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Heat shock protein 9-mediated inflammation reaction in patients with chronic prostatitis with erectile dysfunction

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Abstract. – OBJECTIVE: To investigate the role of heat shock protein (HSP)-9 on the inflammation reaction present in patients with chronic prostatitis with erectile dysfunction (ED).

PATIENTS AND METHODS: A total of 160 participants in the study were assigned to one of four groups of the same size. Group A had patients with chronic prostatitis and ED. Group B had patients with simple chronic prostatitis. Group C had patients with ED. And group D had healthy volunteers. The serum levels of HSP-9, CRP, TNF-α, IL-6 and CD3 in each individual's serum were tested by ELISA. Additionally, the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) and the International Index of Erectile Function-5 (IIEF-5) scores were recorded for each case.

RESULTS: The serum levels of HSP-9, CRP, TNF-α, IL-6 and CD3 in the serum of groups A and B were distinctly higher than those of groups C and D ($p<0.05$). While comparisons between groups A and B, or between groups C and D yielded no significant differences. Nevertheless, the NIH-CPSI scores in the group A were significantly higher (mostly moderately severe) than in the group B (mild to moderate). Furthermore, the IIEF-5 scores in the group A were also significantly higher than those in the group C.

CONCLUSIONS: The serum levels of HSP-9, CRP, TNF-α, IL-6 and CD3 in the sera of patients with chronic prostatitis with ED were clearly increased, reflecting a high degree of inflammation which may be related to the clinical manifestations in patients with chronic prostatitis and ED.

Key Words:
Heat shock protein-9 (HSP-9), CRP, TNF-α, IL-6, CD3, Chronic prostatitis, Erectile dysfunction (ED), NIH-CPSI, IIEF-5, Correlation.

Introduction

Chronic prostatitis is a common genitourinary problem in adult males, nearly 50~65% of males have a history of chronic prostatitis, and in 10-30% of those the disease progresses to prostatic hyperplasia[1]. Additionally, erectile dysfunction (ED) is also present in 40-70% of patients with chronic prostatitis[2]. The determinant factors in the pathogenesis of prostatic hyperplasia include chronic prostatitis, advanced age and high androgen levels. Histopathology studies in chronic prostatitis show increased numbers of T lymphocyte infiltration in prostatic epithelial tissues; activated lymphocytes release a variety of inflammation factors and mediators, which stimulate the proliferation of epithelial and stromal cells, inhibit apoptosis and lead to prostatic hyperplasia[3]. HSP-9 is one inflammatory factor stimulating the serum of other factors by T cells, and plays an important role in the progress of cell signaling pathways and activation of many inflammation factor genes[4]. The quality of sexual life in middle-aged males with chronic prostatitis and ED is adversely affected, and the efficacy of hormone replacement therapies and psychological intervention do little to mitigate the problem. However, approaches targeting the inflammation may result in more effective treatments[5]. Based on this, the aim of this study was to investigate the effects of HSP-9 on the inflammation seen in patients with chronic prostatitis and ED.

Patients and Methods

Patients

A total of 160 individuals were enrolled in the study during the period between January 2015 and January 2016, and were assigned to one of four groups: Group A included 40 patients, admitted to our hospital with the diagnosis of chronic prostatitis with ED and without any previous treatment. The average age of in-

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dividuals was 42.5±13.6 years old, the average course of disease was 1.8±0.6 months and the average education level 16.3±5.5 years. Group B had 40 patients with simple chronic prostatitis; the average age was 44.3±15.7 years old, the average course of disease was 1.5±0.9 months and the average education level 17.2±6.3 years. Group C had 40 patients with simple ED; the average age was 42.8±14.5 years old, the average course of disease was 1.3±0.8 months and the average education level was 16.6±7.0 years. Finally, Group D included 40 healthy volunteers; with an average age of 45.0±16.2 years old, and an average education level of 16.8±6.0 years. The age, course of disease and education level among the groups were all similar and played no role in determining differences amongst groups.

The Ethics Committee of our hospital approved the research and the patients, or their relatives, signed the informed consents. The following were the general inclusion criteria for the study: 1. Age from 18- to 60 years-old. 2. Diagnosis conformed to criteria of chronic prostatitis and ED. The general exclusion criteria were: 1. Presence of prostatic hyperplasia and prostatic cancer. 2. Presence of ED and a severe psychological disorder. 3. Presence of infection or an autoimmune disease. 4. Patient non-complaint or unable to finish the questionnaire.

Chronic prostatitis was confirmed with a detailed medical history, physical examination, and prostatic fluid examination with bacterioscopy, according to the standards for diagnosing chronic prostatitis (II/III type) by the National Institutes of Health (NIH). Exclusion criteria for ED were shown as below: hypertension, diabetes mellitus, liver or kidney dysfunctions; neuropsychic diseases, endocrine diseases; ED secondary to smoking, drinking, drug abuse and primary penis dysfunctions such as Peyronie's disease, phallic shortness, and developmental malformations were also excluded.

We employed the International Index of Erectile Function-5 (IIEF-5) questionnaire to grade each patient with ED (mild: 12-21 points; moderate: 8-11 points; severe: ≤7 points).

Research Methods

For each patient, serum levels of HSP-9, CRP, TNF-α, IL-6 and CD3 in serum were tested by ELISA. 5 ml of fasting blood were taken from the peripheral elbow vein. After a 4000 g centrifugation for 20 minutes, the samples were then preserved in the -20˚C until ready to perform the tests all together. The ELISA kits were bought from R&D Systems (Minneapolis, MN, USA), and the tests were performed with strict adherence to the manufacturer's instructions. The National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) and the International Index of Erectile Function-5 (IIEF-5) scores were obtained by examining each individual in the study. The NIH-CPSI included three parts: pain or discomfort, urinary symptoms and impact on life quality. Total scores within 1-14 points were considered mild, scores within 15-29 points were moderate and within 30-43 points severe.

Statistical Analysis

The SPSS20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data were expressed as mean ± standard deviation, and comparisons among groups used single factor ANOVA analysis. The LSD method and the t-test of independent samples were also used for comparisons. Enumeration data were expressed as number of cases or percentages (%), for comparisons among groups χ^2 test was employed, and ranked data were processed by the rank sum test. A difference with a $p<0.05$ was considered statistically significant.

Results

Comparison of the Serum Levels of HSP-9, CRP, TNF-α, IL-6 and CD3

The serum levels of HSP-9, CRP, TNF-α, IL-6 and CD3 from groups A and B were significantly higher than those from groups C and D ($p<0.05$). However, there was no statistical difference between group A and group B ($p>0.05$). Similarly, the comparison between groups C and D yielded no significant difference ($p>0.05$) (Table I).

Comparison of NIH-CPSI Scores

NIH-CPSI scores in group A were higher than in group B. Group A had mostly moderately severe cases, while group B presented more mild to moderate ones, the differences were statistically significant ($p<0.05$) (Table II).

Comparison of IIEF-5 Scores

IIEF-5 scores in the group A were higher (moderately severe) than in the group C. The differences were significant ($p<0.05$) (Table III).

Table I. Comparison of the expression levels of HSP-9, CRP, TNF-α, IL-6 and CD3 in serum.

Groups	HSP-9 (ng/mL)	CRP (mg/L)	TNF-α (ng/mL)	IL-6 (ng/mL)	CD3 (ng/mL)
A	123.5 ± 35.6	9.2 ± 2.0	42.6 ± 7.7	66.3 ± 14.2	5.6 ± 1.5
B	130.4 ± 40.2	8.6 ± 2.2	38.7 ± 8.0	57.8 ± 13.5	4.8 ± 1.2
C	42.8 ± 12.3	3.4 ± 0.6	10.5 ± 2.3	23.4 ± 6.9	1.3 ± 0.4
D	46.3 ± 14.7	3.2 ± 0.5	8.2 ± 2.4	25.0 ± 6.2	1.0 ± 0.3
F	12.305	8.527	13.421	9.234	8.427
p	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Group A, chronic prostatitis with ED; Group B, simple chronic prostatitis; Group C, simple ED; Group D, healthy volunteers.

Discussion

Studies have shown that both bacterial and non-bacterial chronic prostatitis in patients are related to immune and inflammatory disorders[6]. CRP is a nonspecific acute phase response protein in the body wich has been found high in various diseases. With CRP ≥3.0 mg/l, the urinary symptoms in prostatitis patients are more intense, in particular, urgency becomes apparent[7]. Nonsteroidal anti-inflammatory agents can control inflammation, inhibit the cell proliferation and promote apoptosis[8]. A possible pathogenic mechanism in chronic prostatitis has T lymphocytes up-regulating proinflammatory growth factors and inducing cytokines that lead to hypertrophy of mesenchymal and epithelial cells, and induces matrix formation and angiogenesis. Generated by Th1 cells, IFN-γ, IL-2, TNF-α and FGF-2 take part in inflammation mediated by cells, promoting the secondary secretion of IL-6, IL-8 and IL-15. While IL4, IL-13 and IL-5 (generated by Th2 and negatively regulated by IFN-γ) activate the humoral immunity and participate in antigen antibody reactions and anaphylaxis[9]. Through the loop effect of paracrine and autocrine cells, activated T lymphocytes stimulate the production of IL-2, IFN-γ, IL-6, IL-8, IL-17 and COX-2, which result in a chronic inflammatory immunological response in the local tissues of the prostate gland. Thus ensues the proliferation of mesenchymal, epithelial cells and even fiber muscle cells[10]. The immunological inflammation affecting prostatic mesenchymal cells leads to the long-term existence of a chronic inflammatory immunological response in the gland[11]. CD3 is an antigen differentiated from leukocytes; it connects with the T cell antigen receptor through a salt bridge to participate the signal transduction process in T cells. Multiple studies have demonstrated that 70-80% of activated T cells express CD3 during chronic prostatitis[12,13].

HSPs are a set of highly conserved proteins throughout evolution; they play important roles in maintaining cellular homeostasis, protein syn-

Table II. Comparison of NIH-CPSI scores.

Groups	Cases	Scores	Mild	Moderate	Severe
A	40	26.4 ± 4.5	6 (15.0)	20 (50.0)	14 (35.0)
B	40	17.3 ± 4.3	15 (37.5)	19 (47.5)	6 (15.0)
t (χ^2)		6.857	7.083		
p		0.025	0.029		

Table III. Comparison of IIEF-5 scores.

Groups	Cases	Scores	Mild	Moderate	Severe
A	40	10.5 ± 3.0	10 (25.0)	21 (52.5)	9 (22.5)
C	40	16.6 ± 3.3	23 (57.5)	13 (32.5)	4 (10.0)
t (χ^2)		6.425	8.927		
p		0.030	0.012		

thesis and normal transport of proteins onto the membrane. There is a direct relationship between the level of HSP-9 and that of prostatic specific antigen (PSA) in serum, which has moderately higher sensitivity and specificity in diagnosing prostatic cancer[14]. Molecular research has suggested that the methylation of the 3' terminus in the HSP-9 gene is higher in more severe forms of prostatitis, and probably reaches 20-25% in prostatic cancer[15]. The occurrence of prostate cancer can be closely related to the chronic inflammation of the prostate; therefore, higher levels of HSP-9 are also believed to correlate with cancer led by inflammation[16]. The efficacy of finasteride in treating prostatitis negatively correlates with a reduction in HSP-9 levels[17].

The causes of ED in chronic prostatitis relate to psychology, nerve reflexes and patency of the duct for semen passage. The symptoms of prostatitis itself and the psychological effects caused by prostatitis appear to be the important factors determining ED[18]. Results of the present study demonstrated that the serum levels of HSP-9, CRP, TNF-α, IL-6 and CD3 in groups A and B were higher than those in groups C and D. However there was no statistical difference between A and B or C and D. These results suggested that the abnormal elevation of indicators in serum was related to the occurrence of chronic prostatitis, but not with the occurrence of simple ED. Also, the NIH-CPSI and IIEF-5 scores results seem to argue that inflammation may aggravate the clinical symptoms of chronic prostatitis with ED. An important discovery of our study is that while primary ED has complicated occurrence mechanisms, an intervention against the inflammatory response in patients with chronic prostatitis with ED might probably become an effective target for therapy.

Conclusions

The serum levels of HSP-9, CRP, TNF-α, IL-6 and CD3 in patients with chronic prostatitis with ED were markedly elevated, and the scores of tests for chronic prostatitis and ED symptoms are enhanced, indicating that inflammation level may well be related to the degree of symptoms. However, further studies are still required to verify this hypothesis. Our study may provide a potential therapeutics for chronic prostatitis with ED.

Conflicts of interest

The authors declare no conflicts of interest.

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