Oxidative stress parameters in patients with prostate cancer, benign prostatic hyperplasia and asymptomatic inflammatory prostatitis: A prospective controlled study

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Abstract

Background. The imbalance between oxidant and reductant mechanisms creates a nidus for the etio-pathogenesis of several diseases. In this study, we aimed to compare the oxidative stress (OS) parameters in patients who were diagnosed with prostate cancer (pCa), benign prostatic hyperplasia (BPH) or asymptomatic inflammatory prostatitis (AIP), according to the histopathologic examination of transrectal ultrasonographic prostate biopsy and transurethral prostate resection specimens.

Objectives. In this study, we aimed to compare oxidative stress between histologically proven prostate cancer, hyperplasia and prostatitis.

Material and methods. According to histopathologic examinations, 97 patients were divided into 3 study groups: group 1: pCa (n = 30), group 2: BPH (n = 41), and group 3: AIP (n = 26). Finally, 30 patients were enrolled in a control group. MDA levels, CuZn-SOD, Se-GPx, CAT activities, and trace element levels were evaluated.

Results. A statistically significant difference between prostate cancer and other groups were documented in terms of MDA activity. Contrary to AIP, a statistically significant difference has also been encountered between BPH and the control group. Decreased CuZn-SOD enzyme levels were found in PCa and BPH patients without statistical significance. Increased CAT activity was also documented in PCa, BPH and AIP patients. No significant difference in GPX activity was documented between the groups, except BPH and control group. Trace element levels were low in the patients with prostate cancer and BPH when compared with the control group.

Conclusions. Despite the data regarding OS in PCa patients, there is a paucity of data regarding BPH and especially AIP patients. Our study revealed obvious oxidative stress in BPH and PCa patients as opposed to AIP. Assessing the oxidative stress in these patients may assist in the future prevention, diagnosis and also treatment. However, the question whether the presence of OS-related parameters and drugs could be used for the diagnosis or management of prostatic diseases, needs to be addressed in future larger and better studies with a more rational basis.

Key words: prostate cancer, oxidative stress, chronic prostatitis, benign prostatic hyperplasia, prostate biopsy

Oxidative stress (OS) is defined as the interruption of the balance between oxidant and reductant molecules due to the excessive production of reactive oxygen species (ROS). This imbalance leads to oxidative DNA damage and performs a nidus for the etiopathogenesis of several diseases. There is plenty of data regarding the relationship between ROS and age-related pathologies such as cancer, diabetes or several degenerative disorders.[1] In regard to the prostate, the studies conducted in the last decade have demonstrated that OS is associated with prostate cancer (PCa) development, progression and response to therapy.[1,2] In the last years, a relationship between prostatic inflammation and benign prostatic hyperplasia (BPH) has also been suggested.[3] Conversely, there is a paucity of data regarding the OS parameters in the asymptomatic inflammatory prostatitis (AIP) patients.

In this prospective controlled study, we aimed to compare the OS parameters in patients who were diagnosed with prostate cancer, BPH or AIP according to the histopathologic examination of transrectal ultrasonographic prostate biopsy (TRUS-Bx) and transurethral prostate resection (TURP) specimens.

Material and methods

Subjects and study design

This prospective controlled study was approved by the Ethical Committee of Gülhane Military Medical Academy, protocol number 1491-1175-10/1539, and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all the participants in the study group and before the collection of blood specimens. A total of 127 patients were enrolled to the study to form 3 study groups and a control group. According to the histopathologic examination of TRUS-Bx and TURP specimens, 97 patients were divided into 3 study groups: group 1: prostate cancer (n = 30), group 2: benign prostatic hyperplasia (n = 41), and group 3: asymptomatic inflammatory prostatitis (n = 26). A final group of 30 patients with no lower urinary tract symptoms, normal PSA levels, normal digital rectal examination and no history of previous prostatitis treatment was enrolled as group 4 (control group).

Inclusion and exclusion criteria

Inclusion criteria: TRUS Bx performed on patients due to elevated serum PSA levels or abnormal digital rectal examination and TURP performed on patients due to lower urinary tract symptoms.

Exclusion criteria: Previous history of cancer treatment, presence of liver dysfunction, diabetes mellitus, heart failure or renal failure; smoking; chronic alcohol use, and oral antioxidant supplementation or any mineral supplementation at the moment of the enrollment.

Sample collection and laboratory methods

Blood samples were drawn from the antecubital vein following an overnight fast and distributed into evacuated tubes containing ethylendiaminetetraacetic acid (EDTA). All samples were centrifuged for 10 min at 4000 g and 4°C. After the plasma was separated, the buffy coat was removed and the packed cells were washed 3 times with 2 volumes of isotonic saline. Then, a known volume of erythrocytes was lysed with cold distilled water (1:4), stored in a refrigerator at 4°C for 15 min and the cell debris was removed by centrifugation (2000 g at 4°C for 10 min). Plasma samples and erythrocyte lysates were stored at –70°C until assayed. Copper and zinc-containing superoxide dismutase enzyme (CuZn-SOD), selenium-dependent glutathione peroxidase (Se-GPx) and catalase (CAT) activities were measured in the erythrocyte lysates on a UV-VIS recording spectrophotometer (UV-2100S, Shimadzu Co., Kyoto, Japan). Erythrocyte CuZn-SOD activity was measured as previously described by Eken et al.[4] The measurement of erythrocyte CuZn-SOD enzyme activity was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with INT to form a red formazan dye. CuZn-SOD activity is expressed in U/g Hb. Erythrocyte Se-GPx activity was measured as previously described by Tüzün et al. and expressed in U/g Hb.[5] Erythrocyte CAT activity was measured in hemolysates at 25°C by the method developed by Aebi.[6] The activity is expressed as KU/g Hb. Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances in erythrocyte lysates by the method previously described by Eken et al.[4] After malondialdehyde (MDA) reacted with thiobarbituric acid, the reaction product was followed spectrophotometrically at 532 nm, using tetrametoxypropane as a standard. The results are expressed as nmol/mL. An atomic absorption spectrometer with a Zeaman background correction (PerkinElmer Analyst 800, Shelton, CT 06484-4794 USA) was used to detect the trace element levels: zinc (Zn), copper (Cu), and selenium (Se) in plasma and erythrocyte samples.

Statistical analysis

Statistical Package for Social Sciences (SPSS) v. 15.0 was used as a software package for statistical evaluations. All results were presented as mean ±standard deviation (SD). Conformity to the normal distribution of variables was assessed by the Kolmogorov-Smirnov test. In order to compare the continuous variables among groups, the ANOVA test was used. Pearson's correlation analysis was used to evaluate the relationship between variables. Age-adjusted analysis of covariance was used for comparisons. The values were considered statistically significant, if the p-value was less than 0.05.

Table 1. MDA, antioxidant enzyme and trace element levels according to the groups

	PCa (n = 30)	BPH (n = 41)	AIP (n = 26)	Control (n = 30)
MDA (nmol/mL)	12.26 ±3.08	7.69 ±2.41	6.66 ±1.73	5.95 ±1.11
CuZn-SOD (U/g Hb)	615.29 ±142.28	575.99 ±110.31	647.80 ±39.71	686.57 ±95.61
CAT (KU/g Hb)	62.21 ±10.67	53.31 ±9.67	57.44 ±11.68	50.41 ±9.17
Se-GPx (U/g Hb)	5.18 ±1.18	4.87 ±0.91	5.45 ±0.65	5.83 ±1.17
Cu (µg/mL)	0.56 ±0.11	0.51 ±0.08	0.58 ±0.04	0.63 ±0.08
Zn (µg/mL)	7.89 ±1.53	7.22 ±1.15	8.10 ±0.62	8.68 ±1.11
Se (ng/mL)	98.96 ±19.21	91.08 ±14.16	101.60 ±5.11	111.41 ±11.67

PCa – prostate cancer; BPH – benign prostatic hyperplasia; AIP – asymptomatic inflammatory prostatitis; MDA – malondialdehyde; CuZn-SOD – copper and zinc-containing superoxide dismutase enzyme; Se-GPx – selenium-dependent glutathione peroxidase; CAT – catalase; Cu – copper; Zn – zinc; Se – selenium.

Results

Thirty newly diagnosed men with prostate cancer, 41 men with benign prostatic hyperplasia, 26 men with asymptomatic inflammatory prostatitis, and 30 control subjects were enrolled in the study. Mean patient ages were 65.16 ±8.40, 68.65 ±7.35, 64.76 ±8.09 and 53.93 ±5.41 years in prostate cancer, benign prostatic hyperplasia, asymptomatic inflammatory prostatitis and control groups, respectively. Total serum PSA levels were significantly high in the prostate cancer group when compared to other groups (p < 0.001). On the other hand, there was no statistical difference between the groups in terms of height, weight, body mass index and hemoglobin levels (p > 0.05).

MDA, antioxidant enzyme and trace element levels according to the groups are summarized in Table 1, and the comparison of the groups according to the OS parameters is shown in Table 2. A statistically significant difference between prostate cancer and other groups were documented in terms of MDA and catalase activity (p < 0.05). The only exception was between prostate cancer and AIP group catalase activity. Prostate cancer patients had lower CuZn-SOD levels than control group patients but the difference was not statistically significant (p ≥ 0.05). BPH group had a lower CuZn-SOD, Se-GPx activity and a higher MDA activity when compared with the control group and all differences were statistically significant, except MDA activity (p < 0.05). CAT activity was the second parameter to be found as statistically insignificant. The AIP group had comparable results with those of the control group. CAT activity was found to be significantly high in the AIP group (p < 0.05).

Trace element levels were significantly low in patients with prostate cancer and BPH when compared with the control group, and the differences were statistically significant. The only exception was the Zn levels between the group with prostate cancer and the control group. Again, there was no statistical significance between the AIP group and the control group in terms of trace elements.

Table 2. The comparison of the groups according to OS parameters

Parameters	PCa & BPH	PCa & AIP	PCa & control	BPH & AIP	BPH & control	AIP & control
MDA	<0.001	<0.001	<0.001	0.454	0.068	1
CuZn-SOD	0.762	1	0.130	0.05	0.004	1
CAT	0.003	0.503	0.001	0.692	1	0.137
Se-GPx	1	1	0.184	0.169	0.012	1
Cu	0.099	1	0.043	0.015	<0.001	0.267
Zn	0.119	1	0.135	0.022	<0.001	0.602
Se	0.128	1	0.016	0.021	<0.001	0.116

PCa – prostate cancer; BPH – benign prostatic hyperplasia; AIP – asymptomatic inflammatory prostatitis; MDA – malondialdehyde; CuZn-SOD – copper and zinc-containing superoxide dismutase enzyme; Se-GPx – selenium-dependent glutathione peroxidase; CAT – catalase; Cu – copper; Zn – zinc; Se – selenium; p < 0.05 considered as statistical significance.

Discussion

Oxidative stress is the interruption of the balance between oxidant and reductant mechanisms and the excessive production of ROS. This imbalance leads to oxidative DNA damage and creates a nidus for the etiopathogenesis of several diseases.[1] In relation to the prostate, there is plenty of data regarding the relationship between ROS species, OS and age-related pathologies, such as PCa, BPH and AIP.[1–3] In our prospective controlled study, we aimed to investigated the OS parameters in patients with PCa, BPH and AIP, and compared the results with a control group. All the patients were diagnosed according to the histopathologic examination of transrectal ultrasonographic (TRUS) prostate biopsy (Bx) and transurethral prostate resection (TURP) specimens.

While MDA, hydrogen peroxide, superoxide radical or nitric oxide can be used to evaluate OS status, several endogenous antioxidant enzymes including SOD, GPx, CAT, some trace elements including Cu^{+2}, Zn^{+2}, Se^{+2}, and some molecules like vitamin E, vitamin C, transferrin and ceruloplasmin can be used in order to evaluate antioxidant capacity.[7–10] In our cohort, we analyzed MDA, SOD, GPx, CAT and Cu^{+2}, Zn^{+2}, Se^{+2} to evaluate the OS status.

MDA levels are considered to be a valuable parameter to evaluate lipid peroxidation and OS. MDA is a highly reactive aldehyde and has the potential for DNA damage, probably leading to mutagenic, genotoxic and cytotoxic effects.[11] While Dogru-Abbasoglu et al. did not find a significant difference in MDA levels in comparison to PCa and BPH patients, some other investigators documented significantly increased MDA levels in PCa patients when compared with BPH and control.[12–17] In our study, a statistically significant difference between patients with prostate cancer and other groups was documented in terms of MDA activity. On the contrary, no statistically significant difference was encountered between AIP/control and BPH/control groups.

In the literature in English, there are debatable results regarding antioxidant activity in PCa or BPH patients. While some authors documented decreased activity, others revealed no change.[18–20] In their study, Doğru-Abbasoglu et al. compared PCA and BPH patients in terms of SOD, CAT and GPx activity and showed no significant difference.[11] Aydin et al. conducted a controlled study to evaluate antioxidant activity in the patients with PCa and BPH and revealed that PCa patients had decreased CuZn-SOD enzyme levels when compared with BPH and control.[21] Jun-Fu Zhou et al. also demonstrated significantly decreased CuZn-SOD levels in chronic bacterial prostatitis (category 3), but the literature data regarding AIP patients is lacking.[22] In our study, decreased CuZn-SOD enzyme levels were found to be lower in PCa and BPH patients, but these differences were statistically significant only for BPH and control group patients.

There are also conflicting results regarding CAT and GPx activity in PCa or BPH patients. Some authors demonstrated decreased activity while others showed no difference or increased activity.[21,23–25] In their study, Biri et al. reported increased CAT and GPx activity in PCa patients when compared with BPH and control.[25] They concluded that this increase was due to a rebound effect to increased oxidative stress in order to neutralize it. Our results also revealed increased CAT activity in PCa, BPH and AIP patients when compared with the control group. The difference was statistically significant in PCa patients when compared with BPH and control group patients. But no significant difference in GPx activity was documented between groups, except BPH and control group.

Cu^{+2} and Zn^{+2} catalyze SOD enzyme and their levels are consistent with SOD levels. On the other hand, Zn^{+2} has an additional contribution to hormonal function on prostatic tissue.[26] Yan et al. hypothesized that decreased levels of Zn^{+2} are associated with increased DNA damage.[27] Christudoss et al. and Gomez et al. have found that Zn^{+2} levels were decreased in PCa patients when compared with control.[28,29] In our study, Zn^{+2} levels were decreased in PCa, BPH and AIP groups when compared with control, but a statistically significant difference was observed only between BPH and control groups. Similar results were documented also for Cu^{+2} levels but the statistical significance was seen in both PCa/control and BPH/control groups.

Se^{+2}, a trace element, is found to be protective against several malignancies with respect to several animal and cell culture studies.[30] This is considered to be due to the induction of apoptosis, which prevents cellular proliferation and has a key role for GPx enzyme activity.[31–33] There are some studies showing decreased levels in PCa and BPH patients, in contrast to some showing no difference.[34–37] Our results revealed statistically decreased Se^{+2} levels in PCa and BPH groups when compared with control group.

Conclusions

Oxidative stress, interruption of the balance between oxidant and reductant mechanisms, can lead to several prostatic diseases. Although there is plenty of data regarding OS in PCa patients, there is a lack of data regarding BPH and especially AIP patients. Our prospective controlled study has its unique advantage of evaluating several OS parameters in these patients and showed obvious oxidative stress in BPH and PCa patients as opposed to AIP. Assessing the oxidative stress in these patients may assist in the future prevention, diagnosis and also treatment. Despite our encouraging results, whether the presence of OS-related parameters and drugs could be used for the diagnosis or management of prostatic diseases is something that needs to be addressed in several future larger and better studies with a more rational basis.

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