Clin. Lab. 2023;69:XXX-XXX

ORIGINAL ARTICLE

Study on the Application Value of the Content of Prostatic Exosomal Protein in the Treatment of Chronic Prostatitis

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SUMMARY

Background: **To evaluate the application value of urinary prostatic exosomal protein (PSEP) in the treatment of chronic prostatitis (CP).**
Methods: **We evaluated 174 patients with chronic prostatitis (44 cases of NIH-II, 65 cases of NIH-IIIa, and 65 cases of NIH-IIIb) who had obvious symptoms of chronic prostatitis syndrome and met the diagnostic criteria of National Institutes of Health Prostatitis from May 2018 to February 2021. They were also evaluated according to the clinical treatment's effect after six weeks of treatment. Urine samples of CP patients were collected before treatment and after six weeks of treatment, and the level of PSEP in the urine samples of all patients, before and after treatment, was detected by the ELISA method to evaluate the application value of PSEP in the end of CP curative effect.**
Results: **After six weeks of treatment, the total CPSI score of CP patients decreased significantly, compared to patients before treatment. After six weeks of treatment, the PSEP content in the patients' urine was compared to before treatment. The PSEP levels of CP subgroups decreased significantly ($p < 0.05$): NIH-II group (1.55 ± 1.39 ng/mL vs. 3.09 ± 2.66 ng/mL); NIH-IIIa group (1.68 ± 1.06 ng/mL vs. 3.34 ± 2.69 ng/mL); and NIH-IIIb group (1.57 ± 1.17 ng/mL vs. 3.14 ± 2.81 ng/mL).**
Conclusions: **The concentration of PSEP in the urine of CP patients has a good application value for evaluating clinical treatment's effect on chronic prostatitis, and its concentration level may affect the development and outcome of prostatitis.**
(Clin. Lab. 2023;69:xx-xx. DOI: 10.7754/Clin.Lab.2022.220319)

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Manuscript accepted May 24, 2022

KEYWORDS

prostatic exosomal protein, chronic prostatitis, curative effect

INTRODUCTION

Chronic prostatitis is one of the common diseases of the reproductive system, and it has always been a major problem plaguing men [1]. Due to its complex pathogenesis, stubborn condition, and the special structure of the prostate, clinical treatment is more difficult, and it may cause symptoms, such as male sexual dysfunction

and neurasthenia, which will seriously affect the patient's psychological health, physical health, and overall quality of life [2]. Currently, multiple clinical evaluations and elimination of other conditions must complete the diagnosis and severity evaluation of CP, because a specific biomarker is still unavailable [3]. Because the etiology and pathogenesis of CP are very complicated, their roles and effect are not yet fully understood. Although there are various treatment methods, the overall curative effect and the prognosis is poor. Regular review is usually required to determine the treatment's effect. There is still a lack of detection methods to effectively evaluate the effects of CP treatment. The prostate is a derivative of embryonic Mullerian ducts, lined by cylindrical cells containing apical secretory vesicles. Prostatic corpuscles are small extracellular vesicles secreted by prostatic epithelial cells and subsequently enter prostatic fluid and urine. Prostatic exosomal protein (PSEP) is a protein secreted by the prostatic corpuscle. When CP tissue is infiltrated by inflammatory cells, a variety of pathogenic factors lead to abnormal expression of Wnt signaling pathway, resulting in the release of prostatic corpuscles and changes in their protein phenotypes. The extracellular protein of prostatic corpuscles is secreted into the male reproductive tract, urethra and urine through anatomical channels, termed PSEP. Several studies have shown that PSEP can be used as a marker for the auxiliary diagnosis of chronic prostatitis [4-6]. However, whether it can be used to evaluate the effect of clinical treatment is not clear. This study evaluated the clinical value of PSEP in the treatment effects of CP by measuring the levels of PSEP in the urine of patients with different types of CP, before and after treatment.

MATERIALS AND METHODS

Subjects

This project is a prospective study, the research subjects consisted of 174 patients with chronic prostatitis who had obvious symptoms of chronic prostatitis syndrome and met the diagnostic criteria of the National Institutes of Health (NIH) regarding prostatitis in urology and andrology, from May 2018 to February 2021. After six weeks of treatment, they were evaluated according to the clinical treatment's effect. According to the definition established by the National Institutes of Health [7], the patients were divided into three groups: 44 cases of NIH-II, 65 cases of NIH-IIIa, and 65 cases of NIH-IIIb. The age range of patients ranged from 20 to 65 years, with an average age of 35.81 ± 5.87 years. There was no difference in the distribution of age and body mass index (BMI) among CP patients ($p > 0.05$). All patients were asked for detailed medical history, including medication history and sex life, while also excluding other related diseases that may cause similar symptoms. The patient exclusion criteria are as follows: (1) Patients with other prostate diseases, such as prostate tumors and acute prostatitis; (2) Patients who have taken drugs that affect their immune function before treatment; (3) Patients who are allergic to the drugs used in this study; (4) Patients with urethral stricture and urethritis; (5) Patients with liver and kidney dysfunction; (6) Patients with mental illness; and (7) Patients with poor treatment compliance.

Treatment methods

(1) Treated with traditional Chinese medicine granule formula "Jiawei Baitouweng Decoction" (Jiangyin Tianjiang Pharmaceutical Co., Ltd., batch number: 20110223, specification: 30 g/dose, Jiangsu, China). Recipe composition: Chinese Pulsatilla Root 30 g, Amur Cork-tree Bark 12 g, Ash Bark 12 g, Honeysuckle Flower 20 g, Dandelion 15 g, Hedyotis Diffusa Willd 15 g, Corydalis Yanhusuo 20 g, Danshen Root 15 g, Sanchi 6 g, Prepared Rehmannia Root 15 g, Common Yam Rhizome 15 g, Cornus Officinalis Sieb 12 g. Dissolve the above traditional Chinese medicine with 60℃ warm water and take it orally, 1 dose per day. (2) Treated with Ningbitai Capsules (Guiyang Xintian Pharmaceutical Co., Ltd., approval number: Z200254-42, specification: 0.38 g/capsule, Guiyang, China), orally, 4 capsules/time, 3 times/day. Continuous treatment for 6 weeks.

Efficacy observation indicators and evaluation criteria

The efficacy observation indicators were as follows: after six weeks of treatment for all patients, the effectiveness of the treatment was evaluated through the The National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI), white blood cells in expressed prostatic secretions (EPS-WBC), and lecithin corpus in expressed prostatic secretions (EPS-LC). The NIH-CPSI refers to NIH-CPSI of the National Institutes of Health to score the symptoms of patients before and after treatment, which mainly includes pain symptoms, micturition symptoms, and overall quality of life. The higher the score, the more serious the symptoms are [8]. Efficacy evaluation standard: It was observed that the clinical symptoms disappeared, the texture of the prostate was not significantly abnormal, and the tenderness of the prostate disappeared. In addition, the prostate bacterial examination showed as negative, and the patients' prostate fluid examination and physical sign scores were reduced by more than 90% when compared to before treatment. The white blood cell count during an EPS examination was less than 10/HP, and the number of lecithin corpus (LC) increased, which is curative. At least 40% - 60% of the patients' clinical symptoms were relieved. At the same time, the bacterial test results of the prostate were negative, and the tenderness of the prostate disappeared completely. In addition, the patients' prostate fluid examination and physical sign scores were reduced by 40% to 89%, when compared to before treatment. The white blood cell count during the EPS examination was also reduced when compared to

Table 1. Comparison of CPSI total score, PSEP, EPS-WBC, and EPS-SPL of each group before and after treatment.

Characteristic	NIH-II (n = 44)		NIH-IIIa (n = 65)		NIH-IIIb (n = 65)	
	pre-treatment	post-treatment	pre-treatment	post-treatment	pre-treatment	post-treatment
Age (years)	34.2 ± 9.8	34.2 ± 9.8	33.1 ± 9.1	33.1 ± 9.1	33.5 ± 9.5	33.5 ± 9.5
BMI (kg/m^2)	23.3 ± 2.2	23.3 ± 2.2	22.5 ± 2.0	22.5 ± 2.0	24.4 ± 2.3	24.4 ± 2.3
WBC in EPS (/hpf)	22.9 ± 4.6 *	12.8 ± 1.6	20.9 ± 3.0 *	11.4 ± 1.2	8.1 ± 1.1 *	7.6 ± 1.1
Lecithin corpuscle in EPS (/hpf)	7.8 ± 0.9 *	12.1 ± 1.4	8.3 ± 1.2 *	12.3 ± 1.9	8.7 ± 1.2 *	12.6 ± 2.3
NIH-CPSI	20.5 ± 5.6 *	14.2 ± 4.4	22.3 ± 5.0 *	13.4 ± 3.0	22.0 ± 5.1 *	12.9 ± 3.1
PSEP (ng/mL)	3.09 ± 2.66 *	1.55 ± 1.39	3.34 ± 2.69 *	1.68 ± 1.06	3.14 ± 2.81 *	1.57 ± 1.17

BMI - body mass index, WBC - white blood cell, EPS - expressed prostatic secretions, CPSI - Chronic Prostatitis Symptom Index, PSEP - prostatic exosomal protein, CP - chronic prostatitis, comparison to post-treatment - * $p < 0.05$.

before treatment, with the number of LC also increased. If the clinical symptoms and signs of the patient are not significantly improved or even aggravated, it is considered invalid.

Specimen collection and testing

Specimen collection

The collection of the middle urine of CP patients was in strict accordance with the aseptic operation, and patients were instructed to have a clean diet the night before the urine specimen was collected - alcohol and excretion activities were prohibited. After collection, the specimens were frozen and stored at -80℃ for inspection. All patients must give urine specimens before they can undergo routine EPS examinations, which helps to rule out the influence of prostate massage on urine specimens. The counting of ESP-WBC and ESP-LC was as follows: use prostate massage to collect prostatic fluid and perform microscopic examination of ESP-WBC and ESP-LC to count the EPS that began to flow out. The contents of PSEP in urine samples of chronic prostatitis were detected by ELISA, according to the instructions of the reagents. PSEP detection adopts the ELISA method, and the kit is provided by Jiangsu Taicang Onco Biotechnology Co., Ltd. The absorbance value was read and recorded, which was detected at 450 nm/630 nm dual-wavelength using the Radox RT-6500 microplate reader, then the PSEP concentration in the sample was calculated according to the standard curve.

Statistical analysis

SPSS 23.0 statistical software was used for data processing. The measurement data is expressed as ($\bar{x}$ ± s), and the variance test is used to compare the differences in age, BMI, NIH-CPSI score, EPS lecithin count, and WBC count in EPS. The enumeration data used Pearson's chi-squared test. The nonparametric Mann–Whitney U test was used to compare continuous variables. The statistically significant difference was defined as $p < 0.05$.

RESULTS

Characteristics of selected patients

As seen in Table 1, there were no significant differences in the distribution of age and BMI in the 174 CP patients in NIH-II, NIH-IIIa, and NIH-IIIb ($p > 0.05$).

Each group was compared before and after treatment, based on the total NIH-CPSI score, PSEP, EPS-WBC, and EPS-LC of each group.

The results in Table 1 show that compared with before treatment, after six weeks of effective treatment, the total NIH-CPSI scores of the CP patients were significantly reduced (Figure 1). The difference was statistically significant ($p < 0.05$). The mean total score of NIH-CPSI in patients with NIH-II CP decreased by 6.3 (30.7%), slightly lower than that of NIH-IIIa (8.9, 39.9%) and NIH-IIIb (9.1, 41.4%), but the difference was not statistically significant (Table 1). Similarly, after six weeks of treatment, the total CPSI score of CP patients decreased significantly, compared to patients before treatment. After six weeks of treatment, the PSEP content in the patients' urine was compared to before treatment. The PSEP levels of CP subgroups decreased significantly (Figure 2; $p < 0.05$): NIH-II group (n = 44) 1.55 ± 1.39 ng/mL vs. 3.09 ± 2.66 ng/m; NIH-IIIa group (n = 65) 1.68 ± 1.06 ng/mL vs. 3.34 ± 2.69 ng/mL; and NIH-IIIb group (n = 65) 1.57 ± 1.17 ng/mL vs. 3.14 ± 2.81 ng/mL. The mean number of PSEP in NIH-II, NIH-IIIa, and NIH-IIIb decreased by 1.54 (49.8%), 1.66 (49.7%), and 1.57 (50.0%), respectively.

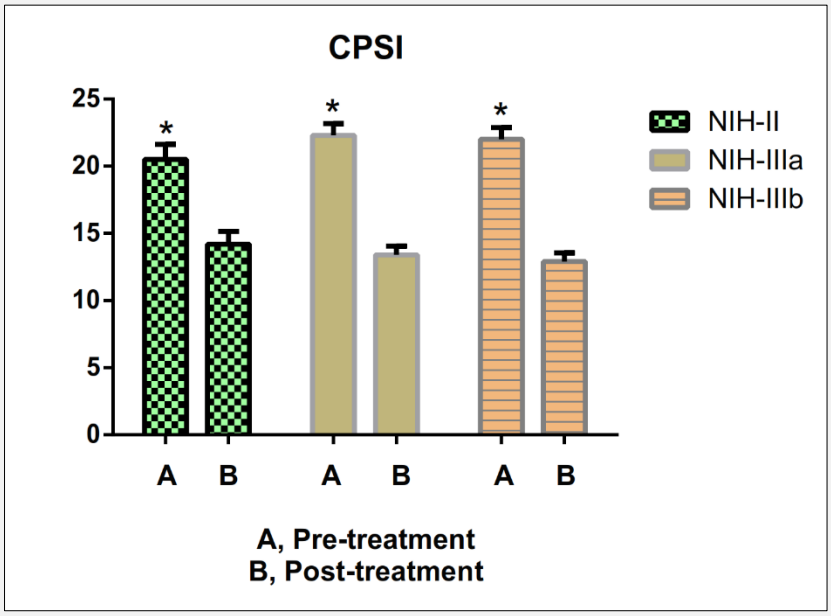

Figure 1. Compared with before treatment, after 6 weeks of effective treatment, the total NIH-CPSI score of patients in CP groups decreased significantly in comparison to post-treatment, * $p < 0.05$.

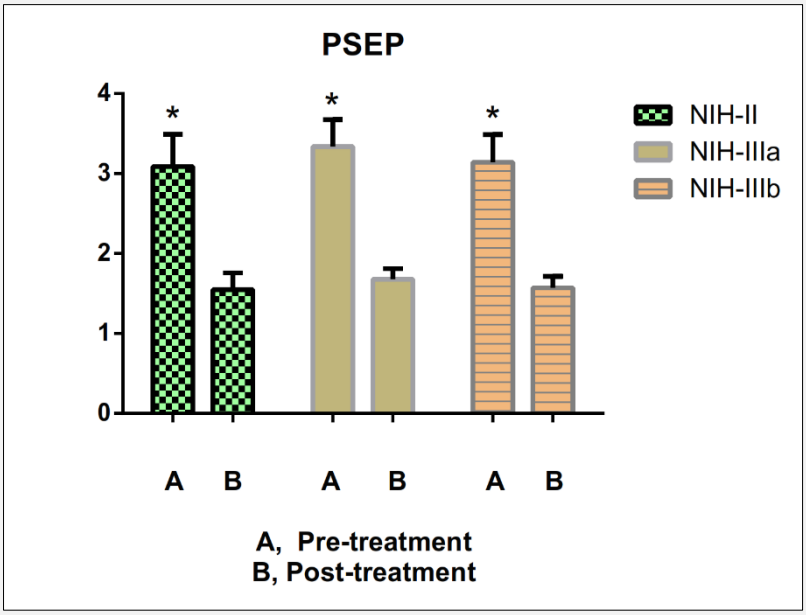

Figure 2. After six weeks of effective treatment, the level of PSEP detected in the middle urine of CP patients in each subgroup decreased compared with that before treatment in comparison to post-treatment, * $p < 0.05$.

There was no difference in the mean decrease level (percentage) of PSEP among subgroups.

The EPS examination results of CP patients showed that with the effective treatment, after six weeks the number of EPS-WBC in each group of CP patients decreased significantly (Table 1; $p < 0.05$). The average number of EPS-WBC in NIH-II, NIH-IIIa, and NIH-IIIb decreased by 10.1 (44.1%), 9.5 (45.5%), and 0.5 (6.2%), respectively. On the contrary, after six weeks of effective treatment, the number of EPS-CL was significantly higher than before treatment ($p < 0.05$). The mean difference in the number of EPS-CL in NIH-II, NIH-IIIa, and NIH-IIIb was 4.3 (55.1%), 4.0 (48.2%), and 3.9 (44.8%), respectively. There was no significant difference in the mean difference (percentage) between EPS-WBC and EPS-CL among the subgroups.

DISCUSSION

Chronic prostatitis is the most common and confusing disease in adult men. Patients may experience urinary discomfort and pain, such as pain in the perineum and pelvis. Some patients may even experience erectile dysfunction, painful ejaculation, and reproductive disorders [9-11]. There are many causes of the disease, involving pathogenic microbial infections, autoimmune and psychophysiological factors, urine reflux in the prostate, local structural changes, and many other aspects [12]. The pathogenesis is complex. So far there is no clear cause that can fully explain the pathogenesis, so there is no clear and unified treatment plan. Most of plans rely on previous treatment experience. The treatment effect on patients is poor, and the prognosis is poor. There is a lack of clinical testing methods to effectively evaluate the effects of CP treatment. Therefore, it is of great clinical significance to actively explore the effective treatment indicators for CP patients. Many studies have shown that there is no correlation between the number of WBCs in EPS and the clinical symptoms of patients [13,14]. However, in this study, it was observed that the number of EPS-WBC in CP patients in NIH-II and NIH-IIIa decreased significantly after treatment when compared to before treatment ($p < 0.05$). Perhaps because the patient had not used antibiotics before, it was observed that WBCs play an important role in the body's defense system when infected. After treatment, the degree of inflammation in the prostate tissue was reduced; additionally, WBCs can decline rapidly in a short period of time. We also found that there was no significant difference in the NIH-CPSI scores after treatment in the three groups, indicating that there was no difference in the improvement of CP in these three groups. It is confirmed that WBCs directly reflect the degree of inflammation and infiltration of prostate tissue, but have no significant correlation with the severity of clinical symptoms [15].

Lecithin corpus (LC), like WBC, is an important part of prostate fluid detection. It is a normal part of an adult male's prostate fluid. It is secreted by prostate epithelial cells and provides nutrients for sperm. It can enhance sperm motility, delay acrosome reaction, protect sperm from acidic environments, and modify biological characteristics and semen composition. It is an indicator of the prostate's secretory function [16,17]. When prostate tissue is inflamed, the glandular epithelial tissue is destroyed, the interstitial tissue around the gland is accompanied by inflammatory cell infiltration, and there are a large number of inflammatory cells and inflammatory exudates in the lumen of the gland. With the continued existence and further development of inflammation, the secretory function of prostate epithelial cells is inhibited, resulting in a decrease in the secretion of lecithin corpuscle coupled with the phagocytosis and clearance of macrophages, resulting in a further decrease in the number of lecithin corpuscle in EPS [18]. However, with the control of inflammation and the improvement of the condition, the function of prostate epithelial tissue is restored, allowing the number of lecithin corpuscle in EPS to gradually increase. In this study, the number of lecithin corpuscle in EPS in CP patients increased significantly after treatment, which was statistically significant compared to before treatment. These results may be related to the reduction of inflammation in CP patients after treatment, along with the recovery of the secretory function of prostate epithelial cells. When the prostate tissue is inflamed, under the effect of WBC phagocytosis, killing, and clearance, it also engulfed lecithin corpuscle and reduced its number. When the inflammation is controlled, as the prostate epithelial cells are repaired, the number of lecithin corpuscle gradually recovers.

Relevant studies have proven that the level of PSEP in urine can be used as an auxiliary index for the clinical diagnosis of CP [4-6], with superior sensitivity and specificity. It also greatly reduces the pain in the diagnosis and treatment of CP patients. When the tissues in CP are infiltrated by inflammatory cells, an active substance called prostate corpuscle is secreted into the male reproductive tract through anatomical channels. It has multiple physiological functions, which can protect sperm in acidic environments, delay acrosome reactions, and enhance sperm viability [19]. The prostatic body can also inhibit the activity of nicotinamide adenine dinucleotide phosphate oxidase of polymorphonuclear leukocytes by transferring cholesterol and sphingomyelin from the prostatic body to the cell membrane, thereby reducing the reactive oxygen species reaction [20]. Since the special lipid structure and composition of the prostate corpuscle are the key factors for its antioxidation and antibacterial function, including whether its exocrine protein can be used as an effective indicator of the therapeutic effect of CP. In this study, the urine PSEP levels of CP patients before and after six weeks of treatment were detected. The results showed that after regular and standardized treatment the PSEP levels in the urine of NIH-II, NIH-IIIa, and NIH-IIIb CP patients decreased significantly. The NIH-CPSI score was

lower than before treatment, the degree of pain was reduced, and the PSEP level was also significantly lower than before treatment, suggesting that the detection of PSEP concentration in the patients' urine may be an indicator of the efficacy of CP. In addition, with the decrease of WBC in EPS, the level of PSEP in urine gradually decreased. These results suggest that PSEP may play a major role in a certain stage of the pathogenesis of prostatitis, and its concentration level may affect the occurrence, development, and prognosis of prostatitis. Also, the blocking treatment of PSEP may improve the pain symptoms of patients, which needs further research. The additional research is needed because when the tissue in CP is infiltrated by inflammatory cells, it will release the prostatic corpuscles, while PSEP is secreted into the male reproductive tract and urethra through the anatomical channel, entering the urine. When the inflammation is controlled or slowed, the body will reduce the release of the prostate corpuscles, so the level of PSEP excreted in the urine will also be reduced accordingly.

Therefore, the morbidity and recurrence rate of CP is high, and regular EPS review is inevitable during the course of treatment. It is a common treatment strategy to guide follow-up treatment based on the results of regular review, which has great clinical significance in the treatment of CP. However, a prostate massage is somewhat invasive, and it will inevitably cause the patient pain and discomfort, which will greatly affect the patient's compliance, ultimately leading to poor treatment for CP patients. PSEP is a prostatic protein that exists in the urine and can only be detected through urine. Therefore, the application of this detection method has very important clinical significance.

There are some limitations in this study. First, due to the particularity of CP disease itself, its therapeutic effect is easily affected by many elements, such as lifestyle habits, mental state, social factors, etc. [21]; therefore, this study cannot completely eliminate these interfering factors. Second, in this study, patients who showed progress after six weeks of treatment were selected for PSEP detection, and the research results did not involve all CP patients. Some CP patients whose symptoms did not significantly improve after treatment and who were evaluated as ineffective were not included in this study.

CONCLUSION

The concentration of PSEP in the urine of CP patients has a good application value for evaluating the clinical treatment effects of chronic prostatitis, and its concentration level may affect the development and outcome of prostatitis. It can provide a novel, simple, non-invasive, and painless detection method for evaluating the therapeutic effect of CP.

Acknowledgment:
The author thanks all participants for their contributions.

Consent to Participate:
All the participants included provided written informed consent and the protocol was reviewed and approved by the Ethics Committee of Guangxi Zhuang Autonomous Region.

Ethical Approval:
All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Source of Funds:
This study was supported by the Key Special Project of China's Key R&D Program "Active Health and Scientific Response to Aging" (No. 2021YFC2009300), Nanning Scientific Research and Technical Development Project (No. 20193103), and the Health Commission of the Guangxi Zhuang Autonomous Region (No. Z-A202 20039).

Declaration of Interest:
The authors declare that they have no competing interests.

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