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Peripheral mononuclear leukocyte DNA damage, plasma prolidase activity, and oxidative status in patients with benign prostatic hyperplasia

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Objectives: Prolidase plays a major role in collagen turnover, matrix remodeling, and cell growth. Benign prostatic hyperplasia (BPH) may be associated with an increased extracellular matrix deposition. Therefore, the present study was designed to investigate the plasma prolidase activity, oxidative status, and peripheral mononuclear leukocyte DNA damage in patients with BPH.

Patients and methods: Twenty-six male patients with BPH and 24 healthy male subjects were included in this study. Blood samples were collected from antecubital vein after an overnight fasting period, and the plasma was separated. Plasma prolidase activity, total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were determined. The peripheral lymphocyte oxidative DNA damage was determined using an alkaline single cell gel electrophoresis assay (comet assay).

Results: The plasma prolidase activity, TOS levels, OSI values, and peripheral mononuclear leukocyte DNA damage were significantly higher ($P < 0.001$), while the TAC levels were significantly lower ($P < 0.001$) in patients with BPH than controls. In BPH patients, the prolidase activity was significantly associated with TAC levels ($r = -0.366$, $P < 0.05$), TOS levels ($r = 0.573$, $P < 0.001$), and OSI ($r = 0.618$, $P < 0.001$) and peripheral mononuclear leukocyte DNA damage ($r = 0.461$, $P < 0.001$).

Conclusions: Our results showed that BPH might be associated with an increased oxidative stress, and also an increased plasma prolidase activity. Increased prolidase activity might play an important role in the etiopathogenesis and/or progression of BPH.

Keywords: Benign prostatic hyperplasia, Prolidase, Peripheral leukocyte DNA damage, Total antioxidant capacity, Total oxidant status, Oxidative stress index

Introduction

Benign prostatic hyperplasia (BPH) is a common health problem among elderly men. It is characterized by an overgrowth of the prostatic epithelium and fibromuscular tissue of the transition zone and periurethral area, and obstructive, irritative lower urinary tract symptoms.[1] The pathogenesis of BPH is not thoroughly understood. Androgens, essential for normal prostate growth and development, play a prominent role. However, there is also evidence that metabolic disturbances may promote prostate hyperplasia and BPH pathogenesis.[2] BPH is a disease associated with aging.[3] Aging is an inherently complex process that is manifested within an organism at genetic, molecular, cellular, organ, and system levels.

Although the fundamental mechanisms are still poorly understood, a growing body of evidence points toward reactive oxygen species (ROS) as one of the primary determinants of aging.[4] ROS are produced in an overwhelming quantity with advanced age. They cause extensive damage to various organs

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and may also be associated with the pathogenesis of BPH. Oxidative stress has a major role in aging process and age-related diseases, such as prostate cancer and BPH.[5] It has been demonstrated that oxidative stress is positively correlated with prostate cancer.[6] Several studies have examined the role of oxidative stress in patients with BPH.[3,6–8] On the other hand, antioxidants are compounds that prevent oxidative reactions. Lower levels of essential antioxidants in the circulation have been found to be associated with an increased risk of cancer.[9] Several studies have shown the altered prooxidant–antioxidant status in the prostatic tissue of men, rats, and permanent cell lines.[6,10]

Prostate growth modulated by age-related changes in endocrine factors and other growth-regulatory factors is likely the cause of cellular proliferation.[11] The extracellular matrix (ECM) consists of collagens, proteoglycans, and glycoproteins. BPH is associated with a proliferation of smooth muscle cells and increased ECM deposition. ECM has been reported to play an important role in epithelial differentiation.[12] BPH is mainly a stromal process with an increased ratio of stromal to epithelial elements, a collagen type III down regulation, and a collagen type I and IV upregulation.[13]

The ECM is degraded by metalloproteinases (MMPs), which results in the release of a large amount of peptides containing proline and hydroxyproline.[14] Among the MMPs, prolidase is a manganese-dependent cytosolic exopeptidase that cleaves imidodipeptides and imidotripeptides with a C-terminal proline or hydroxyproline.[15] Prolidase plays a major role in collagen turnover, matrix remodeling, and cell growth. The final step of collagen degradation is mediated by prolidase, a cytosolic exopeptidase.[14] Prolidase activity has been documented in erythrocytes, leukocytes, plasma, dermal fibroblasts, the kidney, brain, heart, thymus, and uterus.[16] Its activity has been shown to be affected in numerous clinical conditions.[17,18] To the best of our knowledge, the plasma prolidase activity in patients with BPH has not been examined.

The comet assay (single-cell gel electrophoresis) is a well-established genotoxicity test that is simple and sensitive. It has rapidly gained acceptance as a genotoxicity assay.[19] In the literature, limited data are available regarding the leukocyte DNA damage (comet assay) in patients with BPH.[20]

The aim of this study was to investigate the plasma prolidase activity, total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), and peripheral mononuclear leukocyte DNA damage in patients with BPH. We also compared the relationship between oxidant, antioxidant status, and leukocyte DNA damage with prolidase enzyme activity in BPH.

Materials and methods

Subjects

In this prospective study, 26 newly diagnosed male patients with BPH and 24 healthy male subjects were enrolled.

Diagnosis of BPH was performed by history of obstructive or irritative urinary symptoms, measurement of peak uroflow (Qmax; Aymed, Istanbul, Turkey), prostate volume determined by transabdominal ultrasound and symptom score (IPSS questionnaire). Prostate-specific antigen (PSA) levels were evaluated for all subjects enrolled. Histopathological examination in BPH patients was not performed, because it is not appropriate ethically and it is an invasive procedure.

The exclusion criteria were hematuria, urethral strictures, previous prostate surgery, a suspicion of prostate cancer, neurological, psychiatric and malignant diseases, renal and liver diseases, the use of anti-inflammatory or immunosuppressive drugs, and oral antioxidant supplementation.

The control group consisted of 24 healthy subjects (without a history of chronic or recurrent diseases). The control subjects were asymptomatic with an unremarkable medical history and a normal physical examination. Subjects for the control group were chosen among those hospitalized for nonmalignant diseases (e.g., hydrocele, varicocele, and urinary stone disease). All control subjects were lifetime nonsmokers. None of the control subjects had been receiving oral antioxidant supplementation, such as vitamins E or C.

The study was conducted only after voluntary consent was obtained from the subjects. A full explanation of the study protocol was provided to the subjects before their consent was requested. The study protocol was accepted by the Ethical Committee of Yuzuncu Yıl University, Dursun Odabas Medical Center, Van, Turkey.

Blood analysis

Blood samples were collected at rest between 08:00 and 10:00 a.m. from the antecubital vein after an overnight fasting period. Peripheral venous blood samples were placed into heparinized tubes. The blood was centrifuged at 1.409 g for 10 minutes to separate the plasma. The plasma samples were stored at −20°C until the use for the measurement of prolidase activity, and TAC and TOS levels.

Measurement of plasma total antioxidant capacity

The plasma TAC was determined using an automated measurement method developed by Erel.[21] In this method, a hydroxyl radical, which is the most potent biological radical, is produced. In the assay, a ferrous ion solution, which is present in reagent 1, is mixed

with hydrogen peroxide, which is present in reagent 2. The subsequently produced radicals, such as the brown-colored dianisidinyl radical cation produced by the hydroxyl radical, are also potent radicals. Using this method, the antioxidative effect of the sample against the potent free radical reactions that is initiated by the produced hydroxyl radical is measured. The assay has precision values below 3%. The results are expressed as mmol Trolox Eq./l.

Measurement of plasma total oxidant status

The plasma TOS was determined using a novel automated measurement method developed by Erel.[22] The oxidants present in the sample oxidize 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 forms a colored complex with xylenol orange in an acidic medium. The color 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 the micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq./l).

Determination of the oxidative stress index

The percent ratio of the TOS level to the TAC level was accepted as the OSI.[23] OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq./l)/TAC (mmol Trolox Eq./l).

Determination of plasma prolidase activity

The plasma was diluted 40-fold with 2.5-mmol/l Mn^{+2}, 40-mmol/l Trizma HCl buffer (pH: 8.0) and preincubated at 37°C for 2 hours. The reaction mixture containing 30-mmol/l gly-pro, 40-mmol/l Trizma HCl buffer (pH: 8.0), and 100 ml of preincubation serum in 1 ml was incubated at 37°C for 30 minutes. A total of 0.5 ml of 20% trichloroacetic acid solution was subsequently added to stop the incubation reaction. The supernatant was used to measure proline using the method proposed by Myara *et al.*,[24] which is a modification of Chinard's method.[25] The intra-assay coefficient of variation for the assay was 3.8%. The results are expressed as U/l.

Mononuclear leukocyte DNA damage determination using the comet assay

After an overnight fasting, 6 ml of a peripheral blood sample was withdrawn into a heparinized tube from each subject and kept on ice. The mononuclear leukocyte isolation for the comet assay was performed within 2 hours as described elsewhere.[26]

The endogenous DNA damage in the mononuclear leukocyte was analyzed by a comet assay according to Singh *et al.*[27] with minor modifications. After electrophoresis, the slides were stained with ethidium bromide (2 μl/ml in distilled H_2O; 70 μl/slide), covered with a coverslip and analyzed using a fluorescence microscope (Nikon, Olympus BX51, Japan). Images of 50 randomly selected cells (25 cells from each of the two replicate slides) were analyzed visually from each subject as described elsewhere.[26,28] Each image was classified according to the intensity of the fluorescence in the comet tail and was given a value of either 0–3 or 4 (ranging from undamaged (Class 0) to maximally damaged (Class 4) (Fig. 1). Therefore, the total slide score could be between 0 and 200 arbitrary units.

Statistical analysis

The results are expressed as means ± standard deviations (SD). Nonparametric continuous variables were compared by the Mann–Whitney *U*-test. Parametric variables were compared using Student's *t*-test. Qualitative variables were assessed by Chi-square test. Correlation analyses were performed using Pearson's correlation test. Differences were considered to be statistically significant when $P < 0.05$. The data were analyzed using SPSS® for Windows (Version 11.0).

Results

The demographic and clinical data of the subjects with BPH and controls are presented in Table 1. There were no statistically significant differences between the BPH patients and controls with respect to age and body mass index ($P > 0.05$) (Table 1).

IPS score and nocturia were significantly higher in the patients with BPH than the controls (both; $P < 0.001$), while peak uroflow (Qmax) was lower significantly ($P < 0.001$). There was no statistically significant difference between the BPH patients and controls with respect to plasma PSA levels ($P < 0.001$) (Table 1).

The plasma prolidase activity (Fig. 2), TOS levels (Fig. 3), OSI values (Fig. 3), and peripheral lymphocyte DNA damage (Fig. 4) were significantly higher (all; $P < 0.001$), while TAC levels (Fig. 3) were significantly lower in the patients with BPH than the controls ($P < 0.001$) (Table 2).

The plasma prolidase activity was significantly associated with TAC levels ($r = -0.366$, $P < 0.05$), TOS levels ($r = 0.573$, $P < 0.001$), OSI values ($r = 0.618$, $P < 0.001$), and mononuclear leukocyte DNA damage ($r = 0.461$, $P < 0.001$) in BPH patients (Table 3).

Discussion

In this study, we investigated the plasma prolidase activity, mononuclear leukocyte DNA damage, and oxidant and antioxidant status in patients with BPH. We also compared the relationship between oxidant, antioxidant, and leukocyte DNA damage with

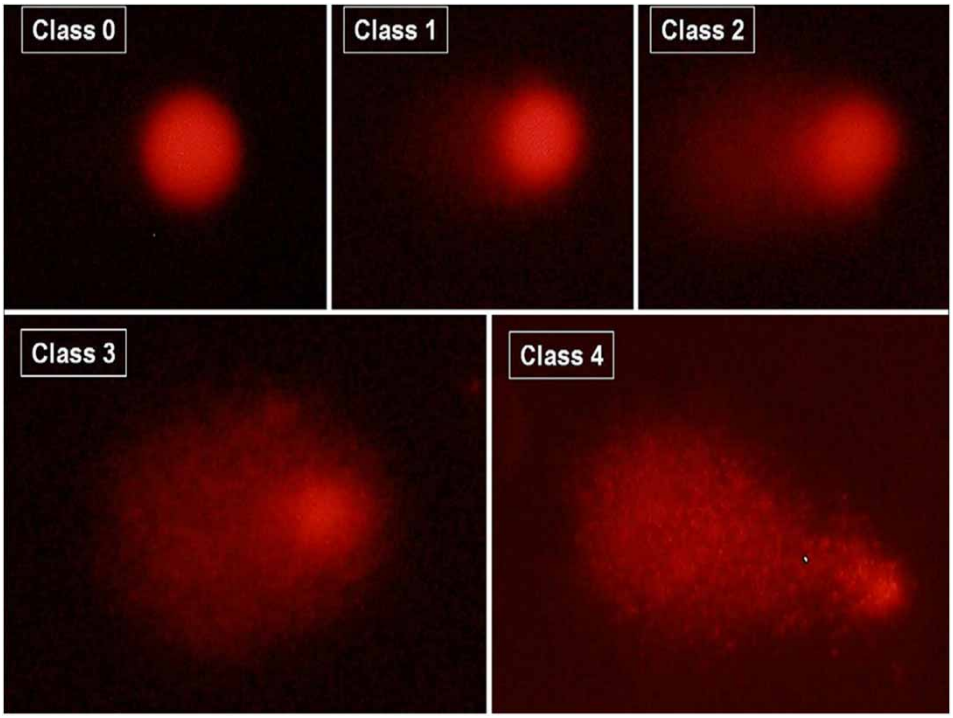

Figure 1 Photomicrographs were showed according to fluorescence intensity in the comet classes (class 0, undamaged; class 4, maximally damaged).

prolidase enzyme activity in BPH. To our knowledge, this paper is the first study that has investigated prolidase enzyme activity in patients with BPH.

In this study, we showed that BPH patients had a significantly higher serum prolidase activity, TOS levels, OSI values, and mononuclear leukocyte DNA damage, while lower TAC levels compared to healthy subjects. In addition, prolidase activity was correlated with TAC levels, TOS levels, OSI values, and mononuclear leukocyte DNA damage in BPH patients. The increased enzyme activity might reflect an increased collagen turnover in BPH patients. An increased prolidase activity due to the increased oxidative stress may also be related to the pathogenesis of BPH.

BPH is one of the most common problems encountered in urology and is the most common benign tumor in men. The process of BPH includes three pathological stages, which are the nodule formation, diffuse enlargement of the transition zone and periurethral tissue, and enlargement of the nodules.[13,29] BPH consists of an overgrowth of the epithelium and fibromuscular tissue of the transition zone and periurethral area. There is a considerable quantity of evidence suggesting that BPH is an endocrine disease. Adipose tissue, which accumulates with age, aromatizes circulating testosterone into estrogen, and it has been hypothesized that alterations in the balance between testosterone and estrogen levels in prostate tissue with age may contribute to BPH.[29]

A comet assay was used to identify the DNA damage in this study. The comet assay has been proposed to be the most sensitive of all procedures for detecting DNA fragmentation. Furthermore, the

Table 1 The demographic and clinical data in benign prostatic hyperplasia and healthy subjects

Parameters	Controls ($n = 24$)	BPH ($n = 26$)	*P*
Age (year)	61.4 ± 2.3	62.2 ± 3.2	ns
Body mass index (kg/m^2)	22.11 ± 1.25	21.23 ± 1.02	ns
PSA (ng/ml)	1.42 ± 0.42	1.43 ± 0.35	ns
IPS score (points)	4.58 ± 1.66	18.23 ± 6.01	0.001
Nocturia	0.58 ± 0.56	3.81 ± 1.67	0.001
Peak flow (ml/s)	16.79 ± 2.02	8.04 ± 2.97	0.001

Values are mean ± SD.
ns = not significant; BPH: benign prostatic hyperplasia; PSA: prostate-specific antigen.

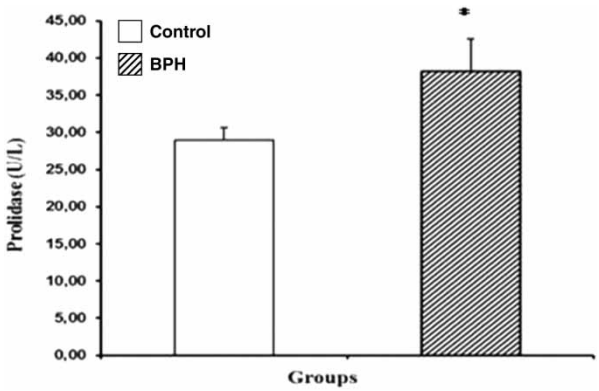

Figure 2 Plasma prolidase activity in benign prostatic hyperplasia and healthy subjects.

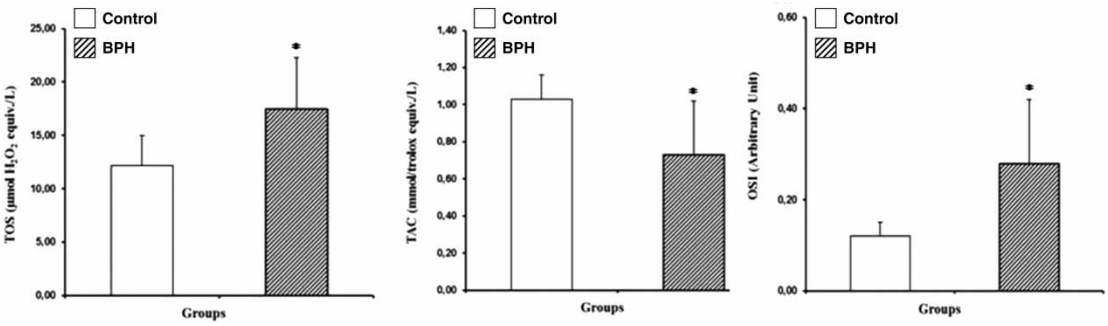

Figure 3 The total antioxidant capacity, total oxidant status, and oxidative stress index in benign prostatic hyperplasia and healthy subjects.

comet assay has been found to be technically suitable for the routine measurement of DNA damage. Damage to DNA is of great importance because of the growing recognition that such damage can both initiate and promote carcinogenesis.[30,31] The single-cell gel electrophoresis (comet) assay is a useful method to quantify DNA damage.[19] Because of the simplicity and sensitivity, the comet assay has rapidly gained acceptance as a genotoxicity assay.[19] In addition, the comet assay has shown that strand breaks arise from DNA damage generated by oxidative stress.[32] Therefore, we used this method to measure the DNA damage in circulating mononuclear leukocytes. More recently, Ahmad *et al.*[20] observed a significant increase in leukocyte DNA damage in patients with BPH patients compared with controls subjects. Similarly, we reported that BPH patients had a significantly higher mononuclear leukocyte DNA damage than in subjects.

Aging, oxidative stress derived from endogenous and exogenous sources, infection, and inflammation are recognized risk factors for BPH and prostate cancer.[33] Chronic inflammation of prostate gland, increased epithelial cell proliferation, and oxidative stress can produce repeated tissue damage and post-translational DNA modifications and can play a role in carcinogenesis.[34] These events may provoke the compensatory cellular proliferation with the risk of simply hyperplasia or prostatic neoplasia.[35] It is well-accepted that regions of prostatic inflammation will generate free radicals. Macrophage and neutrophil infiltration provides a source of free radicals that can induce a hyperplastic or precancerous transformation through the oxidative stress to the tissue and DNA.[35] The oxidative stress and accumulated genomic damage may contribute to prostate carcinogenesis.[36]

The serum concentrations of different oxidant species can be measured separately in laboratories; however, the measurements are time-consuming, expensive, and require complicated techniques. More recently, lipid peroxidation levels were monitored by determining TOS.[23] Moreover, TAC has been used as a measure of antioxidants.[21] Little is known regarding the serum oxidative status and BPH.[2] In that study, Savas *et al.*[2] did not find a significant difference in the plasma TAC, TOS, and OSI levels between BPH patients and controls subjects. Conversely, in the current study, we observed that the serum TOS levels and OSI values were significantly higher, while TAC values were lower in BPH patients than in controls.

The relationship between oxidative stress or antioxidant parameters and BPH has been investigated in

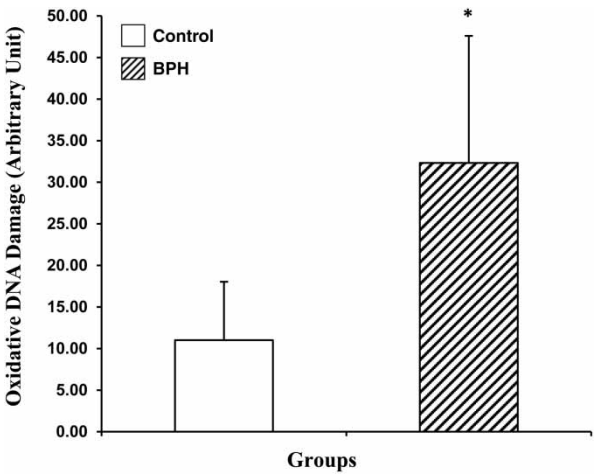

Figure 4 Peripheral lymphocyte oxidative DNA damage in benign prostatic hyperplasia and healthy subjects.

Table 2 The plasma prolidase activity, total antioxidant capacity and total antioxidant status levels, oxidative stress index values, and peripheral lymphocyte oxidative DNA damage in benign prostatic hyperplasia and healthy subjects

Parameters	Controls ($n = 24$)	BPH ($n = 26$)	*P*
TAC (mmol Trolox Eq./l)	1.03 ± 0.13	0.73 ± 0.29	0.001
TOS (μmol H_2O_2 Eq./l)	12.18 ± 2.78	17.47 ± 4.80	0.001
OSI (arbitrary unit)	1.21 ± 0.38	2.85 ± 1.49	0.001
Prolidase (U/l)	28.90 ± 1.74	38.23 ± 4.32	0.001
DNA damage (arbitrary unit)	11.01 ± 7.02	32.33 ± 15.28	0.001

Values are shown as the mean ± SD.
TAC: total antioxidant capacity;TOS: total oxidant status; OSI: oxidative stress index; BPH: benign prostatic hyperplasia.

Table 3 Correlations between oxidant, antioxidant, and DNA damage parameters with prolidase enzyme activity in benign prostatic hyperplasia

Parameters	*r*	*P* value
TAC (mmol Trolox Eq./l)	−0.366	0.05
TOS (μmol H_2O_2 Eq./l)	0.573	0.001
OSI (arbitrary unit)	0.616	0.001
DNA damage (arbitrary unit)	0.461	0.001

TAC: total antioxidant capacity; TOS: total oxidant status; OSI: oxidative stress index.

several studies with conflicting results.[3,6–8] Aydın *et al.*[6] and Aryal *et al.*[7] showed an elevated lipid peroxidation with concomitant antioxidant depletion in BPH. However, some studies have observed no significant difference in the lipid peroxidation or antioxidant system parameters of patients with BPH compared to controls.[3] Similarly, Jung *et al.*[8] have reported that there are no differences between the antioxidant enzyme activities of prostatic epithelial cell cultures of benign and malignant tissue.

ROS may induce oxidative modifications for macromolecules, such as proteins, lipids, carbohydrates, or DNA.[4] ROS have a strong association with many human chronic and degenerative diseases, such as cardiovascular disease, cancer, and immune dysfunction.[37] ROS have also been shown to cause extensive DNA damage, including single-strand breaks, the formation of modified bases, chromosomal damage, and mutations in mammalian cells.[38] Moreover, ROS cause the formation of oxidized bases, single-strand breaks, and crosslinking of DNA.[38]

The interactions between the ECM components, cellular compartments, and hormonal factors suggest a major role of ECM in the development and progression of epithelial abnormalities, such as BPH and cancer.[39] Collagen, the most abundant protein in the body, constitutes more than a quarter of the total body protein.[40] Collagen is responsible for maintaining the architecture and integrity of the connective tissue.[41] Morphometric and histochemical techniques were used to analyze the three major types of collagen (types I, III, and IV) in the human prostate. In BPH tissues, a significant increase in collagen types I and IV and a decrease of type III collagen were observed.[13]

One of the enzymes involved in collagen biosynthesis is prolidase [E.C.3.4.13.9]. Prolidase is a homodimeric iminodipeptidase that releases a carboxy-terminal proline or hydroxyproline from oligopeptides and plays a major role in collagen turnover, matrix remodeling, and cell growth.[14] Moreover, it has been suggested that the prolidase enzyme activity may be a rate-limiting factor in the regulation of collagen biosynthesis.[14] An increased level of prolidase activity has been associated with increased rates of collagen synthesis, although the mechanism for this association is not fully understood. It has been shown that prolidase activity is increased in many clinical diseases, such as chronic liver disease[17] and coronary artery disease,[18] indicating an increased collagen turnover. However, prolidase is a homodimeric enzyme with an activity level that is affected by oxidative stress.[18] Yıldiz *et al.*[18] reported that there is a strong relationship between serum prolidase activity and oxidative stress. However, it is not reported in the medical literature whether there is an association between serum prolidase activity and oxidative status in BPH. We hypothesize that an altered prooxidant–antioxidant balance may lead to an increased prolidase activity and consequently plays an important role in the pathogenesis of BPH.

Our study has several limitations. First, the investigation used a cross-sectional design. Second, the study sample was small, so these observations must be confirmed in a larger patient sample. Therefore, further studies are needed to identify the regulatory mechanisms of BPH-related changes of ECM, which may play a major role in the pathogenesis of BPH. Third, we were unable to perform leukocyte DNA damage, prolidase activity, and oxidative stress levels in prostate tissue of BPH patients due to certain problems.

It has been concluded that the BPH may be associated with an increased oxidative stress and increased serum prolidase activity. These results indicate that an increased prolidase activity seems to be associated with an increased oxidative stress and decreased antioxidant levels in patients with BPH. Therefore, an increased prolidase activity may play a role in the pathogenesis of BPH.

Disclaimer statements

Contributors IG, MA, KC, and HD: Conception and design;

IG, MA, HC, AT, and IM: Analysis and interpretation of the data;

IG, MA, IM, and AK: Critical revision of the article for important intellectual content;

IG, MA, IM, and AK: Final approval of the article;

NP, MG, MU, MK and KT: Collection and assembly of data.

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Conflicts of interest None.

Ethics approval The study protocol was accepted by the Ethical Committee of Yuzuncu Yıl University, Dursun Odabas Medical Center, Van, Turkey.

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