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Increased Oxidative Stress and Damage in Patients With Chronic Bacterial Prostatitis

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Objective To investigate whether chronic bacterial prostatitis (CBP) increases oxidative stress and damage in patients with CBP, and to explore its possible mechanism. **Methods** Eighty patients with CBP and 80 healthy adults as controls were enrolled in a case-control study, in which levels of nitric oxide (NO), vitamin C (VC), and vitamin E (VE) in plasma, as well as malondialdehyde (MDA), activities of superoxide dismutase (SOD), and catalase (CAT) in erythrocytes were determined by spectrophotometry. **Results** Compared with the average values of NO, VC, VE, MDA, SOD, and CAT in the healthy control group, those of plasma NO and erythrocyte MDA in the CBP group were significantly increased (P<0.001), and those of plasma VC and VE as well as erythrocyte SOD and CAT in the CBP group were significantly decreased (P<0.001). Findings from partial correlation analysis for course of the disease and NO, VC, VE, MDA, SOD, and CAT in 80 patients with CBP, adjusted for age, suggested that with prolonged course of the disease, values of NO and MDA were gradually increased (P<0.001), and those of VC, VE, SOD, and CAT were gradually decreased (P<0.05-0.001). The findings from stepwise regression analysis for course of the disease and NO, VC, VE, MDA, SOD, and CAT in CBP group suggested that the model of stepwise regression was $Y = -19.1160 + 0.3112\text{MDA} + 0.0337\text{NO}$, $F = 22.1734$, P<0.001, $r = 0.6045$, P<0.001. The findings from the reliability analysis for VC, VE, SOD, CAT, NO, and MDA in the CBP group showed that the reliability coefficients' alpha (6 items) was 0.7195, P<0.0001, and the standardized item alpha was 0.9307, P<0.0001. **Conclusion** There exist increased oxidative stress and damage induced by chronic bacterial prostatitis in patients, and such a phenomenon is closely related to the course of disease.

Key words: Chronic bacterial prostatitis; Oxidative stress; Oxidative damage; Free radicals; Oxidation; Lipid peroxidation; Antioxidant; Antioxidative enzyme; Nitric oxide; Malondialdehyde

INTRODUCTION

Chronic bacterial prostatitis (CBP) is a torturing disorder which is usually caused by Gram-negative bacilli, especially *E coli*[1]. Some authors have reported that CBP may be associated with free radicals such as superoxide anion and nitric oxide radicals[2-4], reactive oxygen species[5-7], inflammatory cytokines like interleukins[6-9], tumor necrosis factor-alpha (TNF-alpha)[7-9], p53[10], and abnormal metabolism of xanthine[11]. Up to now, there have been neither reports on increased oxidative stress and damage in patients with CBP, nor reports about partial correlation analysis between increased oxidative stress and damage and course of the disease. This paper presents a case-control study designed to investigate whether CBP really increases oxidative stress and damage in patients with CBP and to explore its possible mechanism by comparison between the levels of certain antioxidants and antioxidative enzymes, and malondialdehyde in 80 patients with CBP and 80 healthy adults *via* partial correlation analysis and stepwise regression analysis and reliability analysis.

MATERIALS AND METHODS

Study Design

Acording to the diagnostic criteria[1], 80 patients with CBP and 80 healthy adults were enrolled in a case-control study, in which the levels of nitric oxide (NO), vitamin C (VC) and vitamin E (VE) in plasma, as well as malondialdehyde (MDA), activities of

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superoxide dismutase (SOD) and catalase (CAT) in erythrocytes were determined by spectrophotometry. The differences in the average values of the said biochemical parameters between the CBP group and healthy control group were compared.

Subjects

Eighty patients were randomly selected from 115 patients with CBP confirmed by the diagnostic criteria and the inclusion criteria[1] in the Second Affiliated Hospital, College of Medicine, Zhejiang University, China. Their age ranged 21-30 years, course of disease 1-2 years, systolic blood pressure (SBP) 101-139 mm Hg, diastolic blood pressure (DBP) 70-80 mm Hg, hemoglobin (Hb) 128.5-146.0 g/L, serum albumin (Alb) 36.64-47.86 g/L, and body-mass index (BMI) 20.81 to 24.82.

Eighty adults, whose age, SBP, DBP, nutritional condition, Hb, and Alb were matched with the patients with CBP, were randomly selected from 120 healthy adults in the same Hospital. Their age ranged 21-30 years, SBP 90-138 mm Hg, DBP 68-88 mm Hg, Hb 128.3-147.0 g/L, Alb 36.47-47.57 g/L, and BMI 19.80-24.50.

There were no significant differences in the average values of age, SBP, DBP, Hb, Alb, and BMI, and in nutritional condition, annual earning, education level, profession or occupation, residence region, daily diet (food and drink), or mental status between the two groups by independent-samples *t* test or Pearson chi-square test.

The demographic and other data of the patients with CBP and healthy adults are presented in Table 1.

TABLE 1

Demographic Data and Other Data in Patients With CBP and Healthy Adult Controls

Variables	Men With CBP (n = 80)	Healthy Adult Men (n = 80)	Independent-Samples t Test P Value
Age (Year)	24.2±2.7	24.3±2.8	0.836
Systolic Blood Pressure (mm Hg)	121.1±9.3	120.6±9.0	0.744
Diastolic Blood Pressure (mm Hg)	79.2±5.3	78.6±5.7	0.512
Hemoglobin (g/L)	140.8±4.6	140.6±4.5	0.803
Albumin (g/L)	43.43±2.28	43.62±2.19	0.590
Body-mass Index	23.25±0.98	22.98±1.14	0.116
Course of Disease (Year)	5.9±3.2	—	—
Smoking History	No	No	—
Alcohol Abuse History	No	No	—

The above subjects had no history of disorders in brain, heart, lungs, liver, kidneys, and blood system, circulatory system, respiratory system, *etc*, by routine blood, urine and stool examinations, and radiography, cardiography, and other necessary examinations. They also had no history of hypertension, hyperlipidemia, acute or chronic bronchitis, asthma, autoimmune disease, diabetes mellitus, atherosclerosis, tumors or cancers, subnutrition, malnutrition, or other nutritional diseases.

None of the subjects took any antioxidative supplements, such as vitamin C, vitamin E, β-carotene, *Ginkgo biloba*, and tea polyphenols, or other similar substances during the past month.

Methods

Fasting venous blood samples were collected from all the subjects in the morning. Heparin sodium was added as an anticoagulant, plasma and erythrocytes were separated and stored at –50℃. The blood samples did not undergo any hemolysis as previously described[12-13].

Spectrophotometry for α-naphthylamine coloration was used to determine plasma NO level expressed as nmol/L[14].

Trichloroacetic acid solution was used to precipitate proteins in plasma and to extract VC from plasma. VC in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}. Fe^{2+} reacted with ferrozine formed a colored end product that was detected by spectrophotometry at 563 nm and 10.0 mm cells, and its level was expressed as μmol/L.

Absolute ethanol was used to precipitate proteins in plasma and to extract VE from plasma. VE in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}. Fe^{2+} reaction with ferrozine formed a colored end product that was detected by spectrophotometry at 563 nm and 10.0 mm cells, its level was expressed as μmol/ L.

Spectrophotometry for thiobarbituric acid reactive substances (TBARS) was used to determine erythrocyte MDA level expressed as nmol/g•Hb.

Spectrophotometry for inhibiting pyrogallol auto-oxidation was used to determine erythrocyte SOD activity expressed as U/g•Hb.

Spectrophotometry for coloration of hydrogen peroxide and acetic acid-potassium dichromate was used to determine erythrocyte CAT activity, expressed as K/g•Hb.

The main analytical reagents for the determination of the above biochemical substances and enzymes, namely α-naphthylamine, vitamin C, vitamin E, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazinedisulfonic acid disodium salt (ferrozine), 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid (TBA), Cu•Zn-superoxide dismutase, 1,2,3-trihydroxybenzene (pyrogallol), and catalase were purchased from SIGMA® Chemical Company (USA) and the other analytical reagents were produced in China. Fresh quadruple distilled water was prepared with a quartz glass distilling apparatus. The main analytical instrument was Hewlett Packard 8453-Spectrophotometer (USA).

In the above assays, the same batch number of each reagent, the same quality control, the same laboratory assistant, and the identical analytical apparatus were strictly used for each experiment to decrease errors and bias, and to ensure the analytical quality of determinations.

Statistical Analysis

All the data were statistically analyzed with SPSS 11.0 for Windows statistic software (Serial number: 3805638; License code: 30376 40608 78517 08046 24431 4009) using a Compaq Pentium Ⅳ/2.4 GHz computer. The experimental parameters presented normal distributions by Kolmogorov-Smirnov Z test, and equal variances by Livene's test for equality of variances, and were expressed as $\bar{x} \pm s$, and 95% confidence interval (95% CI). Hypothesis testing methods included independent-samples t test, Pearson chi-square test (χ^2 test), partial correlation analysis, stepwise regression analysis, and reliability analysis. In statistical analysis, the level of hypothesis testing (α) was $\leqslant 0.05$ in order to avoid false positivity (type Ⅰ error), and the power of hypothesis testing (power) was $\geqslant 0.85$ to avoid false negativity (type Ⅱ error).

RESULTS

Compared with the average values of VC, VE, SOD, CAT, NO, and MDA in the healthy control group, those of VC and VE in plasma, as well as SOD and CAT in erythrocytes in the CBP group were significantly decreased ($P<0.001$), and those of NO in plasma and MDA in erythrocytes were significantly increased ($P<0.001$) (Table 2).

The upper limits of 95% confidence interval (95% CI) of the average values of VC, VE, SOD, and CAT in the CBP group were less than the lower limits of 95% CI of those in the healthy control group, and the lower limits of 95% CI of the average values of NO and MDA in the CBP group were greater than the upper limits of 95% CI of those in the healthy control group (Table 2).

TABLE 2

Comparison of Average Values ($\bar{x} \pm s$) of NO, VC, VE, MDA, SOD, and CAT in Patients with CBP and Healthy Adult Controls, and 95% CI

Group	n	Plasma			Erythrocytes		
		NO*	VC*	VE*	MDA*	SOD*	CAT*
		(nmol/L)	(μmol/L)	(μmol/L)	(nmol/g•Hb)	(U/g•Hb)	(K/g•Hb)
Patients With CBP	80	425.2±31.1	47.94±12.00	18.37±4.94	34.25±4.78	1927±224	253.8±69.8
		(418.3–432.1)	(45.27–50.61)	(17.27–19.47)	(33.19–35.32)	(1878–1977)	(238.3–269.3)
Healthy Adult Controls	80	380.2±33.8	54.90±13.46	25.80±4.54	28.47±4.32	2072±226	297.5±77.0
		(372.7–387.7)	(51.91–57.90)	(24.78–26.81)	(27.51–29.44)	(2021–2122)	(280.4–314.6)

Note. Independent-samples t test. Figures in parentheses: 95% CI. *$P<0.001$.

The findings from the partial correlation analysis between course of the disease and VC, VE, SOD, CAT, NO, and MDA in the CBP group adjusted for the age, suggested that with prolonged course of the disease, the values of VC, VE, SOD, and CAT were gradually decreased ($P<0.02$-0.001), and those of NO and MDA were gradually increased ($P<0.001$) (Table 3).

The findings from the stepwise regression analysis between course of the disease and VC, VE, SOD, CAT, NO, and MDA in the CBP group suggested that the model of stepwise regression was $Y = -19.1160 + 0.3112\text{MDA} + 0.0337\text{NO}$, $F = 22.1734$, $P<0.001$, $r = 0.6045$, $P<0.001$ (Table 4).

TABLE 3

Partial Correlation Analysis Between Course of the Disease and NO, VC, VE, MDA, SOD, and CAT in 80 Patients With CBP

Variables Correlated	*n*	Variables Controlled	*r*	*P*
Course and NO	80	Age	0.4486	< 0.001
Course and VC	80	Age	−0.3522	0.001
Course and VE	80	Age	−0.3952	< 0.001
Course and MDA	80	Age	0.4057	< 0.001
Course and SOD	80	Age	−0.2833	0.011
Course and CAT	80	Age	−0.2732	0.015

The findings from the reliability analysis of the levels of VC, VE, SOD, CAT, NO, and MDA used to reflect increased oxidative stress and damage in the CBP group showed that the reliability coefficients' alpha (6 items) was 0.7195, $P<0.0001$, and the standardized item alpha was 0.9307, $P<0.0001$.

DISCUSSION

VC and VE are important antioxidants, SOD and CAT are important antioxidative enzymes in humans. They play a very important role in scavenging excessive superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical (·OH), nitric oxide radical ($NO^{\bullet}$), and other free radicals, as well as excessive reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) in humans[12-24]. NO is one of the very important neurotransmitter molecules, and can directly modify enzymes that produce second messengers[14-15, 18]. MDA is an important metabolic product of peroxidative reactions (auto-oxidation) of lipids exposed to oxygen[12-24]. Therefore, VC, VE, SOD, CAT, NO, and MDA play an important role in normal metabolism of humans[12-24]. Significantly decreased VC, VE, SOD, and CAT, as well as markedly increased NO and MDA may cause metabolic disorders, and increase oxidative stress and damage in humans, inducing a variety of diseases[12-24].

TABLE 4

Model of Stepwise Regression Between Course of the Disease and NO, VC, VE, MDA, SOD, and CAT in 80 Patients With CBP

Model	Coefficients	*t*	*P*	Partial Correlation		ANOVA		*r* Value	
				r	*P*	*F*	*P*	*r*	*P*
Constant	−19.1160	4.4704	<0.001	—	—				
MDA	0.3112	5.0507	<0.001	0.4988	<0.001	22.1734	<0.001	0.6045	<0.001
NO	0.0337	3.5574	<0.001	0.3757	<0.001				

Note. Dependent variable: the course of disease.

The findings in this study suggest that there exist increased oxidative stress and damage in patients with chronic bacterial prostatitis. There may be several interpretations.

The elementary pathologic changes in chronic bacterial prostatitis are inflammation and inflammatory reactions[1]. In chronic bacterial prostatitis, inflammatory reactions and inflammation-induced inflammatory cells, such as macrophages, phagocytes, leukocytes, granulocytes, polymorphonuclear leukocytes, monocytes, lymphocytes, and immunocytes[25-29], generate and release a number of inflammatory mediators and factors, e.g. proinflammatory and inflammatory cytokines like interleukins (IL)[18,27-29], and tumor necrosis factor-alpha (TNF-alpha)[18,28-30], p53[18,31-32], cytochrome P-450[18,33-34], and NADPH-cytochrome P-450[18,33-34], cause abnormal metabolism of hypoxanthine/xanthine oxidase system and/or xanthine/xanthine oxidase system producing many abnormal metabolites[18,34-35], activate and release a large amount of cyclooxygenase-2[4,26], transcription factor nuclear factor-kappa B[26], inducible nitric oxide synthase (iNOS), and a large number of inflammatory oxidants and other chemokines[4,36]. These factors can can generate and release a large amount of $O_2^{\cdot-}$, ·OH, $NO^{\bullet}$, and other free radicals, as well as $^1O_2$, H_2O_2, and other ROS[5,7,25-32,35-36]. Furthermore, prostatodynia, perineal and/or suprapubic pain (chronic pelvic pain syndrome), prostatic hyperaemia and/or hemorrhage, and other prostatic diseases, can also generate and release a large amount of $O_2^{\cdot-}$, ·OH, $NO^{\bullet}$, and other free radicals, as well as $^1O_2$, H_2O_2, and other ROS[1].

A large amount of $O_2^{\cdot-}$, ·OH, $NO^{\bullet}$, and other free radicals, as well as $^1O_2$, H_2O_2, and other ROS as strong oxidants, interacts directly with DNA in humans, damaging DNA, inhibiting and/or depressing DNA replication, and destroys strongly the active sites and active groups in the molecular structures of VC, VE, and other antioxidants, as well as those of SOD, CAT, and other antioxidative enzymes, inactivating and deactivating them[12-24]. A large amount of $O_2^{\cdot-}$, ·OH, $NO^{\bullet}$, and other free radicals, as well as $^1O_2$, H_2O_2, and other ROS also causes oxidative decomposition and peroxidative modification of many organic compounds in humans, further deactivating antioxidants and antioxidative enzymes[12-24]. As a consequence, these changes in biochemistry and biophysics aggravate the reactions of a chain of free radicals and ROS, thereby significantly decreasing the levels of VC and VE in plasma as well as activities of SOD and CAT in erythrocytes, and significantly increasing the level of NO in plasma of patients with CBP. In addition, excessive peroxynitrite anion ($ONOO^-$), a very strong oxidant species generated and released by combined $NO^{\bullet}$ and $O_2^{\cdot-}$, damages DNA and its functions, inactivates and deactivates antioxidants and antioxidative enzymes[12-24], further significantly decreasing VC and VE levels as well as SOD and CAT activities in patients with CBP. Additionally, a large amount of $O_2^{\cdot-}$, ·OH, $NO^{\bullet}$, and other free radicals, and $ONOO^-$, and ROS, accelerates and aggravates lipid peroxidative reactions of polyunsaturated fatty acids, unsaturated phospholipids, glycolipids, cholesterol, other lipids, and other organic compounds containing lipids in blood, tissues, and cellular membranes in humans, leading to significantly increased levels of MDA, conjugated diene, lipid peroxides, and other metabolites from lipid peroxidative reactions in patients with CBP[12-23]. At the same time, significantly decreased plasma VE level per se results in significantly increased levels of MDA, conjugated diene, lipid peroxides, and other metabolites from lipid peroxidative reactions[12-23].

Owing to a close correlation between age and NO, VC, VE, MDA, SOD, and CAT, and the alternated concentrations of $O_2^{\cdot-}$, $NO^{\bullet}$, and other free radicals as well as $^1O_2$, H_2O_2, and other ROS in inflammatory response in humans[3,18,21-23], the partial correlation analysis adjusted for age was used in this study to compute the correlations between the course of disease and NO, VC, VE, MDA, SOD, and CAT for the CBP group in order to eliminate the confounding factor as previously described[3,18,21-23]. The findings from the partial correlation analysis suggest that the values of biochemical substances and enzymes are closely related to the course of disease in the CBP group. When the course of disease was prolonged, the values of VC and VE in plasma, and those of SOD and CAT in erythrocytes in the CBP group were gradually decreased, and those of NO in plasma and MDA in erythrocytes were gradually increased. In other words, the longer the course of disease, the severer the oxidative stress and damage in patients with CBP. In addition, the findings suggest that chronic bacterial prostatitis is a risk factor affecting such patients' physical and mental health.

In this study, the model of stepwise regression between the course of disease and NO, VC, VE, MDA, SOD, and CAT for patients with CBP suggests that there exists a closest correlation between the course of disease and MDA, NO, and that the significantly increased MDA and NO are the main risk factors for increased oxidative stress and damage in patients with CBP[7].

In conclusion, chronic bacterial prostatitis may increase nitric oxide and malondialdehyde, and decrease vitamin C, vitamin E, superoxide dismutase, and catalase. There exists an increased oxidative stress and damage induced by chronic bacterial prostatitis in patients with chronic bacterial prostatitis, and such a phenomenon is closely related to the course of disease.

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