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RESEARCH ARTICLE

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The diagnostic value of urine heat shock protein 70 and prostatic exosomal protein in chronic prostatitis

Yinghua Tang[1] | Aiping Pan[1] | Yonggang Liu[2] | Lianli Yin[3]

[1]Department of Clinical Laboratory, Guangxi Hospital of Traditional Chinese Medicine, The First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning, China

[2]Department of Urology, Nanning Second People's Hospital, The Third Affiliated Hospital of Guangxi Medical University, Nanning, China

[3]Department of Clinical Laboratory, Nanning Second People's Hospital, The Third Affiliated Hospital of Guangxi Medical University, Nanning, China

Correspondence
Yinghua Tang, Department of Clinical Laboratory, Guangxi Hospital Of Traditional Chinese Medicine, The First Affiliated Hospital of Guangxi University of Chinese Medicine, No. 89-9 Dongge Road, Nanning 530023, Guangxi, China.
Email: 271101521@qq.com

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Abstract

Objective: To explore the diagnostic value of the levels of prostatic exosomal protein (PSEP) and heat shock protein 70 (HSP70) in the urine of patients with chronic prostatitis (CP).

Method: Urine samples from 210 CP patients (70 cases of the USA National Institutes of Health Category II [NIH-II], 70 NIH-IIIa, and 70 NIH-IIIb patients) and 70 control subjects were collected between May 2018 and February 2020. The levels of PSEP and HSP70 in urine were detected by enzyme-linked immunosorbent assay. The differences in urine PSEP and HSP70 levels between the groups were analyzed, and receiver operating characteristic (ROC) curves were used to analyze the clinical value of PSEP and HSP70 in the diagnosis of CP.

Results: The PSEP levels of CP patients were significantly higher than those of the control group ($p < 0.001$), but there was no difference in PSEP levels among CP subgroups. The level of HSP70 in the urine of the NIH-II patients was significantly lower than the levels in the NIH-IIIa and NIH-IIIb subgroups and the control group, but there was no difference in HSP70 levels between the NIH-IIIa and NIH-IIIb subgroups and the control group. ROC curve analysis results showed that the area under the curve (AUC) of PSEP for the NIH-II, NIH-IIIa, and NIH-IIIb patients was 0.751, 0.776, and 0.731, respectively. The AUC of HSP70 in NIH-II patients was 0.784, and the AUC of combined detection of PSEP and HSP70 in NIH-II patients was 0.858.

Conclusion: Urine PSEP can be used as a marker for the diagnosis of CP, but it cannot distinguish between the various types of CP, and HSP70 can be used as a diagnostic index for NIH-II classification.

KEYWORDS
chronic prostatitis, heat shock protein 70, prostatic exosomal protein

1 | INTRODUCTION

Chronic prostatitis (CP) is a common but confusing urological disease that seriously affects men's lives and health and cannot be ignored.[1,2] The incidence rate is increasing, and the disease is affecting younger men.[3] Long-term prostatitis affects male sexual function.[4] The etiology and pathogenesis of CP are very complicated. Studies have shown that the detection of heat shock protein 70 (HSP70) levels in the expressed prostatic secretions (EPS) of CP patients suggests that it is involved in the process of inflammation.[5] However, EPS can be obtained by anal prostate massage, which is rather painful, invasive, and intolerable for some patients; also, the amount of time the patient

has been sexually abstinent as well as psychological factors can affect the detection of EPS. Therefore, there is an urgent need to find biomarkers and detection methods that are non-invasive, stable, simple, fast, accurate, and reliable. In recent years, the detection of prostatic exosomal protein (PSEP) in the urine has been reported in the clinical diagnosis of CP,[2,6] but since its diagnostic efficiency and application value are not completely clear, the study of urine PSEP and HSP70 in CP patients has not been reported. In this study, we measured and compared the levels of PSEP and HSP70 in the urine of patients with different types of CP and in control subjects and analyzed their role in the diagnosis and classification of chronic prostatitis.

2 | MATERIALS AND METHODS

2.1 | Subjects

Between May 2018 and February 2020, 210 patients with obvious symptoms of chronic prostatitis syndrome who met the National Institutes of Health (NIH) diagnostic criteria for prostatitis were selected as subjects for the study. Following the NIH classification system,[7] they were divided into three groups: 70 cases with NIH-II, 70 cases with NIH-IIIa, and 70 cases with NIH-IIIb, with an average age of 29.6 years (range: 19–54 years). Seventy men without any clinical symptoms were selected as the control group. In the control group, no abnormalities were detected in the physical examination, EPS routine, urine routine, or urine bacterial culture before and after massage. The control group was 20–48 years old, with an average age of 28.4 years old. All patients provided a detailed medical history, including medication history and sexual life, and patients with related diseases that may cause similar symptoms, such as diabetes, urethritis, epididymitis, varicocele, and rectal and perianal diseases, were excluded.

2.2 | Sample collection

Two-millilitre urine specimens were collected from all subjects (both CP patients and the control group) and stored at −80°C for PSEP and HSP70 testing. The subjects were required to abstain from sex for 3–5 days and to wash and disinfect the head of the penis and outside the urethra before providing the specimens. Urine was taken before and after prostate massage for routine urine and urinary bacteria culture examination. The subjects were asked to position themselves on their chest and knees while a prostatic and anus examination was performed and the prostatic fluid was massaged. This study was conducted under the guidance of the ethics committee of the research institution.

2.3 | Sample testing

Enzyme-linked immunosorbent assay (ELISA) was used according to the reagent instructions to detect PSEP and HSP70 in the urine samples of 210 cases of chronic prostatitis and 70 control subjects. The PSEP kit was provided by Jiangsu Taicang Angke Biotechnology Co., Ltd., and the HSP70 kit was provided by Shanghai Enzyme Link Biotechnology Co., Ltd. The researchers read and recorded the absorbance value of the Radox RT-6500 microplate reader in dual-wavelength mode (450 nm is the test wavelength; 630 nm is the reference wavelength). Then, standard curves were established according to the absorbance values of different concentrations of standard samples, and the patient values were compared with the positive standard well values to calculate the actual concentrations of PSEP and HSP70 of the test samples.

2.4 | Statistical analysis

SPSS 21.0 software (IBM) was used for statistical analysis, and the Pearson chi-square test was used to compare the categorical variables. The nonparametric Mann–Whitney U test was used to compare continuous variables. The Bonferroni correction was applied to comparisons between groups, and $p < 0.0125$ was considered statistically significant. The area under curve (AUC) of the ROC was used to evaluate the specificity and sensitivity of the obtained PSEP and HSP70 measurements to evaluate their clinical value for the diagnosis of CP.

3 | RESULTS

3.1 | Comparison of urine PSEP and HSP70 levels

The baseline characteristics of the study subjects are shown in Table 1; there was no significant difference in age or body mass index distribution between the CP patients and the control group. The PSEP levels of the NIH-II, NIH-IIIa, and NIH-IIIb subgroups of CP patients were significantly higher than those of the control group ($p < 0.001$), but there was no difference in PSEP levels among the CP subgroups ($p > 0.0125$). The level of HSP70 in the urine of NIH-II patients was significantly lower than that of the NIH-IIIa and NIH-IIIb subgroups and the control group, but there was no difference in HSP70 levels between the NIH-IIIa and NIH-IIIb subgroups and the control group (Figure 1). The total imprecision of PSPE and HSP70 evaluated by the calibrator was 13.3% and 11.4%, respectively.

3.2 | The comparison of sensitivity, specificity, positive predictive value, and negative predictive value of PSEP and HSP70

To further evaluate the diagnostic ability of PSEP and HSP70 for CP, the area under the ROC curve was used to evaluate the specificity and sensitivity in CP patients with significantly different expressions of PSEP and HSP70. The results showed that the AUC of

TABLE 1 Baseline characteristics of the patients with CP and controls

Characteristic	CP			Control
	NIH-II	NIH-IIIa	NIH-IIIb	
Age (years)	35 ± 10.3	34 ± 9.2	33 ± 10.1	36 ± 8.9
BMI (kg/m^2)	23 ± 2.3	23 ± 2.1	24 ± 1.9	23 ± 2.0
PSEP (ng/ml)	3.27 ± 3.34*	3.05 ± 3.06*	2.88 ± 3.04*	0.91 ± 0.84
HSP70 (pg/ml)	56.47 ± 4.65**	62.22 ± 5.60	63.28 ± 8.92	64.88 ± 14.48

Note: Comparison to control group, *p < 0.0125; Comparison to NIH-IIIa group, NIH-IIIb group, and control group, **p < 0.0125.

Abbreviations: BMI, body mass index; CP, chronic prostatitis; HSP70, heat shock protein 70; NIH, The National Institutes of Health; PSEP, prostatic exosomal protein.

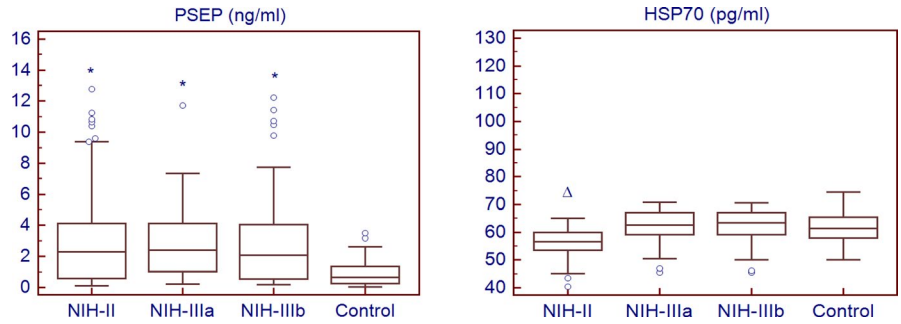

FIGURE 1 The levels of prostatic exosomal protein (PSEP) in the patients with NIH-II, NIH-IIIa, and NIH-IIIb were significantly higher than in the control group (p < 0.001), but there was no significant difference between the CP groups. The levels of heat shock protein 70 (HSP70) in the patients with NIH-II were significantly lower than those of the NIH-IIIa, NIH-IIIb, and control group (p < 0.001), but there was no significant difference between the NIH-IIIa, NIH-IIIb, and control group

PSEP in NIH-II, NIH-IIIa, and NIH-IIIb patients was 0.751, 0.776, and 0.731, respectively. The AUC of HSP70 in NIH-II patients was 0.784 (Figure 2), and the AUC of combined detection of PSEP and HSP70 in NIH-II patients was 0.858 (Figure 3). According to the ROC curve, the cutoff value of PSEP in diagnosing NIH-II patients is 1.89 ng/ml, and the sensitivity, specificity, positive predictive value, negative predictive value, and total diagnosis rate are 61.4%, 84.3%, 79.6%, 68.6%, and 72.9%, respectively. The sensitivity, specificity, positive predictive value, negative predictive value, and total diagnostic rate of HSP70 at a cutoff value of 60.5 pg/ml were 82.9%, 61.4%, 68.2%, 78.2%, and 72.1%, respectively. The sensitivity, specificity, positive predictive value, negative predictive value, and total diagnostic rate of the combined detection of PSEP and HSP70 for NIH-II diagnosis were 91.4%, 85.7%, 86.5%, 90.9%, and 88.6%, respectively. Sensitivity, specificity, and total diagnosis rate are all higher than those of independent testing (Table 2). Similarly, in patients with NIH-IIIa, the cutoff of PSEP is 0.99 ng/ml, and the corresponding detection sensitivity, specificity, positive predictive value, negative predictive value, and total diagnostic rate are 75.7%, 72.9%, 73.6%, 75.0%, and 74.3%, respectively. In the diagnosis of NIH-IIIb patients, the detection sensitivity, specificity, positive predictive value, negative predictive value, and total diagnostic rate of PSEP at a cutoff of 0.99 ng/ml were 67.1%, 72.9%, 71.2%, 68.9%, and 70.0%, respectively.

4 | DISCUSSION

Chronic prostatitis is a common disease in adult men.[8,9] The etiology and pathogenesis of chronic prostatitis are complicated, and laboratory examination methods are limited. More than 10% of adult men have prostatitis-like symptoms, and the quality of life of some of them is seriously affected.[10] Therefore, there is an urgent need to find representative diagnostic markers. Research on the pathogenesis of CP has transitioned from a focus on the performance of cell function in inflammatory response to a focus on the mechanisms that regulate immune response. In this study, PSEP and HSP70 levels in the urine of CP patients were detected, and their clinical value as diagnostic markers of CP was evaluated with the aim of finding a simple, non-invasive, and painless detection method to reduce patients' pain and trauma. The results showed that, compared with the control group, the PSEP levels detected in the samples of the CP subgroups were significantly higher (p < 0.001). ROC curve analysis results showed that the AUC of the NIH-II, NIH-IIIa, and NIH-IIIb subgroups was 0.779, 0.77, and 0.745, respectively. It is suggested that PSEP has operability and value as a clinical diagnosis for chronic prostatitis. However, the expression levels of PSEP in patients with NIH-II, NIH-IIIa, and NIH-IIIb were not statistically different (p > 0.0125). It can be seen that PSEP is not useful for identifying the NIH

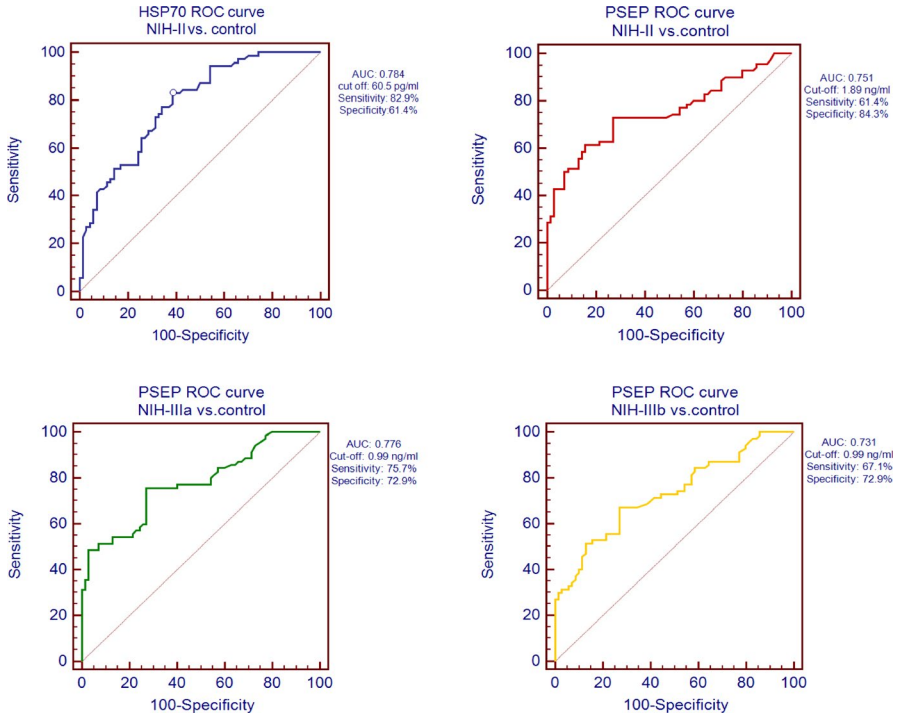

FIGURE 2 The predictive value of urinary prostatic exosomal protein (PSEP) and heat shock protein 70 (HSP70) levels in the diagnosis of chronic prostatitis with NIH-II, NIH-IIIa, and NIH-IIIb

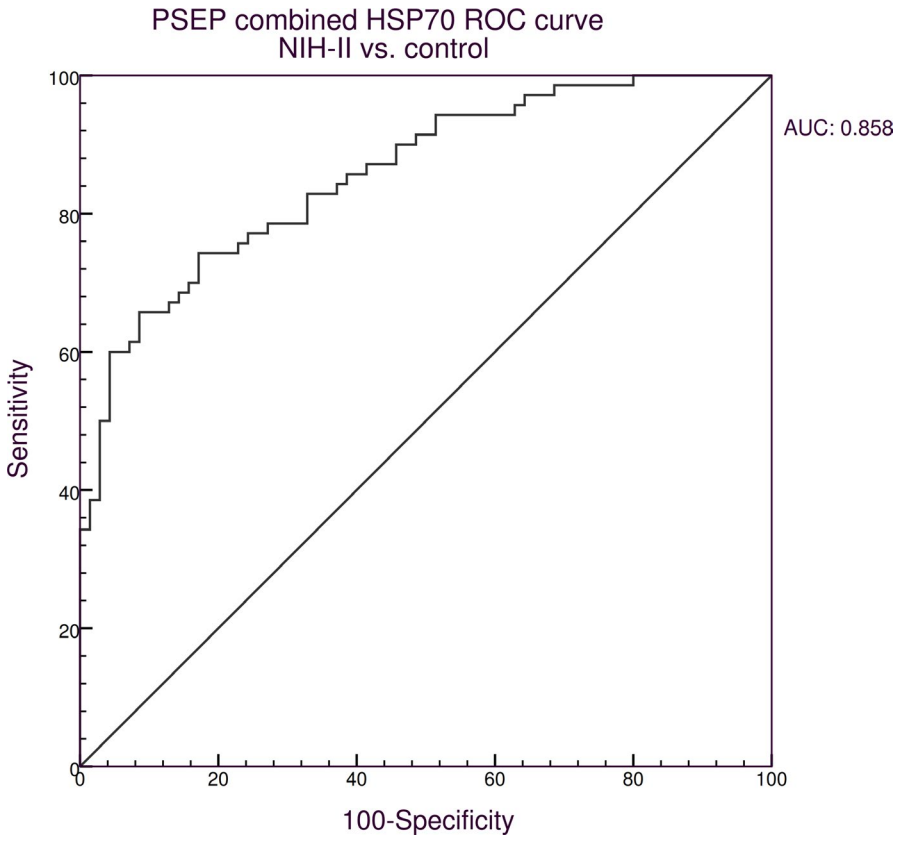

FIGURE 3 The predictive value of combined detection of urinary prostatic exosomal protein (PSEP) and heat shock protein 70 (HSP70) levels in the diagnosis of chronic prostatitis with NIH-II

classification subtypes of CP, which is consistent with our previous report.[11,12] This may be because CP is caused by a variety of etiologies, including complex pathological changes such as immunity, pathogenicity, and neuroendocrine inflammation. The common feature of these pathological changes is the increase in PSEP levels, but there is no difference in PSEP levels among CP subgroups. It is speculated that the secretion concentration does not increase with the increase in the severity of inflammation because when the tissue in CP is infiltrated by inflammatory cells, it releases prostaglandin bodies.[13,14] The release of prostate corpuscles is affected by the pathogenesis or etiology of CP. If the etiology or pathological changes of NIH-IIIb–type CP patients are consistent

TABLE 2 The comparison of sensitivity, specificity, positive predictive value, and negative predictive value of PSEP and HSP70

Test	Group	Sensitivity	Specificity	Positive predictive value (PPV)	Negative predictive value (NPV)	Total diagnosis rate
PSEP	NIH-II	61.4 (43/70)	84.3 (59/70)	79.6 (43/54)	68.6 (59/86)	72.9 (102/140)
	NIH-IIIa	75.7 (53/70)	72.9 (51/70)	73.6 (53/72)	75.0 (51/68)	74.3 (104/140)
	NIH-IIIb	67.1 (47/70)	72.9 (51/70)	71.2 (47/66)	68.9 (51/74)	70.0 (98/140)
HSP70	NIH-II	82.9 (58/70)	61.4 (43/70)	68.2 (58/85)	78.2 (43/55)	72.1 (101/140)
PSEP combined with HSP70	NIH-II	91.4 (64/70)	85.7 (60/70)	86.5 (64/74)	90.9 (60/66)	88.6 (124/140)

Abbreviations: CP, chronic prostatitis; HSP70, heat shock protein 70; NIH, The National Institutes of Health; PSEP, prostatic exosomal protein.

with types IIIa and II, they may release almost the same number of prostate corpuscles, so the level of PSEP excreted in the urine is also similar.

HSP70, also known as the stress protein, is related to a variety of environmental stimuli.[15] HSP70 induced by bacterial infection is an important substance that protects cells from damage to cell function caused by an inflammatory reaction during bacterial infection by refolding and stabilizing denatured proteins.[16,17] Nickerson et al[18] found that the content of inflammatory cytokine HSP70 increased significantly in rats infected with *Escherichia coli*. Zhang et al[19] showed that HSP70 participates in the immune response to bacterial attack and heat stress, and the expression of HSP70 is up-regulated after a bacterial attack. The pathogenesis of bacterial NIH-II is chronic prostatitis caused by bacterial infection. When monocytes and macrophages are stimulated by bacterial toxins, a large amount of HSP70 is synthesized and released. When bacteria invade, a large amount of HSP70 is an important defense response of the host against bacterial infection. In patients with chronic bacterial prostatitis, the cytoprotective effect of HSP70 plays a vital role in regulating the function of cells infected by bacteria, and HSP70 may become an indicator of the severity of CP.

Therefore, this study tried to detect HSP70 levels in the urine of different types of CP patients and control subjects to see whether different subtypes of CP could be identified based on PSEP. The results showed that the expression level of HSP70 in the NIH-II group was lower than that of the NIH-IIIa and NIH-IIIb subgroups and the control group ($p < 0.001$), but there was no significant difference in the expression level of HSP70 between the NIH-IIIa and NIH-IIIb subgroups and the control group ($p > 0.0125$). Interestingly, this result is contrary to the expression level of HSP70 in the prostatic fluid of CP patients reported by Guo et al.[5] The reason may be that when NIH-II patients are infected with bacteria, the surface receptors of monocytes and macrophages recognize the bacteria and release a large amount of HSP70.[20,21] As the main lesion is located in the prostate, a large amount of HSP70 is released into the prostatic fluid to protect the cells by resisting the invasion of bacteria; then, the level of HSP70 is reduced when it is excreted with urine. This hypothesis needs to be confirmed. Also, the sensitivity, specificity, and total diagnostic efficiency were increased by applying the combined detection of PSEP and HSP70 to NIH-II, which were 91.4%, 85.7%, and 88.6%, respectively, and AUC was also increased to 0.858, which was more valuable than a single test.

This study has some limitations. First, the sample size is limited, so the ROC curve was obtained from a few samples. Second, the sensitivity of PSEP and HSP70 can be improved by detecting the initial urine or the urine after ejaculation. However, the secondary antibody in the ELISA kit is an HRP-labeled antibody, and the bacteria in the initial urine may interfere with HRP and produce false-positive results.

In summary, urine PSEP has good application value for the diagnosis of chronic prostatitis, but PSEP cannot distinguish the various subtypes of CP. In contrast, urine HSP70 may become an important basis for distinguishing NIH-II CP patients. Therefore, the levels of PSEP and HSP70 in urine may be reliable biomarkers for the diagnosis of CP, which can effectively improve the accuracy of CP diagnosis. Early diagnosis of chronic prostatitis can be achieved only through urine testing. The application of this detection method may address the shortcomings of the current clinical diagnosis of CP, such as strong subjectivity and certain painful detection methods, and provide a novel, simple, easy, non-invasive, and painless molecular detection method for the diagnosis of CP.

5 | CONCLUSION

Urine PSEP can be used as a marker for the diagnosis of CP, but it cannot distinguish the CP subtypes, and HSP70 can be used as a diagnostic index for NIH-II classification. Both provide a novel, simple, non-invasive, and painless molecular detection method for CP diagnosis.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Yinghua Tang involved in data analysis and manuscript writing. Lianli Yin involved in protocol/project development. Aiping Pan and Yonggang Liu involved in data collection or management.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

ORCID

Yinghua Tang https://orcid.org/0000-0002-5655-9650
Lianli Yin https://orcid.org/0000-0003-4425-1592

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