



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

Shang XJ¹, Zhang GW¹, Hu XN², Yang JJ³, Meng Jie³ and Zeng Yan^{4*}

¹Department of Andrology, Jinling Hospital Affiliated to Nanjing University School of Medicine, China

²Department of Urology, Nanjing University of Chinese Medicine Affiliated Integrated Traditional and Western Medicine, China

³Onco Biomedical technology (Suzhou) CO. LTD, China

⁴Department of Immunology, Jinan Military General Hospital, China

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

OPEN ACCESS

*Correspondence:

Zeng Yan, Department of Andrology,
Jinling Hospital Affiliated to Nanjing
University School of Medicine, Nanjing,
China,

E-mail: zy717@yeah.net

Received Date: 31 Aug 2018

Accepted Date: 18 Sep 2018

Published Date: 21 Sep 2018

Citation:

Shang XJ, Zhang GW, Hu XN, Yang JJ,
Jie M, Yan Z. A Urine-Based Biomarker
for Chronic Prostatitis/Chronic Pelvic
Pain Syndrome: A Retrospective Multi-
Center Study. *Ann Urol Res.* 2018; 2(2):
1016.

Copyright © 2018 Zeng Yan. This is an
open access article distributed under
the Creative Commons Attribution
License, which permits unrestricted
use, distribution, and reproduction in
any medium, provided the original work
is properly cited.

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 prostatitis 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. Our studies highlight the potential value of PSEP as an indicator for CP/CPPS symptoms and disease progression in clinical practice.

Materials and Methods

Subjects

From Sep, 2015 to May, 2018, a total of 372 patients (age ranging from 20 to 61 years old, male) were recruited and diagnosed as having CP/CPPS at the Jinling Hospital Affiliated to Nanjing University school of Medicine, Nanjing University of Chinese Medicine Affiliated Integrated Traditional and Western Medicine, Jinan Military General Hospital. Of the 372 patients, 225 underwent a NIH-CPSI questionnaire survey [19]. For inclusion into this study, CP/CPPS patients must meet the following criteria: male with CP/CPPS

history and clinical symptoms, such as urinary frequency, urgency, and retention, the inflammatory reaction or reflective (perineal pain, abdominal bulge, and discomfort). Some patients presented with premature ejaculation or other symptoms such as infertility. Upon rectal exam, CP/CPPS patients confirmed changes of EPS finding over the normal person, such as WBC and lecithin corpuscle (phosphatidylcholine) in secretion. In addition, routine urinary test or culture showed no significant anomaly of acute inflammatory cell types or other urinary tract infection. NIH-CPSI questionnaire survey was used to report pain, symptoms of abdominal discomfort, finding of urination symptom and quality of life to give rise to a total score.

All research analyses were approved by the Institutional Research Ethics Committee. The written informed consent was obtained from all individuals and Case-Report-Forms (CRF) was administered during outpatient visits to collect the information of age, routine urinary test, EPS and NIH-CPSI etc.

Sample collection

Mid segment urine samples were obtained in the morning and were immediately frozen. They were stored at -20°C until ready for use. Subjects were excluded when there were incomplete clinical data, inadequate quantity of urine samples, or grossly bloody and thick urine or with alcohol consumption.

PSEP assay

The double-blinded PSEP-ELISA assay was performed essentially as described according to the manufacturer's instruction (Onco Biomedical Technology [Suzhou] Co., Ltd) [18]. Void human urine samples were added to the 96-well micro plate trays and incubated at 37°C for one hour. After antibody incubation, the reaction was visualized by the addition of chromogenic Tetramethylbenzidine (TMB). The resulting color development indicates the amount of PSEP in urine samples. The absorbance of the samples was read at 450 nm/630 nm.

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

Table 1: Relationship between urine PSEP level and WBC numbers in EPS.

WBC grade	Case number	PSEP positive	PSEP negative	Positive rate (%)	Mean (x ± s)
WBC±	58	31	27	53.4	2.56 ± 2.62
WBC+	116	76	40	65.5	3.23 ± 3.29
WBC++	75	51	24	68	3.17 ± 2.78
WBC+++	72	55	17	76.4	4.63 ± 3.91
WBC++++	51	42	9	82.4	4.08 ± 2.78

Chi-Square test $\chi^2=13.200$, $p=0.010$

Spearman's correlation coefficient analysis $r_s=0.183$, $p<0.001$

WBC ±: WBC number less than 9/HP

WBC+: WBC number 10-20/HP

WBC++: WBC number 21-30/HP

WBC+++: WBC number 31-40/HP

WBC++++: WBC number >40/HP

Generally $P<0.05$ is statistically significant.

Table 2: Relationship between urine PSEP level and the density of EPS-lecithin corpuscles.

Lecithin grade	Case number	PSEP positive	PSEP negative	Positive rate (%)	Mean (x ± s)
+	89	61	28	68.5	3.37 ± 3.08
++	99	68	31	68.7	3.55 ± 3.38
+++	117	80	37	68.4	3.74 ± 3.48
++++	67	46	21	68.7	3.18 ± 2.69

Chi-Square test $\chi^2=0.003$, $p=0.999$

Spearman's correlation coefficient analysis $r_s<0.001$, $p=0.994$

Table 3: Relationship between urine PSEP level and the NIH-CPSI.

NIH-CPSI	Case number	PSEP positive	PSEP negative	Positive rate (%)	Mean (x ± s)
≤ 15	113	70	43	61.95	3.56 ± 3.60
16-30	89	66	23	74.16	3.39 ± 3.17
>30	23	21	2	91.3	5.20 ± 3.82

Chi-Square test $\chi^2=9.149$, $p=0.0091$

Spearman's correlation coefficient analysis $r_s=0.194$, $P=0.0035$

of documenting WBCs as showed in Table 1.

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 ($\chi^2=13.200$, $p=0.01$). Spearman's correlation coefficient showed a significant rank correlation between EPS-WBC and PSEP concentration either ($r_s=0.183$, $p=0.001$). 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 ($\chi^2=0.003$, $p=0.999$) 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 ($r_s=0.001$, $p=0.994$).

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 ($\chi^2=9.149$, $p=0.0091$). Spearman's correlation coefficient showed a significant rank correlation between NIH-CPSI and PSEP concentration ($r_s=0.194$, $P=0.0035$). 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

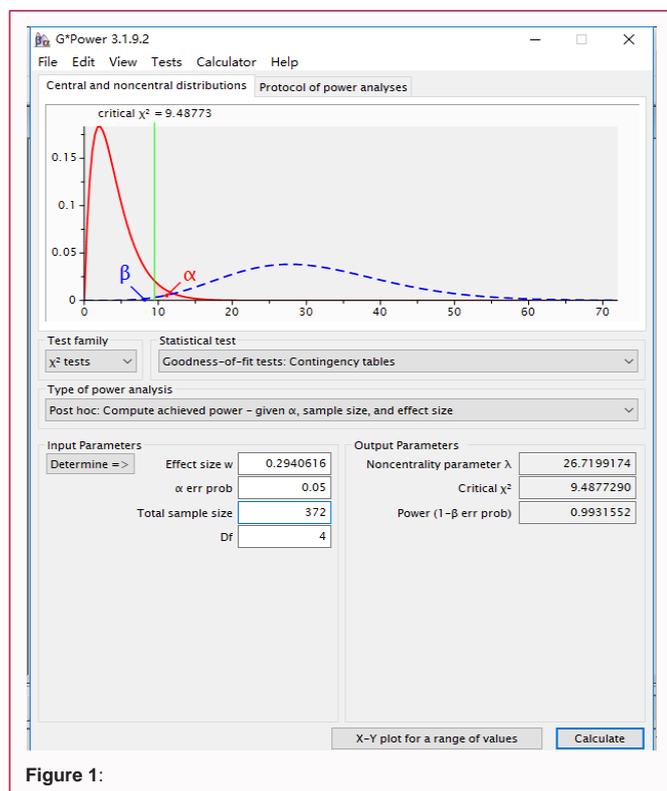


Figure 1:

severity of symptoms and the advanced stages of CP/CPPS in clinical practice.

We have conducted the power analyses by the G power software to assessment the sample size. Power ($1 - \beta$)=0.99, which means that the power of the test is very well and the sample size is enough to have the valid results (Figure 1).

Discussion

CP/CPPS is a frustrating clinical condition for both practitioners and patients. In developing countries, the situation is worse because often CP/CPPS is not correct diagnosed using a combination of multiple methodologies such as NIH-CPSI, EPS, 2- and 4 cup tests, and so on [20]. Rather, clinician experience sometimes dictates the treatment of self-described abdominal and urinary discomfort with frequent prescription of antibiotics to observe treatment response to the anticipated CP/CPPS.

The available PSEP-ELISA assay as a simple, objective, and non-invasive urine test provides the clinician a biological surrogate to assist in the diagnosis of CP/CPPS. In the past couple of years, the PSEP test became adopted in hospitals and clinics in China and received some positive responses. However, the correlation of urine PSEP level with EPS and/or NIH-CPSI was only described in meetings and conferences anecdotally. Therefore the current study is the first to validate that PSEP level is associated with the increasing WBC counts and NIH-CPSI scores. Thus, an increasing PSEP level in the urine can be an indication of severity of CP/CPPS and may guide the optimal treatment plan.

During the chronic prostatic process, leucocytes exudate and swarm to the inflammation region, leucocytes engulf lecithin, making lecithin bodies decrease.

In our current study, we do not find a strong correlation between

urine PSEP level and density of EPS-lecithin corpuscles. 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- α [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 vial 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

- Collins MM, Stafford RS, O'Leary MP, Barry MJ. How common is prostatitis? A national survey of physician visits. *J Urol.* 1998;159(4):1224-8.
- Clemens JQ, Meenan RT, O'Keefe Rosetti MC, Kimes T, Calhoun EA. Prevalence of and risk factors for prostatitis: population based assessment using physician assigned diagnoses. *J Urol.* 2007;178(4):1333-7.
- Polackwich AS, Shoskes DA. Chronic prostatitis/chronic pelvic pain syndrome: a review of evaluation and therapy. *Prostate Cancer Prostatic Dis.* 2016;19(2):132-8.
- Weidner W, Schiefer HG, Krauss H, Jantos C, Friedrich HJ, Altmannsberger M. Chronic prostatitis: a thorough search for etiologically involved microorganisms in 1,461 patients. *Infection.* 1991;19:119-25.
- Schaeffer AJ. Clinical practice. Chronic prostatitis and the chronic pelvic pain syndrome. *N Engl J Med.* 2006;355(16):1690-8.
- Nickel JC, Downey J, Hunter D, Clark J. Prevalence of prostatitis-like symptoms in a population based study using the National Institutes of Health chronic prostatitis symptom index. *J Urol.* 2001;165(3):842-5.
- Tomas D, Kruslin B, Rogatsch H, Schäfer G, Belicza M, Mikuz G. Different types of atrophy in the prostate with and without adenocarcinoma. *Eur Urol.* 2007;51(1):98-103.

8. Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. *Cell Mol Life Sci.* 2018;75(2):193-208.
9. Aalberts M, Sostaric E, Wubbolts R, Wauben MW, Nolte-t Hoen EN, Gadella BM, et al. Spermatozoa recruit prostasomes in response to capacitation induction. *Biochim Biophys Acta.* 2013;1834(11):2326-35.
10. Brody I, Ronquist G, Gottfries A. Ultrastructural localization of the prostasome-an organelle in human seminal plasma. *UPS J Med Sci.* 1983;88(2):63-80.
11. Carlsson L, Pahlson C, Bergquist M, Ronquist G, Stridsberg M. Antibacterial activity of human prostasomes. *Prostate.* 2000;44(4):279-86.
12. Skibinski G, Kelly RW, Harkiss D, James K. Immunosuppression by human seminal plasma- extracellular organelles (prostasomes) modulate activity of phagocytic cells. *Am J Reprod Immunol.* 1992;28(2):97-103.
13. Saez F, Motta C, Boucher D, Grizard G. Antioxidant capacity of prostasomes in human semen. *Mol Hum Reprod.* 1998;4(7):667-72.
14. Saez F, Motta C, Boucher D, Grizard G. Prostasomes inhibit the NADPH oxidase activity of human neutrophils. *Mol Hum Reprod.* 2000;6(10):883-91.
15. Poliakov A, Spilman M, Dokland T, Amling CL, Mobley JA. Structural heterogeneity and protein composition of exosome-like vesicles (prostasomes) in human semen. *Prostate.* 2009;69(2):159-67.
16. Lu Q, Zhang J, Allison R, Gay H, Yang WX, Bhowmick NA, et al. Identification of extracellular delta-catenin accumulation for prostate cancer detection. *Prostate.* 2009;69(4):411-8.
17. Minelli A, Ronquist G, Carlsson L, Mearini E, Nilsson O, Larsson A. Antiprostasome antibody in benign and malignant prostate disease. *Anticancer Res.* 2005;25(6c):4399-402.
18. Zeng Y, Zhang J, Chen YH, Meng J, Liu ZX, Lu Q, et al. Establishment of ELISA detection method for prostatic exosomal protein and its primary evaluation. *J Clinical Med Pharmacy.* 2015;32(10):885-8.
19. Probert KJ, Litwin MS, Wang Y, Alexander RB, Calhoun E, Nickel JC, et al. Responsiveness of the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI). *Qual Life Res.* 2006;15(2):299-305.
20. Yanqun N, Zhangqun Y, Yinghao S, Guang S, Jian H, Chuize K, et al. Manual of diagnosis and treatment of diseases in Department of Urology in China. 2014;568-569.
21. Penna G, Mondaini N, Amuchastegui S, Degli Innocenti S, Carini M, Giubilei G, et al. Seminal plasma cytokines and chemokines in prostate inflammation: interleukin 8 as a predictive biomarker in chronic prostatitis/chronic pelvic pain syndrome and benign prostatic hyperplasia. *Eur Urol.* 2007;51(2):524-33.
22. Aghazarian A, Plas E, Stancik I, Pflüger H, Lackner J. New method for differentiating chronic prostatitis/chronic pelvic pain syndrome IIIA from IIIB involving seminal macrophages and monocytes. *Urology.* 2011;78(4):918-23.
23. Zhu J, Yang C, Dong Z, Li L. The value of neutrophil elastase in diagnosis of type III prostatitis. *Urol J.* 2014;11(3):1666-72.
24. Kagedan D, Lecker I, Batruch I, Smith C, Kaploun I, Lo K, et al. Characterization of the seminal plasma proteome in men with prostatitis by mass spectrometry. *Clin Proteomics.* 2012;9(1):2.
25. Vykhovanets EV, Resnick MI, MacLennan GT, Gupta S. Experimental rodent models of prostatitis: limitations and potential. *Prostate Cancer Prostatic Dis.* 2007;10(1):15-29.