UDK 577. 1 : 61 **ISSN 1452-8258**

J Med Biochem 34: 455–459, 2015

Original paper
Originalni naučni rad

INCREASED ANTIOXIDANT QUALITY VERSUS LOWER QUANTITY OF HIGH DENSITY LIPOPROTEIN IN BENIGN PROSTATIC HYPERPLASIA

POVEĆANI ANTIOKSIDANTNI KVALITET NASPRAM MANJEG KVANTITETA LIPOPROTEINA VELIKE GUSTINE U BENIGNOJ HIPERPLAZIJI PROSTATE

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Summary

Background: Oxidative stress may be involved in the pathogenesis of every human disease. To understand its possible role in benign prostatic hyperplasia (BPH), we measured the overall oxidative status of patients with BPH and the serum activity of the high density lipoprotein (HDL)-related antioxidant enzymes paraoxonase 1 (PON1) and arylesterase (ARE).
Methods: Fifty-six urology outpatient clinic patients with BPH (mean age 64±8.6 years) were prospectively included in the study. Forty volunteer healthy controls from the laboratory staff (mean age 62±10 years) were enrolled for comparison. Serum total antioxidant status (TAS), total oxidant status (TOS), PON1, ARE, and HDL levels were measured by commercially available, ready-to-use kits.
Results: Serum TAS and HDL levels were significantly lower in the BPH group than in the control group (P=0.004 and P=0.02, respectively). No significant between-group differences were observed for TOS levels or PON1 and ARE enzyme activities (P=0.30, P=0.89, and P=0.74, respectively). In the BPH group, the calculated parameters PON1/HDL and ARE/HDL were significantly higher (P=0.02 and P=0.04, respectively).
Conclusions: Our findings agree with the previous reports of impaired oxidant/antioxidant balance in BPH patients. The activities of HDL-related enzymes between groups with sig-

Kratak sadržaj

Uvod: Oksidativni stres može biti uključen u patogenezu svake ljudske bolesti. Da bismo bolje razumeli njegovu potencijalnu ulogu u benignoj hiperplaziji prostate (BHP), merili smo ukupan oksidativni status pacijenata sa BHP i aktivnost u serumu antioksidantnih enzima povezanih sa lipoproteinom visoke gustine (LVG), paraoksonaze 1 (PON1) i arilesteraze (ARE).
Metode: Pedeset šest ambulantnih pacijenata sa BHP (prosečne starosti 64±8,6 godina) prospektivno je uključeno u ovu studiju. Četrdesetoro zdravih ispitanika, dobrovoljaca iz redova laboratorijskog osoblja (prosek starosti 62±10) takođe je uključeno radi poređenja. Ukupni antioksidantni status (UAS), ukupni oksidantni status (UOS), nivoi PON1, ARE i LVG u serumu mereni su pomoću komercijalno dostupnih test paketa.
Rezultati: Nivoi UAS i LVG u serumu bili su značajno niži u grupi sa BHP nego u kontrolnoj grupi (P=0,004 i P=0,02). Nisu uočene značajne razlike između grupa za nivoe UOS ili aktivnosti enzima PON1 i ARE (P=0,30, P=0,89 i P=0,74). Izračunati parametri PON1/LVG i ARE/LVG bili su značajno viši (P=0,02 i P=0,04) u grupi sa BHP.
Zaključak: Naši nalazi slažu se s prethodnim izveštajima o poremećenoj oksidantnoj/antioksidantnoj ravnoteži kod pacijenata sa BHP. Aktivnosti enzima povezanih sa LVG između grupa sa značajno različitim nivoima LVG mogu da

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nificantly different HDL levels may be deceptive; adjusted values may help to reach more accurate conclusions.

Key words: oxidative stress, total antioxidant status, total oxidant status, adjusted paraoxonase and arylesterase, HDL functionality, metabolic syndrome

zavaraju; prilagođene vrednosti pomoći će da se dođe do tačnijih zaključaka.

Ključne reči: oksidativni stres, ukupni antioksidantni status, ukupni oksidantni status, prilagođena paraoksonaza i arilesteraza, funkcionalnost LVG, metabolički sindrom

Introduction

Benign prostatic hyperplasia (BPH) is a nonmalignant overgrowth of prostatic glandular and stromal tissue. The area where the prostate surrounds the urethra is the main cause of clinical manifestations. Enlargement of the gland mechanically blocks the free flow of urine. Depending on the degree of obstruction, patients suffer from highly variable symptoms, collectively called »lower urinary tract symptoms«. BPH is a major concern in the 21st century, a time with the largest senior population in human history. The prostate normally enlarges to some degree in all men with advancing age. Some men with histologically proven BPH have no clinical complications, whereas others experience changes in voiding patterns that lead them to seek medical care and may require surgical treatment. BPH is apparently a chronic indolent disease that manifests over a span of decades (1). Reviews of medical records show evidence of a dramatic rise in the prevalence of histologically proven BPH from approximately 8% of men in their third decade to approximately 90% by their ninth decade.

Oxidative stress is a natural consequence of the use of molecular oxygen. Every human disease is associated with oxidative stress in some way. The human body fortunately is equipped with numerous endogenous scavenging systems. High density lipoprotein (HDL) possesses well-known atheroprotective and antioxidative properties (2). Paraoxonase 1 (PON1) is an HDL-associated enzyme esterase that may contribute to the antioxidant and antiatherosclerotic capabilities of the particle (3). PON1 and PON1-arylesterase (ARE) are two different enzymes. Previous studies have shown that a single gene product in human serum has ARE and PON1 activities. Serum PON1 acts in conjunction with ARE to function as a single enzyme (4).

It is challenging to measure the overall oxidative status in the body; however, several advances have been made. Commercially available laboratory kits have replaced measurements that are time consuming, expensive, or require complicated techniques. The total antioxidant status (TAS) is a useful estimate of the activity of antioxidants in a medium such as serum or plasma. Measurements of the TAS and the total oxidant status (TOS) can provide information on an individual's overall serum antioxidative status, which may include oxidants and antioxidants not yet recognized or easily measured (5, 6).

Life with oxygen is still cost-effective. People want to live healthy, longer lives. Therefore, scientists search for the most feasible methods to measure oxidative stress and related factors, such as antioxidant enzymes. This would assist in understanding the exact pathogenesis of human diseases and, more importantly, would allow the follow up of antioxidative remedies. In this study, we aimed to measure TAS, TOS, levels of HDL and the HDL-related antioxidant enzymes PON1 and ARE in BPH patients.

Material and Methods

Study population and clinical examinations

Fifty-six patients with benign prostatic hyperplasia (mean age 64±8.6 years) who had presented to the Urology Outpatient Clinic were prospectively included in the study. Also, forty volunteer healthy controls among laboratory staff (mean age 62±10 years) were enrolled for comparison.

The patients and controls were evaluated by the same urologist. All subjects gave informed consent before the onset of study. Blood pressure was measured manually with a sphygmomanometer. Hypertension was defined as systolic blood pressure of at least 140 mm Hg, and diastolic blood pressure of at least 90 mm Hg. Body mass index was calculated as weight in kilograms divided by height in meters squared. Those with a known history of any major disease like hypertension, diabetes, cardiac disease, renal, hepatic or endocrine disease were excluded. None of the participants in the present study were using medications including antihypertensive and lipid lowering agents, vitamins or antioxidant drugs. Smokers and alcohol users were also excluded. The subjects were locals with average family income, strongly favoring common dietary habits, and routine daily life. The patients were diagnosed with BPH according to the recommendations of the American Urological Association (7). Final diagnosis of each BPH patient was confirmed by histopathologic evaluation of prostate needle biopsies. This study was performed in accordance with the ethical standards set by the Declaration of Helsinki and was approved by a local ethics committee.

Blood sample collection

Blood samples were obtained after an overnight fasting state. Serum samples were then separated from the cells by centrifugation at 3000 rpm for 10 minutes. Serum HDL was measured freshly. Re-

maining serum portions were stored at –80 °C and used to analyze TAS, TOS, PON1, and ARE.

Measurement of serum HDL

The Ultra HDL assay (Abbott Diagnostics, USA) is a homogeneous method for directly measuring HDL cholesterol concentrations in serum or plasma using an accelerator selective detergent methodology. The method uses a two-reagent format and depends on the properties of a unique detergent. This method is based on accelerating the reaction of cholesterol oxidase with non-HDL unesterified cholesterol and dissolving HDL cholesterol selectively using a specific detergent.

Measurement of HDL-associated antioxidative enzyme activity

PON1 and ARE enzyme activities were measured by using commercially available kits (Relassay®, Turkey). A fully automated PON1 activity measurement method employs two different sequential reagents; the first reagent is an appropriate Tris buffer and it also contains a calcium ion, which is a cofactor of the PON1 enzyme. Linear increase in the absorbance of *p*-nitrophenol, produced from paraoxon, is followed at a kinetic measurement mode. One unit of paraoxonase activity is equal to 1 mmol of paraoxon hydrolysed per liter per minute at 37 °C (8).

Phenylacetate was used as a substrate to measure the ARE activity. PON1, present in the sample, hydrolyses phenylacetate to its products, which are phenol and acetic acid. The produced phenol is colorimetrically measured via oxidative coupling with 4-aminoantipyrine and potassium ferricyanide. Non-enzymatic hydrolysis of phenyl acetate was subtracted from the total rate of hydrolysis. One unit of arylesterase activity is equal to 1 mmol of phenylacetate hydrolysed per liter per minute at 37 °C (9).

Measurements of the total oxidant and antioxidant status in serum

The TAS and TOS of the serum were measured by commercially available kits (Relassay®, Turkey) on an autoanalyzer (Architect® c16000, Abbott Diagnostics), using automated colorimetric measurement methods developed by Erel (5). In the TAS method, antioxidants in the sample reduce the dark blue-green colored 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical to a colorless reduced ABTS form. The change of absorbance at 660 nm is related with the total antioxidant level of the sample. Using this method, the antioxidative effect of the sample against the potent free radical reactions initiated by the produced hydroxyl radical is measured (5).

In the TOS method, oxidants present in the sample oxidize the ferrous ion–chelator complex to ferric ion. The ferric ion makes a colored complex with chromogen 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 (6).

Statistical analysis

Statistical analyses were carried out using the statistical software SSPS. In normally distributed groups the results were presented with mean and SD, otherwise with medians and interquartile ranges. The significance of the differences between groups was determined by Student's unpaired t-test for normal distributions, and by the Mann–Whitney U-test in abnormal distributions. Pearson correlation coefficient and Spearman correlation coefficient were used to test the strength of any associations between different variables. P value equal to or less than 0.05 was accepted as the significance level.

Results

Data of the BPH patients and controls are summarized in *Table I*. The patients and controls were age

Table I Serum TAS and HDL were significantly lower in BPH group compared to the controls. PON1 and ARE enzyme activities showed no significant difference while enzyme activity per HDL concentration calculations showed higher activity in BPH patients.

Parameter	Patients (n=56)	Controls (n=40)	P
Age, (years)	64±8	62±10	0.34
BMI (kg/m^2)	27.3±3.8	26.7±3	0.42
PON1 (U/L)	77±39	76±46	0.89
ARE (kU/L)	131±63	135±65	0.74
TOS (μmol H_2O_2 Equiv./L)	4.36 (4–4.8)	4.7 (4–5.4)*	0.30**
TAS (nmol Troloks/L)	1.19±0.25	1.35±0.29	0.004
HDL (mmol/L)	0.90±0.36	1.06±0.25	0.02
PON1/HDL	2.7±2.3	1.9±1.1	0.02
ARE/HDL	4.4±3.2	3.3±1.5	0.04

The values are presented as mean±SD for normal distributions, median (IQR) for abnormal distributions. *abnormally distributed data, **: Mann-Whitney U-test was used.

matched as planned. Mean BMI of both groups showed no statistical difference, while HDL levels were significantly lower in the BPH group ($p=0.02$).

Serum TAS was significantly lower in the BPH group compared to the controls ($p=0.004$). There were no significant differences in TOS levels, and PON1, ARE enzyme activities between groups ($p=0.30$, $p=0.89$, $p=0.74$, respectively). When the calculated parameters were compared: PON1/HDL, and ARE/HDL were significantly higher in the BPH group ($p=0.02$, $p=0.04$, respectively).

Positive correlations were observed between ARE/HDL and TAS in both BPH and control groups ($r=0.35$ $p=0.008$, $r=0.32$ $p=0.04$, respectively); between ARE and TAS in BPH group ($r=0.66$ $p<0.0001$).

Discussion

Our findings are best discussed on two levels. First, the TAS and TOS measurements provided information on the subjects' overall oxidative balance. We observed that the patients with BPH tended to have similar oxidant levels as controls but failed to generate a corresponding antioxidant defense. Thereby, we considered them as being under a higher oxidative burden. Second, antioxidant enzyme activities were not significantly different between the groups, despite the first finding. To complicate the situation further, HDL (the protein related to these enzymes) was markedly lower in patients with BPH. At this point, we asked ourselves a crucial question: are these results related to each other in some way or not?

Since the introduction of the oxidative stress hypothesis, it is well known that HDL is associated with antioxidants and active antioxidant enzymes with high antioxidant potential (3). Among all HDL-related antioxidants, PON1 has attracted significant interest as the protein that is responsible for most antioxidant properties of HDL. Experimental studies have indicated that impaired PON1 activity leads to dysfunctional HDL, which indicates a low quality, »out of power« particle (3). PON1 is synthesized in the liver and secreted into the bloodstream where it is capable of breaking down artificial and naturally occurring compounds. PON1 is a multifaceted, multifunctional enzyme that also manifests ARE activity (4). Serum ARE activity has strong correlations with multiple systemic measures of oxidative stress (10). The HDL particle is susceptible to structural modifications that are mediated by various mechanisms (e.g., oxidation, glycation, or enzymatic degradation) that affect its functional properties (3).

Because of the close relationship between HDL particles and related antioxidants, a low level of HDL should be taken into account when interpreting PON1 and ARE activities. In the present study, the activities of both enzymes were similar in patients and controls; however, the serum TAS and HDL concentrations of the patients were significantly lower. We recognize this situation as compensation. The activities of the antioxidant enzymes were apparently modified to compensate for the low HDL levels in the patients with BPH.

To provide evidence for this hypothesis, we divided the measured enzyme activity by the serum HDL concentration to establish a numerical value for enzyme activity per HDL mass. The results supported our suggestion in that both parameters showed statistically significant differences. We actually employed these parameters in a previous study in which we measured oxidative stress in bladder cancer patients; however, we could not fit the results to any situation (11). In the current study, we are confident about the feasibility of these parameters.

Oxidative stress has already been defined as a culprit in the BPH pathogenesis (12–14). A handful of studies in the literature have demonstrated higher levels of oxidants or lower levels of enzymatic or nonenzymatic antioxidants in patients with BPH compared to controls. There are also studies on experimental therapies of antioxidant supplements (15, 16). The use of antioxidant supplements for oxidative stress seems quite reasonable, and may in fact be *too* reductive. Simple solutions can sometimes cause complications. In 2011, a 10-year follow up report on SELECT (the Selenium and Vitamin E Cancer Prevention Trial) showed an increased risk of developing prostate cancer, which dampened the exuberant welcome of antioxidant therapies (17). In 2012, we also observed higher PON1 enzyme activity in prostate cancer patients and indicated the need for closer scrutiny of the antioxidant medications. We warned about the potential unwanted consequences of these therapies, such as promoting carcinogenesis (18).

In summary, we observed a diminished TAS in BPH patients. These patients also had lower HDL levels compared to controls. We defined the adjusted HDL-related enzyme activity as the enzyme activity per HDL concentration. We accepted the statistical results of these parameters as the body's effort to compensate for the decreased quantity of HDL by increasing the antioxidant quality of the particle. Apparently, HDL is capable of achieving appropriate modifications in response to internal and environmental changes. A modern approach in disease therapy is to support these »good« modifications. After all, a novel concept of HDL functionality presents the superiority of measuring particle quality over quantity, in line with our findings (3). Studies such as ours will inspire future ones to search for enhancement of HDL functionality. Of note, we suggest using adjusted values when interpreting the activity of HDL-related antioxidant enzymes between groups with significantly different HDL levels.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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Received: March 15, 2014

Accepted: April 27, 2014