Evaluation of Oxidative Stress Status and Antioxidant Capacity in Patients with Localized Prostate Cancer and Benign Prostatic Hyperplasia

Lokalize Prostat Kanseri ve Benign Prostat Hiperplazi Hastalarında Oksidatif Stres Durumu ve Antioksidan Kapasitenin Değerlendirilmesi

Localize Prostate Kanseri ve Oksidatif Stres / Localized Prostate Cancer and Oxidative Stress

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Özet

Amaç: Bu çalışma, lokalize prostat kanseri (PCa) ve benign prostat hiperplazili (BPH) hastalarda oksidatif stres durumu ve antioksidan kapasitenin değerlendirilmesi amacıyla yapılmıştır. Gereç ve Yöntem: Bu prospektif çalışmaya, tamamı histolojik tanılı 24 lokalize PCa ve 36 BPH'lı erkek hasta dahil edilmiştir. Lokalize PCa ve BPH tanılı hastaların sabah açlık kan örneklerinden, total oksidan durum (TOS), total antioksidan kapasite (TAC), paraoxonase1 (PON1), arylesterase ve total thiol düzeyleri araştırılmış ve karşılaştırılmıştır. Bulgular: Hastaların ortalama yaşı BPH grubunda 67 ± 12 yıl, localize PCa grubunda ise 68 ± 8 yıl idi (p>0.05). Elde edilen verilere göre, tüm oksidatif stress parametreleri ve antioksidan kapasite değerleri açısından iki grup arasında istatistiksel anlamlı fark olmadığı tespit edilmiştir (tüm parametreler için p>0.05). Tartışma: Çalışmamızda elde ettiğimiz sonuçlara dayanarak, serumda çalışılan bu parametrelerin localize PCa ve BPH'lı hastaların ayırıcı tanısında bir belirteç olarak kullanılamayacağını göstermiştir.

Anahtar Kelimeler

Lokalize Prostat Kanseri; Oksidatif Stres; Antioksidan Kapasite

Abstract

Aim: The aim of this study was to evaluate the oxidative stress status and antioxidant capacity in patients with localized prostate cancer (PCa) and benign prostatic hyperplasia (BPH). Material and Method: The prospective study consisting 24 men with PCa and 36 men with BPH. PCa or BPH were diagnosed histologically. Blood samples were obtained following an overnight fasting state. We evaluated and compared the total oxidant status (TOS), total antioxidant capacity (TAC), paraoxonase1 (PON1), arylesterase and total thiol levels of patients with PCa and BPH. Results: The mean age of BPH group was 67 ± 12 years and PCa group was 63 ± 8 years (p>0.05). No statistically significant differences were detected between the groups in terms of oxidative stress parameters and antioxidant capacity measured in the serum of patients including PON1, TOS, TAC, arylesterase and thiols levels (p>0.05 for all parameters). Discussion: Our results demonstrated that evaluation of these parameters in the serum of BPH and localized PCa patients may not be used as a marker to discriminate between these diseases.

Keywords

Localized Prostate Cancer; Oxidative Stress; Antioxidant Capacity

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Introduction

Prostate adenocarcinoma (PCa) is the most seen cancer in men and is one of the leading causes of death due to cancer. Although its etiology doesn't known exactly it has been suggested that hormonal and genetic factors, race, socioeconomic status, diet rich in fat and infections have role in pathogenesis [1]. Furthermore, androgens may play a role in smooth muscle proliferation associated with the development of PCa. So men at an increased risk of PCa may also display heightened risk of cardiac disease [2]. The mechanism of prostate carcinogenesis is not completely understood, but evidence recommends that oxidative stress plays a role [3]. Relationship between prostate cancer risk and oxidative stress has been well-recognized. There is considerable evidence indicating oxidative stress contributes to the etiology and pathogenesis of the prostate cancer [4;5].

Paraoxonase1 (PON1) and arylesterase, a high-density lipoprotein (HDL)-bound enzyme system, are well-known antioxidant molecules. This enzyme system protects low-density lipoprotein (LDL) and HDL from oxidation by hydrolyzing activated phospholipids and lipid peroxide products thus prevent atherosclerosis [6;7]. Thiols are endogenous molecules that contain the sulfhydryl group (-SH) attached to a carbon atom. They support aerobic cells to maintain a reducing state, despite an oxidizing environment [8]. These molecules are extremely efficient antioxidants and they protect cells from results of damage occurred by free radicals, due to their ability to react with the latter [9]. Both intracellular and extracellular redox states of thiols play a critical role in the determination of protein structure and function, regulation of enzymatic activity of transcription factors and antioxidant protection [10].

We evaluated and compared the serum total oxidant status (TOS), total antioxidant capacity (TAC) and PON1 levels of patients with localized PCa and benign prostatic hyperplasia (BPH). Additionally, arylesterase and thiols levels were compared between these two groups.

Material and Method

The prospective study consist 24 men with localized PCa and 36 men with BPH symptoms but without any clinical or histological evidence of PCa. A complete medical history and physical examination were provided by all study participants. Prostate cancer or BPH were diagnosed histologically with specimens obtained by biopsy. Abdominal and pelvic computed tomography and bone scan was performed in all patients with PCa. Patients with medical conditions that alter oxidative status, such as another malignant disease, active inflammatory disease, vascular disease, ischemic disease, diabetes mellitus, thyroid disease and severe dysfunction of the heart, liver or kidney, were excluded from study. In addition, the metastatic PCa patients were also excluded from this study. The study was approved by the Ethical Committee of Yıldırım Beyazıt University.

Blood samples

Blood samples were obtained following an overnight fasting state. Samples were withdrawn from a cubital vein into blood tubes and immediately stored in ice at 4 °C. The serums was then separated from the cells by centrifugation at 1000 g for 10 min and were directly frozen and stored at -80 °C until analysis.

Measurement of total oxidant status (TOS)

Serum TOS levels were determined using a novel automated measurement method, developed by Erel [11]. In this method, oxidants present in the sample oxidise the ferrous ion–o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per litre (μmol H2O2 equiv. l^{-1}).

Measurement of total antioxidant capacity (TAC)

Serum TAC levels were determined using a novel automated measurement method, developed by Erel [12]. In this method, hydroxyl radical, which is the most potent radical, is produced via Fenton reaction. In the classical Fenton reaction, the hydroxyl radical is produced by mixing of ferrous ion solution and hydrogen peroxide solution. In the most recently developed assay by Erel, same reaction is used [12]. In the assay, ferrous ion solution, which is present in the Reagent 1, is mixed with hydrogen peroxide, which is present in the Reagent 2. The sequential-produced radicals such as brown-coloured dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. In this assay, antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has got excellent precision values,

which are lower than 3%. The results are expressed in mmol Trolox equiv. l^{-1}.

Measurement of paraoxonase-1 (PON1) activities

PON1 activity was determined using paraoxon as a substrate and measured by increases in the absorbance at 412 nm due to the formation of 4-nitrophenol as already described [13]. The activity was measured at 25 ºC by adding 50 μl of serum to 1ml Tris-HCl buffer (100 mM at PH 8.0) containing 2 mM CaCl2 and 5.5 mM of paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm. Enzymatic activity was calculated by using molar extinction coefficient 17100 M-1 cm^{-1}.

Measurement of arylesterase activities

Arylesterase activity was measured using phenylacetate as a substrate. Serum was diluted 400 times in 100 mM Tris-HCl buffer, pH = 8.0. The reaction mixture contained 2.0 mM phenylacetate (Sigma Chemical Co) and 2.0 mM CaCl2 in 100 mM Tris-HCl buffer, pH = 8.0. Initial rates of hydrolysis were determined by following the increase of phenol concentration at 270 nm at 37 °C on a CE 7250 spectrophotometer (Cecil Instruments Limited, UK) [14]. Enzyme activities were expressed in international units (U) or kilo units (kU) per 1 litre of sera.

Measurement of total thiol activities

Serum total thiol concentration or sulfhydryl groups (SH) were measured by the methods originally described by Elman and

modified by Hu [15;16]. Here, thiols interact with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), forming a highly colored anion with maximum peak at 412 ńm (e412 = 13,600 M^{-1} cm^{-1}). Here, this method was adapted to an automated biochemistry analyzer (Advia 1800; Siemens, Germany) [17].

Lipid profile analysis

The levels of triglyceride, total cholesterol (TC), HDL and LDL were determined using a colorimetric method (Advia 1800; Siemens) with an automatic analyzer (Siemens, Germany).

Statistical Analysis

SPSS version 11.5 software (Chicago, Illinois, USA) was used for data analysis. Parameters were expressed as mean ± standard deviation. Shapiro-Wilk test was used for a normal distribution before analysis. . Independent samples t test and Mann Whitney U test were used to compare in Group 1 and Group 2 according to normal distribution or not. P-values less than 0.05 were considered significant.

Results

A total of 60 men were enrolled in this study. Biopsy specimens were obtained from all patients. In 24 of these men the biopsies were positive for prostatic adenocarcinoma, and 36 men had benign hyperplasia. The mean age of BPH group was 67±12 years and PCa group was 63±8 years (p=0.955). The mean total prostate specific antigen (PSA) was 5.98 ± 2.01 and 34.25 ± 43.68 ng dl-1 in BPH and PCa patients, respectively. The mean prostate volume of BPH cases was 76.25 ± 35.62 while PCa cases was 51.71 ± 30.86 ml. The mean total PSA level and prostate volume were statistically significant between these two groups (p<0.001 and p=0.002, respectively). According to prostate biopsy results; Gleason grade 3+2 in 1 patient, 3+3 in 12 patients, 3+4 in 10 patients and 4+4 in 1 patient was recorded. No statistically significant difference was found in lipid profile, oxidative stress status and antioxidant capacity parameters between PCa and BPH groups (Table 1, Figure 1 and 2).

Table 1. Comparison of biochemical parameters and statistical results of two groups.

	BPH Mean ± standard deviation (n = 36)	PCa Mean ± standard deviation (n = 24)	P
Age	63.33±8.40	68.54±7.5	0.955[1]
TAC (mmol Trolox equiv. L-1)	2.73±1.11	2.56±0.49	0.846[1]
Total thiol (mm)	893.10±477.70	785.21±269.22	0.675[1]
Arylesterase (U l-1)	342.22±28.94	327.01±18.3	0.960[1]
HDL (mg dl-1)	40.58±7.36	38.28±8.62	0.734[1]
LDL (mg dl-1)	87.46±26.96	104.95±38.78	0.771[1]
Total cholesterol (mg dl-1)	172.81±30.37	164.0±49.24	0.728[1]
Triglyceride (mg dl-1)	116.11±45.39	142.83±48.43	0.910[1]
TOS (µm)	52.31±92.60	35.42±37.99	0.662[2]
Paraoxonase-1 (U l-1)	152.94±89.71	171.49±101.59	0.407[2]
Total PSA (ng dl-1)	5.98±2.01	34.25±43.68	<0.001[2]

[1]: Student's t test was used. [2]: Mann-Whitney U test. P<0.05 significantly value. TAC: total antioxidant capacity; TOS: total oxidant status; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

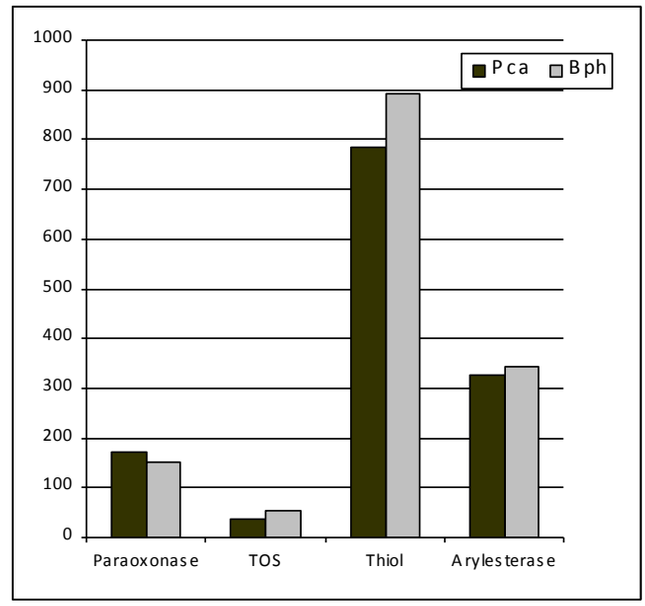

Figure 1. Total oxidant status and antioxidative capacity parameters in two groups.

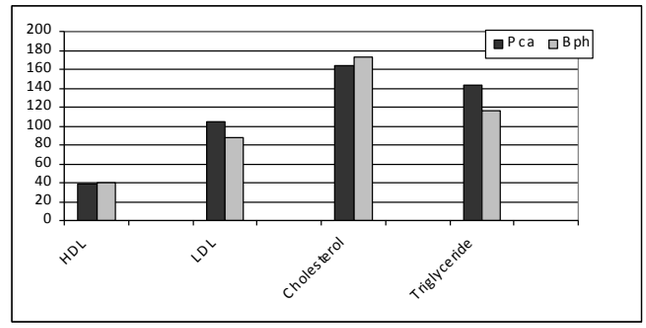

Figure 2. Lipid profile in PCa and BPH groups.

Discussion

Prostate cancer is a progressive disease in which tumor cells under oxidative stress may manifest continuous genetic changes and repairs that correlate with increasing frequencies of chromosomal abnormalities and mutations [18]. Many factors, especially aging, cigarette smoking, dietary and environmental toxicants are associated with increased reactive oxygen species (ROS) generation. In many susceptible individuals and in association with decreased cellular antioxidants, these ROS generated by redox cycling express oncogenes, damage cellular membrane by lipid peroxidation, interact with proteins, and cause progression of mutant clones [19].

Although other studies used parameters such as malondialdehyde (MDA) and catalase for evaluating oxidative stress and antioxidant capacity, to the best of our knowledge, no published study evaluated all of the oxidative stress and antioxidant parameters including PON1, TOS, TAC, arylesterase and thiols levels in patients with BPH and localized prostate cancer. As an example, Arsova-Sarafinovska et al. have reported significant increase in serum malondialdehyde (MDA) level compared to BPH patients; when enzymatic antioxidants decreased [3]. Their findings were also in agreement with the earlier reports of Yılmaz et al., on the elevated lipid peroxidation with concomitant antioxidant depletion in the prostate cancer [20].

Yossepowitch et al. also have observed a significant increase in MDA level and decrease in uric acid level in advanced PCa patiants, while no change in localized PCa patients [21]. These findings confirm previous observations and indicate that advanced prostate cancer but not localized PCa is associated with a state of high oxidative stress [22-24]. Similarly; another study have shown that no significant change was found in lipid peroxidation or antioxidant systems in the plasma of patients with BPH and prostate cancer [25]. Also, it has been reported that there was no difference in the antioxidant enzyme activities of prostatic epithelial cell cultures from benign and malignant tissues [26]. On the other hand; in a different study, malignant epithelial cells in prostatic adenocarcinoma have been founded to express lower levels of antioxidant enzymes than does benign prostatic epithelium [27].

Battisti et al. showed changes in oxidative stress biomarkers and antioxidant defenses parameters with having bone metastases or not, receiving treatment or not and gleason scores in patients with PCa [28]. Furthermore; in another study, it was founded a significant increase in oxidative stress group of patient those who were treated with androgen deprivation therapy and stated that antioxidant usage was preventing to progress to castration resistant prostate cancer stage [29]. These studies indicate that the oxidative stress level is parallel to disease stage.

In the present study, no statistically significant difference was founded in lipid profile, oxidative stress status and antioxidant capacity parameters between localized PCa and BPH groups (Table 1). This condition may be due to our study which contains only localized PCa patients but not advanced PCa.

Conclusions

In this study, levels of oxidative stress status and antioxidant capacity in the serum of BPH and localized PCa patients were compared. According to our results, no significant change was founded in oxidative stress or antioxidant capacity parameters in the serum of BPH or localized PCa patients. The results of the present study indicate that evaluation of the oxidative stress status and antioxidant capacity parameters in the serum of BPH and localized PCa patients may not be used as a marker to discriminate between these diseases. Further research is warranted to evaluate the relation to oxidative stress and prostate cancer with patients with all stage of disease and higher number of cases.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. De Marzo AM, Coffey DS, Nelson WG. New concepts in tissue specify for prostate cancer and benign prostatic hyperplasia. Urology 1999;53(3 Suppl 3a):29–40.
2. Weisman K, Larijani G, Goldstein M, Goldberg ME. Relationship between benign prostatic hyperplasia and history of coronary artery disease in elderly men. Pharmacotherapy 2000;20(4):383-6.
3. Arsova-Sarafinovska Z, Eken A, Matevska N, Erdem O, Sayal A, Savaser A et al. Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. Clin Biochem 2009;42(12):1228-35.
4. Chomyn A, Attardi G. MtDNA mutations in aging and apoptosis. Biochem Biophys Res Commun 2003;304(3):519–29.
5. Dakubo GD, Parr RL, Costello LC, Franklin RB, Thayer RE. Altered metabolism and mitochondrial genome in prostate cancer. J Clin Pathol 2006;59(1):10–6.
6. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998;101(8):1581–90.
7. Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM et al. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. J Clin Invest 1995;96(6):2882–91.
8. Chung KY, Lee SJ, Chung SM, Lee MY, Bae ON, Chung JH. Generation of free radical by interaction of iron with thiols in human plasma and its possible significance. Thromb Res 2005;116(2):157-64.
9. Sen CK. Redox signaling and the emerging therapeutic potential of thiol antioxidants. Biochem Pharmacol 1998;55(11):1747-58.
10. Wlodek L. Beneficial and harmful effects of thiols. Pol J Pharmacol 2002;54(3):215-23.
11. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38(12):1103-11.
12. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37(4):277-85.
13. Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet 1983;35(6):1126-38.
14. Haagen L, Brock A. A new automated method for phenotyping arylesterase (EC 3.1.1.2) based upon inhibition of enzymatic hydrolysis of 4-nitrophenyl acetate by phenyl acetate. Eur J Clin Chem Clin Biochem 1992;30(7):391-5.
15. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82(1):70-7.
16. Hu ML. Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol 1994;233:380-5.
17. da Costa CM, dos Santos RCC, Lima ES. A simple automated procedure for thiol measurement in human serum samples. J Bras Patol Med Lab 2006;42(5):345-50.
18. Dizdaroglu M, Jaruga P. Mechanisms of free radical-induced damage to DNA. Free Radic Res 2012;46(4):382-419.
19. Kovacic P, Somanathan R. Recent developments in the mechanism of anticancer agents based on electron transfer, reactive oxygen species and oxidative stress. Anticancer Agents Med Chem 2011;11(7):658-68.
20. Yilmaz MI, Saglam K, Sonmez A, Gok DE, Basal S, Kilic S et al. Antioxidant system activation in prostate cancer. Biol Trace Elem Res 2004;98(1):13-9.
21. Yossepowitch O, Pinchuk I, Gur U, Neumann A, Lichtenberg D, Baniel J. Advanced but not localized prostate cancer is associated with increased oxidative stress. J Urol 2007;178(4 Pt 1):1238-43.
22. Eichholzer M, Stahelin HB, Gey KF, Ludin E, Bernasconi F. Prediction of male cancer mortality by plasma levels of interacting vitamins: 17-year follow-up of the prospective Basel study. Int J Cancer 1996;66(2):145-50.
23. Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH et al. Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. Cancer Res 1999;59(6):1225-30.
24. Chan JM, Stampfer MJ, Ma J, Rimm EB, Willett WC, Giovannucci EL. Supplemental vitamin E intake and prostate cancer risk in a large cohort of men in the United States. Cancer Epidemiol Biomarkers Prev 1999;8(10):893-9.
25. Doğru-Abbasoğlu S, Aykaç-Toker G, Koçak T, Unlüer E, Uysal M. Antioxidant enzyme activities and lipid peroxides in the plasma of patients with benign prostatic hyperplasia or prostate cancer are not predictive. J Cancer Res Clin Oncol 1999;125(7):402-4.
26. Jung K, Seidel B, Rudolph B, Lein M, Cronauer MV, Henke W et al. Antioxidant enzymes in malignant prostate cell lines and in primary cultured prostatic cells. Free Rad Biol Med 1997;23(1):127-33.
27. Baker AM, Oberley LW, Cohen MB. Expression of antioxidant enzymes in human prostatic adenocarcinoma. Prostate 1997;32(4):229-33.
28. Battisti V, Maders LD, Bagatini MD, Reetz LG, Chiesa J, Battisti IE et al. Oxidative stress and antioxidant status in prostate cancer patients: relation to Gleason score, treatment and bone metastasis. Biomed Pharmacother 2011;65(7):516–24.
29. Shiota M, Song Y, Takeuchi A, Yokomizo A, Kashiwagi E, Kuroiwa K et al. Antioxidant therapy alleviates oxidative stress by androgen deprivation and prevents conversion from androgen dependent to castration resistant prostate cancer. J Urol 2012;187(2):707-14.

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