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The application of IL-10 and TNF-α in expressed prostatic secretions and prostatic exosomal protein in urine in the diagnosis of patients with chronic prostatitis

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Abstract

Background: The aim of this study was to investigate the expression of tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) in expressed prostatic secretions (EPSs) of patients with chronic prostatitis (CP) and the expression of prostatic exosomal protein (PSEP) in urine, and to evaluate its correlation with the condition.

Methods: Urine samples from 310 patients with CP (101 National Institutes of Health [NIH] II, 112 NIH IIIa, and 97 NIH IIIb, classified according to the US National Institutes of Health) and 110 control group subjects were collected. The samples were tested for PSEP by enzyme-linked immunosorbent assay (ELISA). At the same time, EPSs in 60 patients from 310 patients with CP and 20 control group subjects were collected. The levels of IL-10 and TNF-α in the collected samples that EPS were determined by double antibody sandwich ELISA. SPSS 23.0 statistical software was used for statistical analysis of the measured data.

Results: The level of PSEP in patients with CP was significantly higher than that in the control group ($P < .001$). The levels of TNF-α and IL-10 in the EPS of patients with NIH II and NIH IIIa CP were higher than those of the patients with NIH IIIb and the control group ($P < .001$). There was a positive correlation between PSEP and IL-10 and TNF-α, while TNF-α and IL-10 were also positively correlated.

Conclusion: PSEP, TNF-α, and IL-10 may serve as a basis for the classification diagnosis of CP. Their combination can provide more accurate diagnostic information for clinical CP typing.

Abbreviations: AUC = area under the curve, BMI = body mass index, CP = chronic prostatitis, CT = computed tomography, ELISA = enzyme-linked immunosorbent assay, EPS = expressed prostatic secretions, IL-10 = interleukin-10, MRI = magnetic resonance imaging, NIH = National Institutes of Health, PLSs = prostatitis-like symptoms, PMN = polymorphonuclear leukocytes, PSEP = prostatic exosomal protein, ROC = receiver operating characteristic, ROS = reactive oxygen species, TNF-α = tumor necrosis factor alpha.

Keywords: chronic prostatitis, interleukin-10, prostatic exosomal protein, tumor necrosis factor alpha

1. Introduction

Chronic prostatitis (CP) is a common disease in adult males. It also occurs in young men aged 20 to 40 years old. It is mainly characterized by pain, urinary tract symptoms, reproductive system symptoms, and depression. In 1998, the US National Institutes of Health (NIH) classified CP into 3 types. These are NIH II: chronic bacterial prostatitis; NIH III: CP/chronic pelvic pain syndrome, which is divided into 2 subtypes, inflammatory (IIIa) and noninflammatory (IIIb), according to the white blood cell count in expressed prostatic secretions (EPSs) and semen; and NIH IV: asymptomatic inflammatory prostatitis. This is defined by the presence of leukocytes in seminal secretions or by inflammatory infiltrates detected in histologic specimens, as well as absence of other typical genitourinary tract symptoms. At present, the etiology and pathogenesis of CP are complicated, the clinical manifestations lack specificity due to individual differences, and there is no objective and accurate diagnostic method. The clinical diagnosis and treatment effects have been unsatisfactory.[1] However, it has been reported that the changes that occur in cytokines can be detected in many inflammatory pathologic processes.[2–4] When the prostate tissue is infiltrated by inflammatory cells, various active substances are released. As a result, prostaglandins are released into the posterior urethra and then excreted in the urine. The prostate corpuscle is also one of substances that is released.[5]

In this study, we measured and compared the levels of the tumor necrosis factor alpha (TNF-α), interleukin-10 (IL-10), and prostatic exosomal protein (PSEP) in the urine of various types of patients with CP and normal human EPS, and then analyzed the role of these in the diagnosis, classification, and treatment of CP.

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2. Materials and methods

2.1. Subject

2.1.1. Medical history inquiry. All patients were asked for detailed medical history, including medication history and sexual life, and excluded other related diseases that may cause similar symptoms, such as diabetes, urethritis, epididymitis, varicocele, rectal, and perianal lesions.

2.1.2. Grouping of study subjects. The subjects were grouped according to the NIH classification criteria for prostatitis.[6] The results were: 101 patients with NIH II CP, EPS with leukocytes ≥10/HP, lecithin ≤++, middle urinary bacteria before prostate massage both culture, and microscopy were negative. The urine culture was positive in the 1st stage after massage. There were 112 cases of NIH IIIa CP group, all of which had clinical symptoms. The urine culture was negative before and after massage. The leukocytes of EPS were ≥10/HP, the lecithin ≤++, and the bacterial culture was negative. In the NIH IIIb CP group, 97 cases were negative for urinary bacterial culture before and after prostate massage. The number of leukocytes in EPS microscopy was ≤10/HP, and lecithin ≤++ to +++.

2.1.3. Sample collection. A total of 310 patients who had significant CP syndrome and met US NIH prostatitis diagnostic criteria were selected as subjects from urology and male clinics from May 2017 to June 2018, and they were divided into 3 groups according to the definition of the NIH: 101 cases of NIH II, 112 cases of NIH IIIa, and 97 cases of NIH IIIb. The patients were from 18 to 56 years of age with an average age of 30.1 years. A total of 110 people who had undergone a health checkup which revealed no clinical symptoms were selected as the control group. For these subjects, physical examination, routine EPS examination, routine urinalysis, and urine bacterial culture before and after prostate massage were normal, and they were from 20 to 45 years of age with an average age of 29.5 years. Morning urine samples were collected from all patients with CP and the control group, and they were stored at –80°C for PSEP detection. At the same time, only 60 of the 310 patients agreed to undergo EPS examination, 60 patients from the 310 patients with CP (20 cases of NIH II, 20 cases of NIH IIIa, and 20 cases of NIH IIIb), and 20 subjects from the control group were examined for TNF-α and IL-10 in the EPS. Both the study subjects and the control group required abstinence for 3 to 5 days. Cleaning and disinfecting the penis head and the external urethra were performed before taking the specimen. Routine urinalysis results and urine bacterial cultures were examined before and after prostate massage. When collecting prostatic fluid, the patient assumed the chest and knee position, an anal prostate examination was performed, and the prostate was massaged to express prostatic fluid. The EPS that flowed out was collected in a 1.5-mL centrifuge tube and stored at –80°C until examination. The study was carried out in accordance with the ethics committee of the Institute and Ethical Committees.

2.2. Sample detection

An indirect enzyme-linked immunosorbent assay (ELISA) was used to detect the PSEP content of urine samples of the 310 cases of CP and the 110 control subjects. The PSEP kit was provided by Jiangsu Taicang Angke Biotechnology Co, Ltd (Jiangsu, China). The collected urine samples were tested according to the PSEP kit operating instructions. The absorbance values on the BIO-RAD 680 microplate reader in dual wavelength mode (450 nm as the test wavelength and 630 nm as the reference wavelength) were read and recorded. Then, a standard curve was established according to the absorbance values of the standard of different concentrations, and the patient's value was compared with the positive standard hole value to calculate the actual concentration of the PSEP of the test sample.

Similarly, the levels of IL-10 and TNF-α in 60 cases of CP and 20 cases of control EPS were detected by double antibody sandwich ELISA. The ELISA reagent was purchased from Beijing Jingmei Biological Engineering Co, Ltd (Beijing, China). The *A* value was read at 450 nm using the fully automatic microplate reader, and a standard curve was drawn. The corresponding TNF-α concentration was calculated on the standard curve based on the *A* value of each specimen.

2.3. Statistical treatment

All statistical analyses were conducted using the IBM SPSS ver 20.0 (IBM, Armonk, NY) software. Categorical variables were compared using Pearson Chi-squared test. The paired mean values were also compared using the nonparametric Mann–Whitney *U* test. Partial correlation coefficient was used to assess the association among the IL-10, TNF-α, and PSEP levels in the CP and control groups; $P < .0125$ was considered statistically significant. The area under a receiver operating characteristic (ROC) curve was used to assess the specificity and sensitivity of the obtained IL-10, TNF-α, and PSEP measurements to compare their ability to diagnose CP.

3. Result

The baseline characteristics of the CP group and the control group are included in Table 1. There was no difference in age and body mass index (BMI) distribution between the CP group and the control group. PSEP levels in the NIH II, NIH IIIa, and NIH IIIb groups were 3.19 ± 3.04, 2.90 ± 2.83, and 2.85 ± 2.94 ng/mL, respectively. Compared with the 0.90 ± 0.86 ng/mL of the control group, the PSEP levels of the NIH II, NIH IIIa, and NIH IIIb groups in the CP group were significantly higher than in the control group ($P < .001$), but no difference in PSEP was found between the CP groups ($P = .60$, $P = .30$, $P = .63$). Similarly, the levels of TNF-α in the NIH II, NIH IIIa, NIH IIIb, and normal controls were 117.57 ± 39.19, 96.03 ± 34.69, 37.09 ± 10.26, and 25.4 ± 10.70 pg/mL, respectively. The IL-10 levels were 358.10 ± 34.04, 344.87 ± 32.35, 268.67 ± 35.7, and 221.34 ± 35.19 pg/mL, respectively. The levels of TNF-α and IL-10 of EPS in the patients with NIH II and NIH IIIa were significantly higher than those of the NIH IIIb group and the control group ($P < .001$), but there was no significant difference between the NIH IIIb group and the control group ($P = .18$, $P = .28$). Similarly, there was no significant difference between the NIH II and NIH IIIa groups ($P = .09$, $P = 1.00$; Table 1, Fig. 1).

After adjusting for age and BMI in a multivariate analysis, partial correlation coefficient analysis showed a positive correlation between IL-10 and TNF-α in the patients with EPS ($r = 0.578$, $P < .001$; Fig. 2). There was a positive correlation between PSEP and TNF-α and IL-10 ($r = 0.624$, $P < .001$; $r = 0.501$, $P < .001$, respectively; Fig. 2). To further evaluate the predictive ability of IL-10, TNF-α and PSEP to CP, the ROC curves and areas of area under the curve (AUC) analysis of IL-10, TNF-α and PSEP in patients with NIH II were 1.00, 1.00, and

Table 1

Baseline characteristics of the patients with CP and controls.

Characteristic	CP			Control
	NIH-II	NIH-IIIa	NIH-IIIb	
Age, yrs	35 ± 13	37 ± 12	35 ± 12	37 ± 8
BMI, kg/m^2	25 ± 3	24 ± 2	25 ± 3	23 ± 2
PSEP, ng/mL	3.19 ± 3.04*	2.90 ± 2.83*	2.85 ± 2.94*	0.90 ± 0.86
TNF-α, pg/mL	117.57 ± 39.19*,†	96.03 ± 34.69*,†	37.09 ± 10.26*	25.4 ± 10.70
IL-10, pg/mL	358.10 ± 34.04*,†	344.87 ± 32.35*,†	268.67 ± 35.7*	221.34 ± 35.19

BMI = body mass index, CP = chronic prostatitis, IL-10 = interleukin-10, NIH = National Institutes of Health, PSEP = prostatic exosomal protein, TNF-α = tumor necrosis factor alpha.

* Other groups comparison to control group, $P < .05$.

† Other groups comparison to NIH-IIIb group, $P < .05$.

0.779, respectively; Youden index reached a maximum at a cut-off value of 296.63 pg/mL, 62.49 pg/mL, 0.97 ng/mL, respectively. The ROC curves and areas of AUC analysis of IL-10, TNF-α, and PSEP in patients with NIH IIIa were 0.99, 1.00, and 0.770, respectively; Youden index reached a maximum at a cut-off value of 284.20 pg/mL, 42.07 pg/mL, and 1.03 ng/mL, respectively. The ROC curve and area of AUC analysis of IL-10, TNF-α, and PSEP in patients with NIH IIIb were 0.81, 0.80, and 0.745, respectively. Youden index reached a maximum at a cut-off value of 226.66 pg/mL, 26.46 pg/mL, and 1.00 ng/mL, respectively (Fig. 3).

4. Discussion

The CP is one of the most common diseases in adult males, and it seriously affects the physical and mental health and life quality of the patients. It is a common but very confusing urologic disease.[7] The rate has been increasing year by year, and the patients are gradually becoming younger.[8] Long-term prostatitis will affect male sexual function and is often associated with erectile dysfunction.[9–11] Lotti et al[12] reported that infertile men had higher symptoms of prostatitis than fertile men. The etiology and pathogenesis of CP are very complicated. The clinical symptoms are related to the season, diet, sexual activity, genitourinary tract

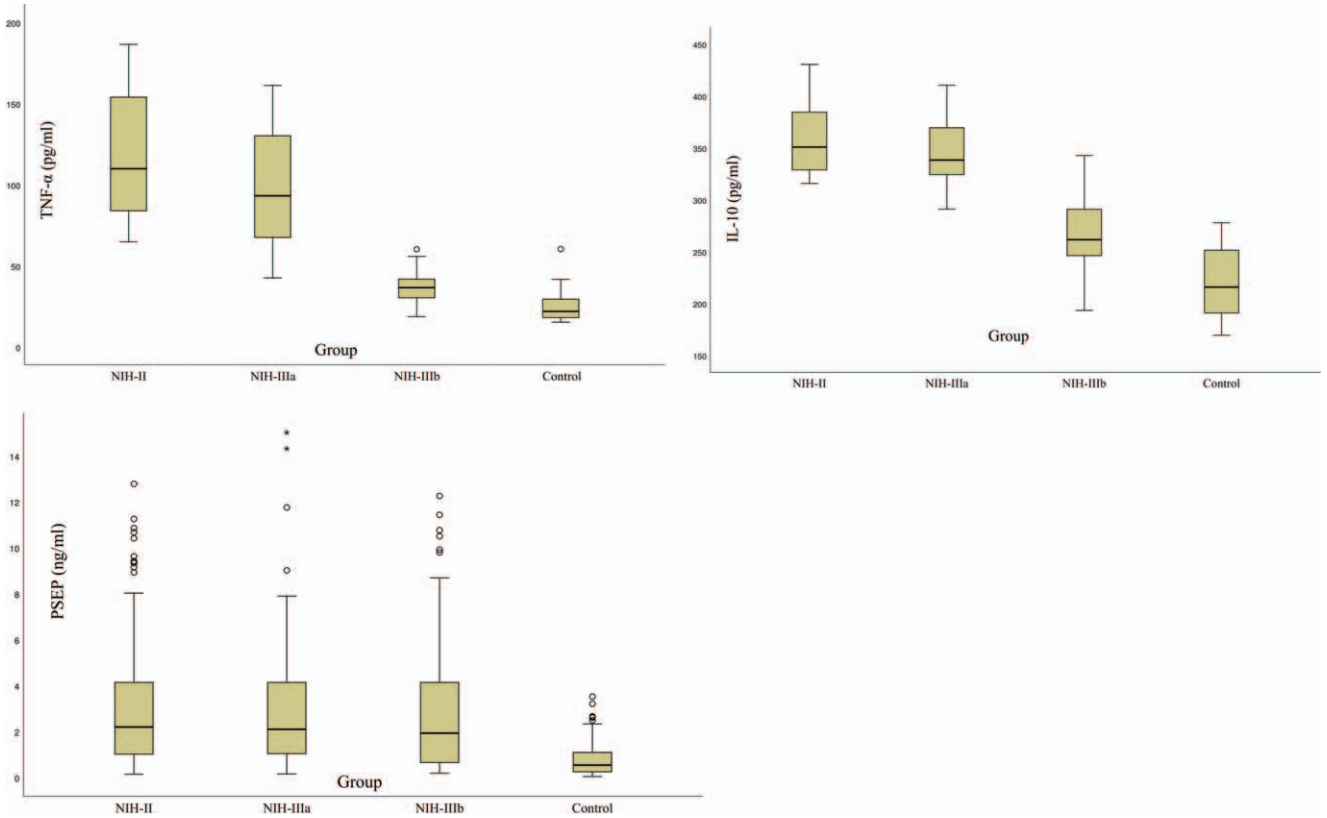

Figure 1. Compared with the control group, the prostatic exosomal protein (PSEP) levels of the National Institutes of Health (NIH) II, NIH IIIa, and NIH IIIb groups in the chronic prostatitis (CP) group were significantly higher than in the control group ($P < .001$), but no difference in PSEP was found between the CP groups ($P = .60$, $P = .30$, $P = .63$). The levels of tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) of expressed prostatic secretions (EPS) in the patients with NIH II and NIH IIIa were significantly higher than those of the NIH IIIb group and the control group ($P < .001$), but there was no significant difference between the NIH IIIb group and the control group ($P = .18$, $P = .28$).

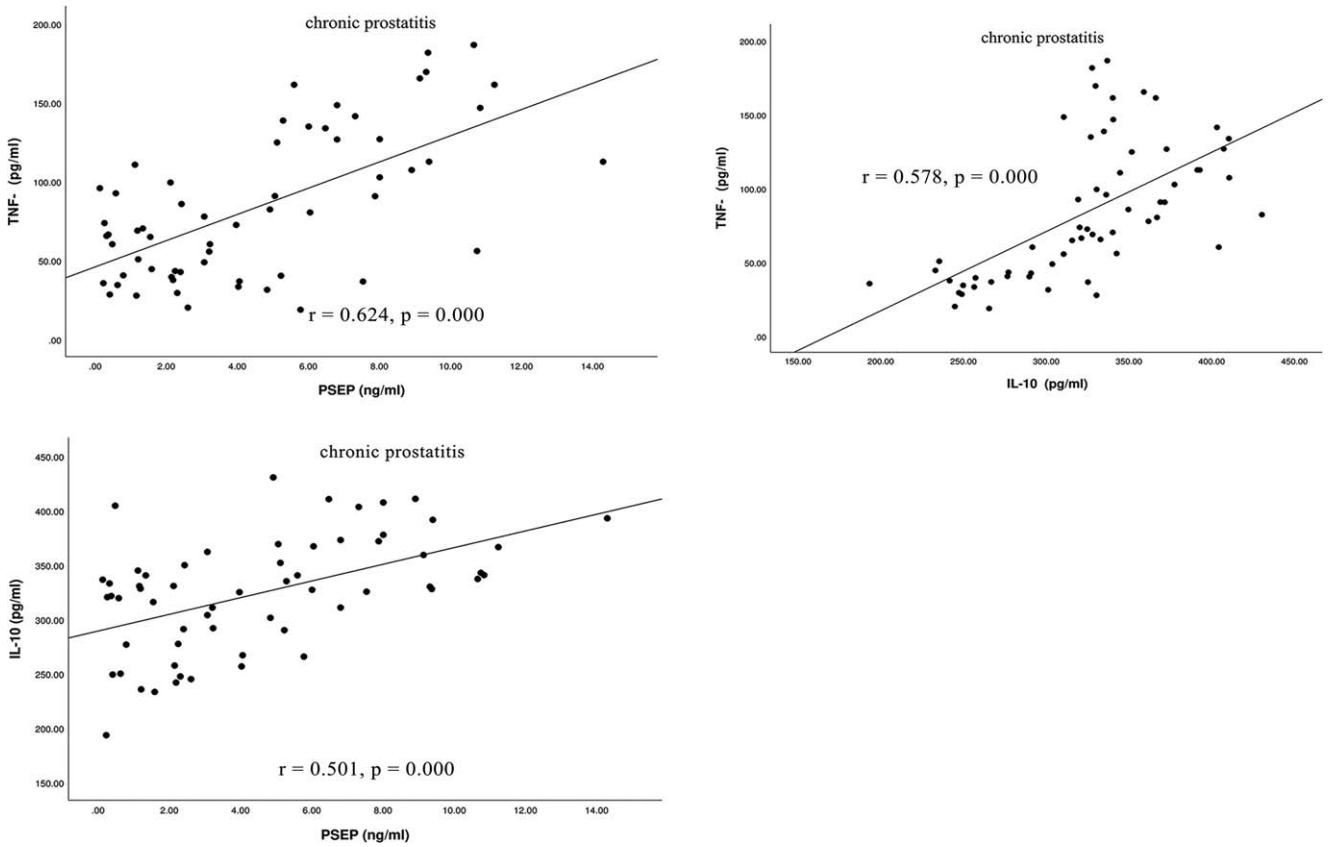

Figure 2. Correlation between prostatic exosomal protein (PSEP), tumor necrosis factor alpha (TNF-α), and interleukin-10 (IL-10) in chronic prostatitis. (1) There was a positive correlation between PSEP and TNF-α ($r = 0.624$, $P < .001$). (2) There was a positive correlation between PSEP and IL10 ($r = 0.501$, $P < .001$). (3) There was a positive correlation between IL-10 and TNF-α ($r = 0.578$, $P < .001$).

inflammation, and mental psychology. Many other conditions may present the same symptoms as CP, such as pelvic organ cancer, prostate abscess, urine tract infection, urethral stricture, benign prostatic enlargement, urinary calculi, epididymal orchitis, and bladder dysfunction,[13] which further increases the difficulty of CP diagnosis. Laboratory tests play an important role in the diagnosis of CP. The current methods for its clinical diagnosis include prostate gut diagnosis, routine EPS examination, routine urinalysis, urine sediment examination, pathogen examination, and B-ultrasound, computed tomography, magnetic resonance imaging, and other imaging examinations.[14,15] It can be regarded as a symptomatic disease. The patient often complains of more symptoms with no specificity, clinical diagnosis is often too subjective, and providers can have limited laboratory and imaging methods.[16,17] Also, as the white blood cell count in EPS is not related to the severity of the subjective symptoms of the patient, the clinician cannot evaluate the patient's condition, which presents great challenges in clinical work. Thus, we urgently need to find a more representative diagnostic marker for CP.

In recent years, the role of cytokines as the main regulatory mediator in the process of inflammation in the pathogenesis of CP has received much attention.[18] The interaction and equilibrium relationship between these cytokines may ultimately affect the process and outcome of inflammation.[19] Studies[20] have shown that the therapeutic response is related to the expression of cytokines. Compared with white blood cells, cytokines can respond to patients' condition changes sooner and more accurately. On the contrary, when the CP tissue is infiltrated by inflammatory cells, various active substances and chemokines are released, and an active substance called prostatic corpuscle is secreted into the male reproductive tract through the anatomical channel.[21] Prostate corpuscle has multiple physiologic functions. With a combination of CD59, CD52, and CD55, a series of biochemical reactions can protect sperm in an acidic environment, delay acrosome reaction, and enhance sperm motility. The prostatic corpuscle also inhibits the activity of nicotinamide adenine dinucleotide phosphate enzymes in polymorphonuclear (PMN) leukocytes, resulting in a decrease in reactive oxygen species (ROS),[22,23] and has antiviral and antibacterial characteristics. ROS is the main cause of idiopathic male infertility. Forty percent of semen samples from infertile patients have increased ROS, and prostatic bodies inhibit the activity of nicotinamide adenine dinucleotide phosphate oxidase of PMN through transferring cholesterol and sphingomyelin from the prostate to the cell membrane, thereby reducing ROS response.[24] The exocytic protein of the prostatic body is secreted into the male reproductive tract, the urethra, and into the urine through an anatomical channel. In recent years, antiprostaglandin antibodies have also been reported as markers for the diagnosis of prostate cancer and metastatic prostate cancer.[25] Therefore, we have reason to believe that PSEP are potential diagnostic markers for CP. In this study, we examined TNF-α, IL-10, and PSEP levels of urine in ESP and evaluated their clinical value as diagnostic markers for CP.

The TNF-α is a monokine that increases the phagocytic capacity of neutrophils; enhances antibody-dependent cell-

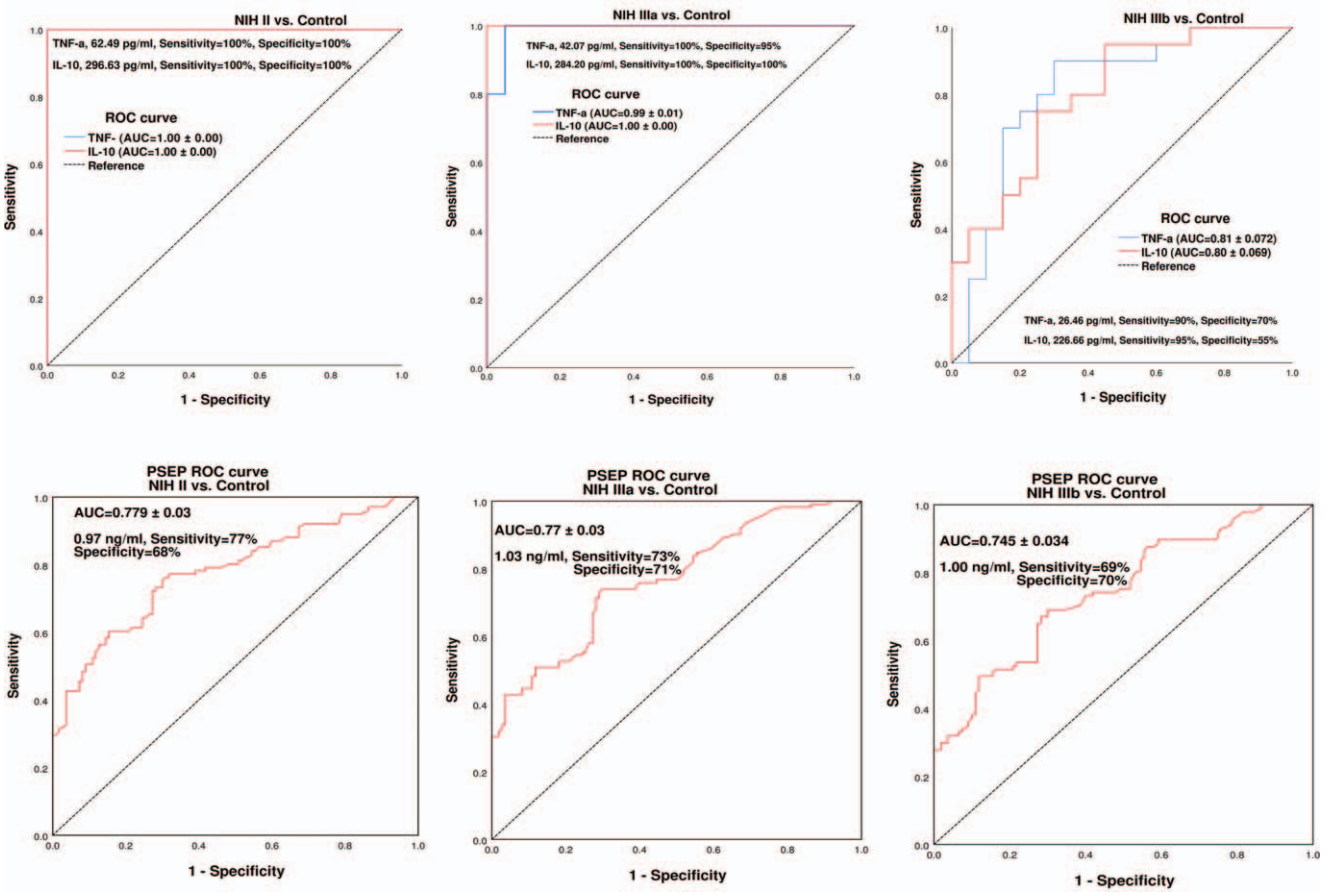

Figure 3. The predictive value of urinary prostatic exosomal protein (PSEP) and seminal interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-α) levels in the diagnosis of chronic prostatitis with National Institutes of Health (NIH) II, NIH IIIa, and NIH IIIb.

mediated cytotoxicity; promotes the expression of endothelial cells, major histocompatibility antigen I, and intercellular adhesion molecule-1; and promotes the adhesion of neutrophils to endothelial cells, thereby stimulating the local inflammatory response and promoting the inflammatory response. IL-10 is a multicell, multifunctional cytokine that regulates cell growth and differentiation, participates in inflammatory responses and immune responses, inhibits inflammatory responses, and promotes tissue repair and regeneration as an antiinflammatory cytokine. The results of this study showed that the levels of antiinflammatory cytokine IL-10 and proinflammatory cytokine TNF-α in the EPS of patients with NIH II and NIH IIIa CP were higher than those of the NIH IIIb and control groups, similar to the results of other researchers,[26,27] suggesting that TNF-α and IL-10 may be involved in the pathogenesis and immunopathologic processes of prostatitis. Moreover, the cut-off values of TNF-α in patients with NIH-II, NIH IIIa, and NIH IIIb were 62.49, 42.07, and 26.46 pg/mL, respectively. The cut-off values of IL-10 in patients with NIH-II, NIH IIIa, and NIH IIIb were 296.63, 284.20, and 226.66 pg/mL, respectively. Thus, it can provide a good basis for various types of diagnosis of patients with CP. He et al[28] showed that the levels of TNF-α and IL-10 in EPS of patients with CP were NIH II > NIH IIIa > NIH IIIb, which was consistent with our results. While there was no significant difference in the expression of TNF-α and IL-10 in the EPS of patients with NIH II and NIH IIIa CP, it is speculated that the pathologic changes and inflammatory reactions of NIH II and NIH IIIa CP may be the same, and the pathogeny and pathogenesis may be similar. In fact, the incidence of some patients with NIH IIIa CP may be related to microbial infections that have not been cultivated or are not yet recognized by conventional methods, which can be confirmed by the results of a method for the determination of endotoxin levels in EPS and initial urine after prostate massage in patients with CP,[29] or detection of gram-negative bacteria 16S rRNA in EPS by the polymerase chain reaction method.[30] All these techniques are needed to find better indicators. Similarly, there was no significant difference in the levels of TNF-α and IL-10 between the patients with NIH IIIb and the control group, but there was a statistically significant difference between patients with NIH IIIb and patients with NIH II and NIH IIIa CP. It is speculated that the prostate of the NIH IIIb CP may have small lesions. Also, factors other than the prostate are involved, such as the muscles and tension of the muscles surrounding the prostate, as well as mental and psychologic abnormalities, but they do not cause excessive TNF-α and IL-10 to participate in inflammation process. Therefore, TNF-α and IL-10 may be simple markers of CP II and IIIa, while may not distinguish IIIb from healthy controls reliably. In the results of this study, the levels of urinary PSEP were compared between groups. The comparison showed that the levels of PSEP detected in the subgroups of patients with CP were significantly higher than those in the control group, but did not seem to be able to identify CP subtypes for NIH classification, similar to the results of other researchers.[31] The results of ROC

curve analysis showed that the areas under NIH II, NIH IIIa, and NIH IIIb curves were 0.779, 0.77, and 0.745, respectively. The diagnostic efficiency is moderate. In the patients with NIH II, the PSEP cut-off value of 0.97 ng/mL corresponds to a detection sensitivity of 77% and a specificity of 68%. In the patients with NIH IIIa, the PSEP cut-off value of 1.03 ng/mL corresponds to a detection sensitivity of 73% and a specificity of 71%. In the patients with NIH IIIb, the PSEP cut-off of 1.00 ng/mL corresponds to a detection sensitivity of 69% and a specificity of 70%, suggesting that PSEP as a clinical test for CP has operability and good clinical value. In addition, previous studies have suggested that TNF-α, as a multifunctional cytokine, is involved in the pathogenesis of various diseases.[32] PSEP is only reported in prostate diseases and has excellent specificity. PSEP, TNF-α, and IL-10 can be used for clinical diagnosis of CP and may be one of the evaluation indicators for the treatment of CP, due to adding PSEP can be effectively divided into IIIb and healthy controls. Therefore, their combined detection is more helpful for the diagnosis of CP.

In addition, the correlation analysis of TNF-α, IL-10, and PSEP showed a positive correlation between TNF-α levels and IL-10 in patients with EPS ($r=0.578$, $P<.001$). It is speculated that the increase of TNF-α in the patient may lead to an increase in the secretion of the antiinflammatory cytokine IL-10, thereby suppressing excessive inflammatory reaction to achieve the balance in the body. However, in this study, its correlation coefficient was not high. We believe that this may be the result of the body's immune response; the production of cytokines is not a continuous process, and the 2 must be completely synchronized.

After adjusting for age and BMI in a multivariate analysis, there was a positive correlation between urinary PSEP levels and TNF-α and IL-10 levels in EPS ($r=0.624$, $P<.001$; $r=0.501$, $P<.001$, respectively). The correlation was not high; thus, we believe that CP is caused by a variety of factors, including complex pathologic changes such as pathogenic, immune, and neuroendocrine inflammation. The common feature of these pathologic changes is the increase of the PSEP level, but no difference was found among the subgroups of CP. It is speculated that the secretion concentration does not increase to a higher value as the severity of inflammation increases. Because when the tissue in CP is infiltrated by inflammatory cells, prostatic corpuscles are released, and the release of prostatic bodies is affected by the pathogenesis or cause of CP. If the cause or pathologic changes in patients with NIH IIIb CP are consistent with those of NIH IIIa and NIH II, they may release almost equal amounts of prostatic corpuscles, and the amount of exosomal protein excreted by the prostatic corpuscles in the urine is similar. Therefore, PSEP does not seem to be able to identify CP subtypes for NIH classification. In contrast, the expression of TNF-α and IL-10 in the EPS of patients with NIH IIIa and NIH IIIb CP was statistically significant, suggesting that they may become an important basis for the diagnosis of CP typing. The results of ROC analyses showed that the levels of TNF-α and IL-10 are sensitive and specific for the diagnosis of CP in patients. They might be suitable diagnostic markers of CP. Therefore, the combination of TNF-α, IL-10, and PSEP levels can distinguish CP types effectively and improve the accuracy of CP diagnosis.

There are some limitations in this study. First, the study was conducted in an environment with a high burden of prostatitis, which might not be applicable to other population of the results of the study; therefore, the data may not fully represent the situation in other countries. Second, the sample size of this study was limited. TNF-α and IL-10 is based on a small sample, with only 60 patients and 20 subjects testing TNF-α and IL-10 at the same time. Therefore, the cut-off value and the ROC curve are obtained from a small number, which is an important limitation of the study, and should be further verified in future research. In addition, another limitation is that the subjects of the study are not continuous. In addition, Lotti et al[16] pointed out that prostatitis-like symptoms (PLSs) represent a large group of painful and frustrating reproductive system diseases, not only from the prostate, but also from other parts of the male reproductive tract. Therefore, PLS should be distinguished from CP. However, this study did not distinguish between PLS and CP subjects very well, and the result may not fully represent patients with CP. This is another important limitation of this study.

5. Conclusion

In general, there is still no single test that is sufficient for the diagnosis of all patients with CP. The changes in TNF-α, IL-10, and PSEP levels play an important role in the pathogenesis of prostatitis, and they can be used as a basis for the diagnosis and classification of prostatitis. If one could measure TNF-α and IL-10 in the clinical laboratory, they can be an easy marker for CP types II and IIIa, but may not reliably distinguish IIIb from healthy controls. The addition of PSEP can potentially distinguish IIIb from healthy controls. When the diagnosis is difficult, they can be combined with each other, providing the doctor with CP typing diagnosis information that is more accurate, so that patients can receive effective treatment in the shortest possible time.

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