summarized in Table 1. The study was conducted with 42 individuals – 22 in the control group and 20 in the prostate cancer group. Sixpeoples in the control group were in the 45-50 age group, 11 individuals in the 51-60 age group, and 5 individuals in the 61-72 age group. Two peoples in the prostate cancer group were in the 45-50 age group, 7individuals in the 51-60 age group, and 11 individuals in the 61-77 age group. No statistical difference was observed between age and thiol/disulfideequilibrium parameters. All the cancer patients were diagnosed with adenocarcinoma and half of them received surgical treatment while the other half underwent various treatments. A family history of prostate cancer was present in 30% of patients.It was observed that, in the control group, 5 individuals had PSA levels below 0.5 ng/ml and 17 individuals had PSA levels ranging between 0.6 and 3.0 ng/ml. In the prostate group, 4 patients had PSA levels below 0.5 ng/ml, 8 patients had PSA levels between 0.6 and 3.0 ng/ml, 6 patients had PSA levels between 3.1 and 6.0 ng/ml, 1 individual had a PSA level between 6.1 and 10.0 ng/ml and 1 individual had a PSA level above 10.0 ng/ml (Table 2).

No significant difference was observed between groups. It was detected that PSA activity was statistically significantly different from the control group (Table 3). As a result of the Mann-Whitney U Test, PSA values as well as TT,–SH, and –S–S– and the ratios of SS/(SH + SS) and SS/SH, the ratios of SH/(SH + SS) values are presented in Table 3 and 4 (Table 3 and 4). According to the test results, IMA values were measured as 5.97±3.83 ABSU (Absorbance unit) in the prostate cancer group and 4.48±5.10

ABSU in the control group; albumin values were calculated as 5.75±2.39 g/dL in the prostate cancer group and 5.76±2.49 g/dL in the control group. No statistically significant difference was found. Native thiol levels were 272.6±47.49 µmol/L in the prostate cancer group and 312.06±83.45 µmol/L in the control group while total thiol levels were 310.78±53.39 µmol/L in the prostate cancer group and 356.96±85.82 µmol/L in the control group, and native thiol and total thiol levels were significantly low (P <0.05). Disulfide values were 19.01±7.92 µmol/L in the prostate cancer group while they were calculated as 22.45±7.49 µmol/L in the control group (Table 3).

Table 1. Descriptive statistics based on age between Volunteers.

Age Range		
Control (n: 22)		
45-50	(6)	27.7%
51-60	(11)	50%
61-72	(5)	22.72%
Mean age		60.09
Prostate Cancer (n: 20)		
45-50	(2)	10%
51-60	(7)	35%
61-77	(11)	55%
Mean age		67.32
Family History		
Positive		30%
Negative		45%
Unknown		25%

Table 2. PSA level of Volunteers.

Psa Level	**n**
Control	Total :22
below 0.5 ng /ml	5
0.6 - 3.0 ng /ml	17
3.1- 6.0 ng /ml	-
6.1 - 10.0 ng /ml	-
up to 10 ng /ml	-
mean of PSA	1.21
Prostate Cancer	Total :20
below 0.5 ng /ml	4
0.6 to 3.0 ng /ml	8
3.1 to 6.0 ng /ml	6
6.1 to 10.0 ng /ml	1
up to 10 ng /ml	1
mean of PSA	2.56

Table 3. PSAactivity and thiol/disulfide levels

	Control	**Prostate cancer**
Native thiol (µmol/L)	312.06±83.45	272.76±47.49*
Total thiol (µmol/L)	356.96±85.82	310.78±53.39*
Disulfide (µmol/L)	22.45±7.49	19.01±7.92
IMA (ABSU)	4.48±5.10	5.97±3.83
Albumin (g/dL)	5.76±2.49	5.75±2.39
PSA total	0.76±0.60	2.83±2.63*

Mann-Whitney U Test was performed. * P <0.05 was considered significant according to the control.

Table 4. Descriptive statistics and comparison of Disulfide /native thiol; Disulfide /total thiol, and Native thiol /total thiol variables by group and PSA activity

	Control	**Prostate cancer**
Index 1 Disulfide /native thiol*100	7.80±3.82	7.11±3.18
Index 2 Disulfide /total thiol*100	6.58±2.63	6.09±2.43
Index 3 Native thiol /total thiol*100	86.83±5.27	87.81±4.85
PSA total	0.76±0.60	2.83±2.63*

Mann-Whitney U Test was performed. * P <0.05 was considered significant according to the control.

Discussion

Prostate cancer is a common disease. PSA is used in monitoring itsfollow-up and treatment. Although PSA level varies in prostate cancer cases, its sensitivity and specificity are considered to be limited since it may be high in prostatitis, prostatic intraepithelial neoplasia (PIN),and benign prostate hyperplasia (BPH) cases [4,7,8,12]. However, PSA is used in the follow-up of prostate cancer patients receiving surgical treatment, radiotherapy, hormonal therapy, or chemotherapy and thus it is still an important follow-up method and there is a need for supporting biomarkers [4,7,8]. Since free radicals are highly active, they cause a chain reaction of radicals by influencing other molecules. Thiols are compounds containing sulfhydryl groups that exhibit a protective effect against any damages caused by these radicals by reacting with radicals. Lipids and proteins can be degraded, especially after the removal of thiol groups. It has been reported that this thiol-disulfide balance that is impaired in diseases such as cancer is important. [3,13-17]. In a study, it was reported that changes in thiol level could be associated with the severity of stroke [16]; thiol-disulfide equilibrium might be a prognostic biomarker in small cell lung cancer [14] and it could offer new treatment alternatives for the effects of oxidative stress in ankylosing spondylitis [15]. In the present study, it was detected that native thiol and total thiol levels decreased in patients with prostate cancer compared to the control group. This result was statistically significant (p<0.05). It is pointed out

in a study that lower thiol levels may be associated with aortic aneurysm development and may increase after surgical therapy [18]. It is expressed that decreased thiol levels as a result of increased oxidative stress may be one of the important factors in development and progression of diabetic nephropathy [19] and, in a study conducted with patients exposed to mercury, a significant correlation is reported between, native thiol, total thiol and disulfide levels and urinary mercury levels [20]. In our study, increased oxidants in prostate cancer patients may have caused a decrease in native thiol and total thiol levels. Highernative thiol and total thiol levels in healthy controls indicate that antioxidant capacity cannot be overlooked. If we had had the chance to examine the thiol levels of prostate cancer patients before the onset of disease, we could have demonstrated its effect on cancer development more clearly. Low disulfide levels were reported in studies on colorectal, bladder, and kidney cancers [13], while a study on thyroid cancer revealed no change in disulfide level [3]. It was reported that patients with lichen planus had no marked change [21], whilethiol/disulfide equilibrium changed in favor of disulfide in patients with familial hypercholesterolemia [17]. In the present study, there was no significant changein the disulfide level compared to the control group. The antioxidant capacity of thiols is determined according to the disulfide chains contained in their structures [22]. The absence of any changes in the disulfide level in our study suggests that such structures still have protective roles against oxidants in prostate cancer patients known to have no other chronic diseases. Considering that prostate cancer has a longer life cycle than other cancers, it is possible to conclude that disulfide structures remain undegraded, thus, an increased oxidant activity does not occur in addition to the destruction caused by cancer cells and cancer progression is not very fast as a result.This can be viewed as a positive result in terms of the patient's life expectancy.

It is reported in several other studies that no significant difference was detected in thiol-disulfide equilibrium values of patients with OSAS (obstructive sleep apnea syndrome) [23]; patients with idiopathic sudden sensorineural hearing loss (ISSNHL) and increased oxidation following treatment responded better to the treatment [24]. No change was observed in thiol-disulfide equilibrium in studies conducted on patients with acute appendicitis and patients with fibromyalgia; and dynamic thiol/disulfide homeostasis was unaffected in studies conducted on patients with alopecia areata [25-27].In a study with smokers, it was reported that thiol-disulfide equilibrium mostly changed in favor of disulfide compared to the healthy group [28]; on the other hand, no significant change was detected in thiol-disulfide homeostasis in various studies performed on patients with retinal vein occlusion [29] and epilepsy patients [30], colon cancer patients [31],meme cancer [32] and healthy volunteers.

In our study, although we assumed a relationship between thiol-disulfide homeostasis and PSA levels in the prostate cancer group and control group, no relation was found ($p>0.05$).A literature review shows that thiol-disulfide equilibrium in prostate cancer patients is investigated for the first time and our study is the first report in the literature addressing this subject. Although there are plenty of studies on thiol-disulfide balance, no study is available in the literature on patients with prostate cancer.

Furthermore, we were unable to detect any relationship in the initial stages of this study due to the shortage of participating patients. However, we expanded the scope of our study by increasing the number of participants. Although our findings failed to indicate a marked relationship between PSA and thiol-disulfide equilibrium, they demonstrated a significant relationship between native thiol and total thiol levels in the prostate cancer group.

As for the limitations of our study,we could not perform an evaluation based on Gleason scoring because the first clinics to which the patients were admitted were different from each other. Also, we failed to compare total PSA / free PSA levels and thiol-disulfide equilibrium due to the mismeasurement of free PSA levels in some patients. Our study aimed to demonstrate the relationship of PSA measurement with a biomarker that would assist in the diagnosis and follow-up of prostate cancer. Although we were unable to show thiol-disulfide homeostasis as an early biochemical prognostic factor in prostate cancer patients, it has been an exciting journey to express this relationship, albeit partially. Further investigation is needed with prospectively designed studies and larger series of patients to obtain better results.

Conflict of interests

The authors declare that they have no conflict of interest and any financial disclosures.

Financial Disclosure

All authors declare no financial support.

Ethical approval

It was performed after approval from the Research Ethics Committee of Dumlupınar University in Turkey .

References

1. Bray F, Jemal A, Grey N, et al. Global cancer transitions according to the Human Development Index (2008–2030): a population-based study. Lancet Oncol. 2012;13:790-801.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, CA: A Cancer. J Clinic. 2017;67:7-30.
3. Ozdemir A, Erel O. Thıol-Dısulfide Balance: Could be a new marker for Thyroid Cancer? J Cancer Therap (JCT). 2018;9.
4. Akinloye O, Adaramoye O, Kareem O. Changes in antioxidant status and lipid peroxidation in Nigerian patients with prostate carcinoma. Polskıe Archıwum Medycyny Wewnętrznej (Polish Arch Intern Med). 2009;119.
5. Tatar M, Tatar F, Colorectal cancer in Turkey: current situation and challenges for the future. Eur J Health Econ. 2010;10:99–105.
6. Mottet N, Bellmunt J, Bolla M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, diagnosis, and local treatment with curative intent. Europ Urol. 2017;71;618–29.
7. Akbayır S, Muşlu N. Prostate-specific antigen and derivatives in prostate cancer diagnosis. Türk Klinik Biyokimya Derg. 2016;14:189-204.
8. Vickers AJ, Savage C, O'Brien MF, et al. Systematic review of pretreatment prostate-specific antigen velocity and doubling time as predictors for Prostate Cancer. J Clin Oncol. 2009;27:398–403.
9. Fi L, Yang XW, Krausz KW, et al. Modulation of Colon Cancer. Nutmeg J. Proteome Res. 2015;14:1937−46.

10. Sen CK, Packer L. Thiol homeostasis, and supplements in physical exercise. Am J Clin Nutr. 2000;72:653–69.

11. Horwich A, Hugosson J, de Reijke T, et al; Panel Members; European Society for Medical Oncology. Prostate cancer: ESMO Consensus conference guidelines. Ann Oncol. 2013;24:1141-62.

12. Yeniyol CO, Bozkaya G, Çavuşoglu A, et al. The relation of prostate biopsy results and ratio of free to total PSA in patients with a total PSA between 4–20 ng/mL. Internat Urol Nephrol. 2001;33:503–6.

13. Hizal M, Sendur MAN, Bilgin B, et al. Evaluation of dynamic serum thiol/disulfide homeostasis in locally advanced and metastatic gastric cancer. J Oncol Sci. 2018;4:1-4.

14. Dirican N, Dirican A, Şen O, et al. Thiol/disulfide homeostasis: a prognostic biomarker for patients with advanced non-small cell lung cancer? Redox Report. 2016:21.

15. Dogru A, Balkarli A, Yildirim Cetin G, et al. Thiol/disulfide homeostasis in patients with ankylosing spondylitis. Bosn J Basic Med Sci. 2016;16:187–92.

16. Bektas H, Vural G, Gümüşyayla Ş, et al. Dynamic thiol-disulfide homeostasis in acute ischemic stroke patients. Acta Neurol Belgica. 2016;116:489–94.

17. Şimşek O, Çarlıoğlu A, Alışık M, et al. Thiol/Disulfide balance in patients with familial hypercholesterolemia. Hindawi Cardiol Res Practice. Volume 2018, https://doi.org/10.1155/2018/9042461.

18. Akkuş O, Kaypaklı O, Koca H, et al. Thiol/disulphide homeostasis in thoracic aortic aneurysm and acute aortic syndrome. Biomark Med. 2018;12:349-58.

19. Eren MA, Koyuncu I, İncebıyık H, et al. The evaluation of thiol/disulfide homeostasis in diabetic nephropathy. Turk J Endocrinol Metab. 2018;22:22-3.

20. Neşelioğlu S,Alışık M, Bal C, et al. Dynamıc thıol-dısulfıde homeostasıs in mercury-exposed patıents. 2017;2:51.

21. Kalkan G, Emre S, Alisik M, et al. Dynamic thiol/disulfide homeostasis in patients with lichen planus. J Clin Lab Anal. 2018 ;3:e22642.

22. Ozdemir A (2019). Tiyoller. İstanbul-Türkiye: İstanbul Medikal Sağlık ve Yayıncılık.

23. Arguder E, Kılıç H, Parlak E, et al. Thiol disulfide homeostasis in obstructive sleep apnoea syndrome (OSAS)- Preliminary report. Europ Respirat J 2017;50:PA2318.

24. Solak I, Arslan A, Kutlu R, t al. Effect of hyperbaric oxygen therapy on thiol/disulfide homeostasis in patients with idiopathic sudden sensorineural hearing loss. Fam PractPalliat Care. 2018;3:133-8.

25. Kilinc F, Sener S, Akbas A, et al. Investigation of dynamic thiol-disulfide homeostasis in alopecia areata patients. Brit J Med Medical Res. 2017;21:1-7.

26. Yuzbaşıoğlu Y, Ercan Haydar G, Otal Y, et al. Thiol–disulfide homeostasis in acute appendicitis. Med J Islamic World Acad Sci. 2017;25:114-7.

27. Fidan F, Alkan BM, Uğurlu FG, et al. Dynamic thiol/disulphide homeostasis in patients with fibromyalgia. Arch Rheumatol. 2017;32:112-7.

28. Solak I, Cetinkaya CD, Gederet YT, et al. Effects of smoking on thiol/disulfide homeostasis. Eur Rev Med Pharmacol Sci. 2018;22:2477-82.

29. Polat OA, Kurt A, Kılıç R, et al. Is There Any Association Between the retinal vein occlusion and the thiol-disulfide homeostasis which is an oxidative stress indicator? Turkiye Klinikleri J Ophthalmol. 2019;28:23-8.

30. Gümüsyayla Ş, Vural G, Bektaş H, et al. Evaluation of dynamic thiol-disulphide homeostasis in patients with epilepsy. Epilepsy. 2016;22:86-92.

31. Ozdemir A, Dilli UD, Ozdemir D, et al. Levels of thiol–disulfide in Colorectal Cancer. Anadolu Kliniği TıpBilimleri Dergisi. 2019;24.

32. Ozdemir A, Dilli UD, Ozdemir D, et al. The association between thiol-disulphide balance and Ca-15-3 Levels in patients with Breast Cancer. Kocatepe Med J. 2020;21:12-6.