



Antioxidant enzyme profile in chronic pelvic pain syndrome patients

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Abstract

Chronic pelvic pain syndrome (CPPS) is a common and serious health problem affecting the quality of life in men. According to National Institute of Health classification system abacterial chronic pelvic pain has two sub category which are defined as 3a and 3b. The major difference between these two groups inflammation status. In this study we aim to investigate the antioxidant enzyme levels such as Glutathion peroxidase (Gsh-Px), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation products in CPPS patients. All enzyme levels were determined in patients and disease free control group blood samples by spectrophotometrically. In our study, the enzyme levels were not a good indicator for comparison of 3a or 3b type CPPS patients. However, Gsh-Px, SOD and lipid peroxidation products were significantly different from disease free control samples. Although high generation of H₂O₂ due to SOD activity, we did not detect any change in CAT activity in patients blood samples compare to control group.

Key words: Prostatitis, superoxide dismutase, catalase,

glutathion peroxidase, lipid peroxidase.

Introduction

Chronic prostatitis is a major healthcare issue, and management of chronic prostatitis symptoms is still poor (Potts and Pasqualotto, 2003). Considerable confusion exists about the clinical findings associated with prostatitis and the effect of these conditions on male reproductive physiology. Chronic prostatitis is defined by lower urinary tract symptoms, predominantly with pain. This diagnosis is made when neurologic, infectious, and urologic disease (e.g. benign prostatic hyperplasia or kidney stones) has been excluded. Symptoms in patients with chronic prostatitis may last several weeks or more and may recur periodically for many years. The National Institutes of Health (NIH) classification and definition of the four categories of prostatitis defines category 3a as an inflammatory chronic pelvic pain syndrome with white blood cells (WBCs) in semen, expressed prostatic secretions (EPSs), or voided bladder 3 (VB3) (1995). Category 3b is defined as non inflammatory chronic pelvic pain syndrome without WBCs in semen, EPSs, or VB3 (Orsilles and Piante-Depaoli, 1998). However, the presence of inflammatory cells in EPSs correlates poorly with the presence of inflammatory cells in semen.

Predominantly neutrophils were found as a response to generation of reactive oxygen species (ROS) in semen (Krieger *et al.*, 1996). Increased levels of free radicals may cause defect spermatozoa and impair sperm function in infertile men (Roberts and Jacobsen, 2000). There is still controversy concerning the gold standard treatment for this chronic disease, thus a proper diagnostic technique for inflammatory and non inflammatory CPPS still remains to be defined (Shahed and Shoskes, 2000). It may be caused by auto-immune response disorders or another factor (Orhan *et al.*, 2001). In recent studies, ROS levels of prostatitis in patients' semen samples do not verify the illness. So it is the cause reason of DNA damages and other signaling pathway inducers performing activity. Previous data established that the total antioxidant

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Table 1. Patient and healthy group characteristics.

	Type 3a (n=40)	Type 3b (n=31)	Control (n=36)	Total (n=107)
Age (Mean, range)	33.7 (22-54)	36.35 (24-63)	32.44 (21-58)	34.04 (21-63)
Smoking				
Yes	28/40 (70 %)	18/31 (58.1 %)	20/36 (55.5 %)	66/107 (61.6 %)
No	12/40 (30 %)	13/31 (41.9 %)	16/36 (44.5 %)	41/107 (38.4 %)
PSA levels ng/dl				
(Mean- range)	1.043 (0.458-2.84)	1.048 (0.54- 2.94)	0.995 (0.324-2.58)	1.028 (0.324-2.94)
Other medical problems*	3 (7.5 %)	4 (12.9 %)	2 (5.5 %)	9(8.4 %)

*Hepatitis, HIV, hypertension, cardiovascular disease.

status of prostatitis patients is not in the normal range. Accelerated ROS amount cause the over expression of antioxidant enzymes. Although many studies, the exact mechanism of antioxidant status of prostatitis is still under debate (Potts and Pasqualotto, 2003).

In this study we aim to answer the roles of antioxidant enzyme levels whether may have a role in inflammatory type of CPPS.

Material and methods

Patients, definitions and selection criteria

Out of a study group comprising 71 patients who met the diagnostic criteria for chronic prostatitis; 40 met the criteria for NIH category 3a and 31 met that NIH criteria category 3b, classified clinically on the basis of four-glass test results (Table1). Apart from these 71 patients, a further 12 were excluded from the study due to their incompatibility with the set criteria. The following criteria were approved for research studies on chronic nonbacterial prostatitis/CPPS (Chronic prostatitis workshop, 1995). The Helsinki Declaration was strictly observed regarding the use of human samples. Also, all studies were undertaken with the approval and institutional oversight of the Institutional Review Board for Ethics of Human Subjects. All 71 patients were later compared on the basis of Endtz test results. 36 healthy donors with normal semen characteristics as proven by various reports were

recruited as controls. Donors with a history of genitourinary infection, symptoms, or instrumentation were excluded from the study. 46 of 71 patients were (65 %) smoker and 7 of 71 (10 %) gave up smoking in 5 years and the rest never used. The case and control group were not used to take antioxidant pills regularly and they were eating vegetables and meat products regularly.

Semen analysis

Semen samples were collected by masturbation into sterile containers after at least 72 hours of sexual abstinence. After liquefaction, semen specimens were evaluated for semen volume, appearance, and viscosity. Semen characteristics (concentration, motility, and morphology) were examined in accordance with WHO criteria (WHO laboratory manual, 1999). Computer assisted semen analyzer were loaded for 5µl for each semen sample and motile cells were examined (Table 2).

White blood cells

The presence of white blood cells (WBCs) in semen specimens were assessed by the myeloperoxidase (Endtz) test (Shekarri *et al.*, 1995). A 20µl volume of liquified semen was placed in a 1.5ml cryogenic vial, followed by 20 µl of phosphate buffer saline (pH 7.0) and 40µl of benzidine solution. The sample was mixed, allowed to sit for 5 min and examined for cells that had

Table 2. Comparison of semen characteristics between controls and patients with chronic pelvic pain prostatitis with or without leukocytospermia.

Variable	Controls (n= 36)	CPPS (Endtz negative) (n= 60)	CPPS (Endtz positive) (n= 11)
Concentration (x 10 ⁶ /ml)	59.9 ± 1.2	37.6 ± 0.9	40.1 ± 2.3
Motility (%)	60.2 ± 4.3	51.0 ± 6.7	47.2 ± 5.0
WHO morphology (%)	63.9 ± 2.4	31.3 ± 2.7	29.6 ± 1.3

Data presented as mean ± SE

CPPS= Chronic pelvic pain syndrome

stained brown, indicating cells positive for peroxidase. Leukocytospermia was defined as 1 x 10⁶ or more WBC/ml of semen (Table 2).

Antioxidant enzyme activities in blood

Blood samples were centrifuged for 2 min at 1500 rpm at room temperature and plasma was separated into a clean tube. 200µl blood plasma samples were analyzed each enzyme activity spectrophotometrically (Shimadzu, Japan). Glutathione peroxidase (Gsh-Px) and superoxide dismutase (SOD) enzyme activity were determined according to manufacturer instructions using specific kit (Ransel and Ransod, RANDOX, England). Catalase activity (CAT) was measured in hemolysates at 25°C by the method of Aebi (1984). The decomposition rate of the substrate H₂O₂ was monitored spectrophotometrically at 240 nm for 30 s. The activity is expressed as mU/L. 1 U is equal to 1 µmol of H₂O₂ decomposed/min. Lipid peroxidation was estimated by measurement of thiobarbituric acid

reactive substances (TBARS) in erythrocyte lysates by the method previously described by Aydin *et al.* (2001). After the reaction of MDA with thiobarbituric acid, the reaction product was followed spectrophotometrically at 532 nm, using malondialdehyde as a standard. The results are expressed as nmol/mL (Table 3).

Statistical analysis

The enzyme activity results were expressed as mean ± standard error (SEM). All experiments were replicated in triplicate. Student t-test was used for comparison between NIH classified. CPPS patients and control subjects. The 0.05 level was selected as point of the minimal statistical significance

Results

We determined lipid peroxidation by TBARS method, SOD and related Gsh-Px enzyme activity and CAT activity in serum samples. Antioxidant defense levels in serum of control, type 3a and 3b CPPS patients are

Table 3. TBARS and antioxidant enzymes levels in blood samples of study groups.

Parameters	Controls (n = 36)	CPPS 3a (n = 40)	CPPS 3b (n = 31)
TBARS (nmol/mL)	51.21 ± 4.22	58.11 ± 7.29 ^a	62.20 ± 6.01 ^{b,c}
Gsh-PX (U/mL)	8.91 ± 2.03	6.30 ± 2.01	5.28 ± 2.32 ^{b,c}
SOD (U/mL)	108.21 ± 19.16	90.21 ± 8.31 ^a	78.87 ± 6.67 ^{b,d}
CAT (MU/L)	160.89 ± 34.90	155.55 ± 26.27	151.92 ± 35.19

Values are indicated by mean ± SD.

^a As compared with controls, *P* < 0.05.

^b As compared with controls, *P* < 0.001.

^c As compared with the CPPS 3a group, *P* < 0.05

^d As compared with the CPPS 3a group, *P* < 0.001

^e As compared with controls, *P* < 0.01.

summarized in Table 3. Lipid peroxidation was 51.21 nmol/ml in control patients. This enzyme activity was increased in CPPS patients. In type 3a lipid peroxidation was 58.11 nmol/ml and in type 3b, this level was estimated as 62.20 nmol/ml. There was slightly significant difference between type 3a and type 3b ($p=0.045$). When we compared the activity of lipid peroxidation between control and CPPS patients, the significant difference was calculated ($p=0.034$). In general, antioxidant levels of control patients had always the highest enzyme activity for Gsh-Px and SOD. SOD enzyme activity was decreased approximately 17 % in type 3a and 27 % in type 3b patients. When we investigated the Gsh-Px activity, we estimated concomitant results for this enzyme with SOD activity. The Gsh-Px enzyme activity was lower in type 3a patients by 30 % compare to control group

This enzyme activity was decreased by 40 % in type 3b patients. When all results were calculated for SOD and Gsh-Px enzyme analysis, a significant ($p<0.05$) reduction of levels for each case was obtained significantly correlated ($p<0.001$). CAT activity determined to understand the how diminished SOD activity induce H_2O_2 production in patients samples. However CAT activity was found stable and there was no significant difference between all groups ($p=0.078$).

Discussion

A wide variety of reactive oxygen and nitrogen species can attack DNA directly and form mutagenic lesions. Radical oxygen species may also cause the formation of DNA adducts indirectly by initiating autocatalytic lipid peroxidation, which generates a large variety of potential genotoxic breakdown products, including alkoxyl radicals (LO), peroxy radicals (LOO), and aldehydes, such as MDA (Dotan *et al.*, 2004; Meagher and Fitzgerald, 2000). Antioxidant enzymes such as SOD, peroxidases and CAT act as sensitive regulators to protect cells from damage by oxygen free radicals (Potts and Pasqualotto, 2003; Kinnula *et al.*, 2004; Stoeckelmacher, 2002). Recent studies on colorectal, lung, breast, bladder and gastrointestinal cancers, asthma, *Diabetes mellitus*, schizophrenia, parkinson revealed a decreased antioxidant activity in these patient samples compared to disease free controls (Pociot *et al.*, 1993; Tikly *et al.*, 2004).

In this study, we determined the antioxidant profile of patients who have CPPS 3a or 3b compare to control group. We checked the Gsh-Px results which were upregulated in disease free conditions. It is well

known that, Gsh-Px enzyme activity is related to SOD enzyme production. Substrate of Gsh-Px is H_2O_2 mainly produced as end product of SOD activity. We have found elevated lipid peroxidation or TBARS activity in CPPS 3a and 3b groups versus controls. Our results supported previous reports (Pasqualotto *et al.*, 2000). The protection ability of high antioxidant enzyme levels in patient profile has diminished but these results do not provide for a satisfactory classification reason between type 3a and 3b because among these groups the differences in enzyme activity has no significance ($p>0.05$). Nevertheless control samples were remarkably different from patient enzyme activity ($p<0.05$). SOD enzyme levels were concomitantly changed with Gsh-Px levels in both groups. There was no significant difference for CAT activity between type 3a and 3b CPPS.

We could speculate that the circulating antioxidant enzymes might be used up in the attempt to counteract the enhanced lipid peroxidation in the CPPS 3a and 3b. Another speculation is that the enhanced lipid peroxidation occurs as a consequence of the insufficient power of a depleted antioxidant defense system for a prolonged time. Furthermore, as Gsh-Px and SOD are themselves susceptible to oxidation by the oxidative reactive molecules and lipid peroxides, they could be inactivated by their own substrates (Piogele *et al.*, 1990). Blum and Fridovich (1985) have found that Gsh-Px activity may be inactivated in oxidative stress conditions by superoxide anion and toxic ligands such as MDA could partially inhibit Gsh-Px activity. Another reason might be occurred by non-regular diet which has depicted trace elements such as Cu, Mn, Zn, and Se could lead to the inactivation of the antioxidant enzymes (Gate *et al.*, 1990). Arisan *et al.*, (2006) suggested that low antioxidant profile in CPPS might be a reason genetic allelic variation which was occurred on transcription site of key enzymes such as Manganase-SOD. Diminished Gsh-Px activity in CPPS patients correlated with SOD activity decreament might be a reason of calculated high radical oxygen species in CPPS. It has been obviously understood that alone normal CAT activity was probably unable to detoxify H_2O_2 in to H_2O completely. An accumulation of H_2O_2 might occur, resulting in higher production of $\cdot OH$ radical. This highly reactive oxidant molecule binds and oxidizes DNA, lipid, and proteins, and it reacts with structures from its close neighborhood. Any oxidative lesion that is not repaired can lead to mutations, increasing the risk for many chronic illnesses (Arisan *et al.*, 2006). Additionally, lowered

SOD activity could result in accumulation of highly diffusible and potent superoxide anion, which causes deleterious effects at sites far from the tissue.

However, this stability might not be very important to prevent high radical oxygen species attack because of other enzymes disability. On the other hand, our results are compatible with previous reports which prove that patients with Tardive dyskinesia (TD) had a lower SOD activity than those without TD (Zhang *et al.*, 2002). However, these reports have not shown the relationship between specific MnSOD activity and the occurrence of TD, and not all studies did a clear identification of the relationship between polymorphism and enzyme activity.

In conclusion, we assessed the relationship between CPPS and control group focusing on oxidant and antioxidant profile balance. We assumed that antioxidant injury caused by the impaired antioxidant enzyme production, might contribute to adverse developments in the pathogenic cascade of CPSS patients. Our findings lead to the suggestion that oxidative disorder-linked medical health problems can be associated with genetic risk factors such as mutation on gene expression profile.

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