A Urine-Based Biomarker for Chronic Prostatitis/Chronic Pelvic Pain Syndrome: A Retrospective Multi-Center Study

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Abstract

Prostatitis, especially Chronic Prostatitis (CP) or Chronic Pelvic Pain Syndrome (CPPS) is one of the most common diseases of male urinary-genital system. Not only CP/CPPS has detrimental effects on male urinary and reproductive functions, but it can also result in strong mental distress because of its prolonged disease course. There are currently no objective diagnostic criteria for CP/CPPS and no accepted therapies that cure the disease. Its diagnosis depends heavily on self-described symptoms and a questionnaire survey for National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) as well as the exclusion of other urinary tract diseases. Therefore, a simple and efficient biological surrogate would significantly improve the diagnosis and aid in drug development and optimized treatments. PSEP (Prostate Exosomal Protein)-ELISA assay is a recently developed test that can quantify PSEP from the void urine. Multi-center clinical studies validated that CP/CPPS patient’s present elevated PSEP level in urine when compared to the healthy men. Our previous work showed that the content of PSEP in chronic prostatitis sample was greater than 1.2 ng/ml which was much higher than the normal control. In this study, we further investigated the relationship between PSEP in urine and expressed prostatic secretion (EPS) indexes as well as NIH-CPSI in CP/CPPS patients. From the claims data obtained from the three hospitals, we identified 372 patients with chronic prostatitis diagnosed from 2015 to 2018. Controls comprised 60 men randomly selected from health examination center in these hospitals. All samples conform to the ethical requirements of the hospital. Our study demonstrated a correlation between the increase of PSEP level and NIH-CPSI scores. Also the correlation was found between the PSEP level and EPS indexes. These findings highlight the potential of PSEP as a viable indicator of symptomatic progression of CP/CPPS. Applications of PSEP assay may guide drug discovery and lead to the better treatment to improve patient’s quality of life.

Keywords: Chronic prostatitis (CP); Prostate exosomal protein (PSEP); Chronic pelvic pain syndrome (CPPS); Urine-based biomarker

Introduction

Chronic Prostatitis (CP) or Chronic Pelvic Pain Syndrome (CPPS) is one of the most common diseases in young and middle-age men and accounts for up to 30% of the outpatient male seen in the urological clinics [1]. On the basis of epidemiological survey, 4.5~10% of male population presents symptoms of prostatitis worldwide and 50% of men suffer from prostatitis at some points during their life time [2,3]. Thus, CP/CPPS is of paramount importance as a medical problem in international health care. However, despite the intense research in the past decades, the etiology and pathogenesis of CP/CPPS is still unclear. In addition, the clinical manifestation of CP/CPPS lacks specificity making clinical diagnosis and treatment very challenging [4,5].

Currently, the diagnosis of CP/CPPS has included a combined process of recording clinical symptoms and signs, routine urine test, or culture as well as the Express Prostatic Secretion (EPS) which can be obtained by performing a rectal exam with massage on the prostate [6]. But this is a clinical process which requires qualified doctor to operate and often disturb to the patients. In addition, EPS index may exclude other potential pelvic pain associated urological disorders. Most practice clinics and hospitals in our country (for example, the Jinling hospital in Nanjing; the Taicang people’s hospital and Military general hospital in Jinan) carry out the National Institutes of
Health Chronic Prostatitis Symptom Index (NIH-CPSI) to document the patient symptoms and responses to diagnose CP/CPPS. In this process, the patient has to answer many questions. The doctor should calculate the NIH-CPSI score according to the medical history and clinical symptoms. Therefore, it is quite urgent to identify and introduce a viable tool of CP/CPPS surrogate for diagnosis.

In addition, studies have indicated increased risks of Prostate Cancer (PCa) for men with history of prostatitis compared with that of the case control. For example, Tomas et al found the atypical hyperplasia in epithelial cells with dark, swelling, and prominent nucleoli in the tissue slide showing lesion of inflammatory atrophy. Inflammatory atrophy can provide a favorable breeding ground for PCa development [7].

Exosomes are small, membrane-bound storage vesicles that mediate transport of a cytosolic cargo between the cells and to the extracellular space [8]. Exosomes are produced in many cell types including the prostate epithelial cells where they are termed prostasomes [9]. They can also be excreted to the interstitial tissue compartments when infiltrating leucocytes accumulate in response to inflammation. Thus, prostasomes can be found in seminal plasma and urine [10]. Prostasomes have been reported to elicit antioxidant effects, antibacterial activity, and immunomodulation [11,12]. It has been proposed that prostasomes may have the ability to reduce the production of Reactive Oxygen Species (ROS) [13]. Studies also suggested that prostasomes inhibit the NADPH oxidase activity of polymorph nuclear neutrophils by lipid transfer from prostasomes to the plasma membrane of these cells [14]. The molecular composition of human prostasomes is varied and consists of hundreds of known and unknown proteins. Prostate diseases such as prostate cancer, Benign Prostatic Hyperplasia (BPH), and prostasitis present unique phenotypes at the level of their respective prostasomal proteomes [15].

Recently, antibodies against human prostasomes were generated and found to be reactive to urine samples of CP/CPPS patients. The proteins that are immune reactive to the antibodies were designated as Prostatic Exosomal Proteins (PSEPs) [16,17].

A multi-center clinical trial performed in China indicated that CP/CPPS patients present elevated PSEP in the void urine when compared to the healthy men [18]. Subsequent applications of PSEP test confirmed the utility in many clinics across China; however, these applications have not addressed the relationship between PSEP test and current methods of diagnosing CP/CPPS. In this study, we intended to be the first to elucidate the potential relationship between PSEP in urine samples and EPS indexes such as White Blood Cells (WBC) and lecithin corpuscles as well as NIH-CPSI. The results were calculated respectively. Contingency tables and Spearman’s correlation coefficient were used to test for independence between PSEP positive/negative status and concentration with Chi-square test statistics by SAS9.4 (The SAS software was developed by The State University of North Carolina, U.S.A in 1966) for each individual factor including WBC and lecithin corpuscle and NIH-CPSI. Data were stratified by WBC and lecithin corpuscle in secretion and NIH-CPSI respectively according to different classification methods. The mean of PSEP concentration and detection rate of PSEP were calculated respectively. Contingency tables and Spearman’s correlation coefficient were used to test for independence between PSEP positive/negative status and concentration.

Statistical analysis

The statistical analysis was performed in a blinded manner. For the cross-sectional study analysis, a database was established to collect all information from each patient including age, routine urinary test, EPS such as WBC and lecithin corpuscle in secretion as well as NIH-CPSI. Data were stratified by WBC and lecithin corpuscle in secretion and NIH-CPSI respectively according to different classification methods. The mean of PSEP concentration and detection rate of PSEP were calculated respectively. Contingency tables and Spearman’s correlation coefficient were used to test for independence between PSEP positive/negative status and concentration with Chi-square test statistics by SAS9.4 (The SAS software was developed by The State University of North Carolina, U.S.A in 1966) for each individual factor including WBC and lecithin corpuscle and NIH-CPSI. Data were stratified by counting method to minimize potential confounding factors when testing for association between PSEP and CP/CPPS status. The differences were considered significant when p<0.05. We conduct power analyses for the assessment of our sample size by G power software.

Results

Relationship between urine PSEP level and EPS-WBC number with “+/-” as indicator of disease severity

All 372 patients were documented with EPS-WBC number in their Case Report Forms (CRFs). They were stratified by this method
They were divided into different groups according to their WBC number in EPS. WBC number less than 9 under the high power microscope is considered as negative or set as ±; WBC number 10-20 is set as +; WBC number 21-30 is set as ++; WBC number 31-40 is set as +++; WBC number >40 is set as ++++. As is shown in Table 1, with the increase of EPS-WBC number, the positive rate of PSEP showed a trend of increase. The mean PSEP concentration appeared to increase as well. PSEP concentration in urine sample change significantly when we analyzed the dataset with Contingency tables chi-square test (χ²=13.200, p=0.01). Spearman’s correlation coefficient showed a significant rank correlation between EPS-WBC and PSEP concentration either (rs=0.183, p<0.001). These data suggested that, in the current cohort of 372 patients, there was statistically significant correlation between the number of WBC and the concentration of PSEP in urine of CP/CPPS patients.

Relationship between urine PSEP level and EPS-lecithin corpuscles

Although the vitality EPS examination has been questioned in clinical practice, EPS is still widely used clinically because there is no ideal specific diagnostic marker. We therefore examined EPS-lecithin corpuscles for all patients. All 372 patients had records of EPS-lecithin corpuscle in their CRFs. They were stratified by the grade of EPS-lecithin corpuscle density as show in Table 2. In the normal EPS, a full field of lecithin corpuscles was being seen under the high power microscope, which was designated as ++++. The density of EPS-lecithin corpuscles lower than 50% (++) per vision field under the high power microscope is viewed as a sign of CP/CPPS in urological clinics. Form Table 2, the data showed that there was no statistical significance (χ²=0.003, p=0.999) between the density of lecithin corpuscles and PSEP concentration in the urine of CP/CPPS patients when we analyzed them with contingency tables chi-square test. Also the Spearman’s correlation coefficient showed no significant rank correlation between the two either (rs=0.001, p=0.994).

Relationship between urine PSEP level and NIH-CPSI

The chronic prostatitis symptom index developed by the NIH of United States (NIHCPSI) is an established scoring method to record the symptoms of the patients. According to the severity of symptoms, NIH-CPSI is divided into mild (1-14 points), moderate (15-29 points) or severe (30-43 points) [19]. In General, increases of NIH-CPSI were used as the indication that CP/CPPS becomes more pronounced with more severe symptoms.

From the 372 CP/CPPS patients, 225 patients had NIH-CPSI records. The correlation between urine PSEP level in urine and NIH-CPSI was examined. As shown in Table 3, the rising NIH-CPSI was correlated with the increase in the number of patient with positive rate of PSEP. We analyzed them with Contingency tables chi-square test (χ²=9.149, p=0.0091). Spearman’s correlation coefficient showed a significant rank correlation between NIH-CPSI and PSEP concentration (rs=0.194, P=0.0035). Although the correlation between NIH-CPSI and PSEP is weak, these data suggest that an increased PESP concentration in urine sample is correlated with the severity of symptoms.
During the chronic prostatic process, leucocytes exudate and swarm to the inflammation region, leukocytes engulf lecithin, making lecithin bodies decrease. We noticed this result and had extensive discussions with other clinicians. Nevertheless, we believe that the further study may be needed to evaluate this relationship more closely in a study with a larger sample size.

There is intense research ongoing to identify better and more practical biomarkers for CP/CPPS. Studies have shown that inflammatory cytokines in seminal plasma of CP/CPPS patients are increased significantly, such as IL-1, IL-6, IL-8, IL-10, and TNF-a [21]. Polymorph nuclear (PMN) elastase in EPS was also shown to be significantly higher in IIIa in comparison to that of IIIb [22,23]. The presence of other pathogens than bacteria, such as Chlamydia, is associated with increased WBC counts and pain severity in men with CP/CPPS. Perhaps the most thorough survey of the protein biomarkers came from mass spectrometry of seminal plasma proteome of prostatitis patients [24]. This study identified 418 proteins associated with prostatitis versus 280 present in the healthy individuals with 1662 proteins present in both populations. While these are encouraging steps towards the development of viable biomarkers for CP/CPPS, they are either derived from EPS or require sophisticated equipment to perform analysis. Therefore, they are not of practical value for general clinical application at this moment.

While PSEP-ELISA assay is simple to perform on voided urine, much remains to be learned. For example, it would be important to validate our study by more independent hospitals and clinics around the world for different ethnic background. There are reports that in some regions, particular pathogens may be more closely related with CP/CPPS. In addition, the mechanism of PSEP involvement in CP/CPPS is completely unknown. It would be important to understand why PSEP is elevated in CP/CPPS and whether it is causative or it is a mere biomarker surrogate. The understanding of PSEP biology would also be important for drug development as well. For example, there are many animal models that are currently being employed to investigate the etiology and drug response of experimental prostatitis in animal models [25]. PSEP may be used to monitor the disease course and drug treatment outcomes.

**References**


