The Value of Neutrophil Elastase in Diagnosis of Type III Prostatitis

Jun Zhu, Changhai Yang, Zhichun Dong, Liming Li

Department of Urology, Tianjin Medical University General Hospital, 154 Anshan Road, Heping District, Tianjin 300052, China.

Corresponding Author:

Changhai Yang, MD
Department of Urology, Tianjin Medical University General Hospital, 154 Anshan Road, Heping District, Tianjin 300052, China.

Tel: +86 22 6553 9807
Fax: +86 22 6553 9807
E-mail: ychanghai@yahoo.com

Purpose: To explore the value and significance of neutrophil elastase (NE) in diagnosis of type III prostatitis.

Materials and Methods: The prospective study recruited 123 patients diagnosed with type III prostatitis (IIIA, 36 cases; IIIB, 87 cases) and 84 healthy controls, between April 2008 and July 2012. NE concentrations in expressed prostatic secretions (EPS), EPS routine examination, bacterial culture and The National Institute of Health Chronic Prostatitis Symptom Index (NIH-CPSI) score were detected in all the subjects. Difference of NE, CPSI score, and withe blood cell (WBC) count between 2 or more than 2 groups and relationships between NE concentrations and WBC count were all analyzed.

Results: There was significant difference in levels of NE ($P < .05$) between IIIA and IIIB groups, and obviously positive correlation between the level of NE and number of leukocyte in type IIIA prostatitis group was observed ($P < .05$). The values of CPSI score between IIIA and IIIB groups was statistically significant ($P = .037$). The levels of leukocyte mount, NE and CPSI were statistically significant between IIIA and the control group ($P < .05$). NE concentration and CPSI score were statistically significant between IIIB and control group ($P < .05$), while the numbers of leukocyte was not statistically significant ($P = .360$).

Conclusion: The level of NE in EPS is a significant indicator in diagnosis of type IIIA and IIIB prostatitis.

Keywords: prostatitis; classification; diagnosis chronic disease; leukocyte elastase; chemistry.

INTRODUCTION

Prostatitis is an inflammation of the prostate and chronic prostatitis is the most common urologic disease in men less than 50 years old, accounting for approximately 8-25% of the urology outpatients.[1,2] In the US, over 2 million patient-visits per year are a result of prostatitis.[3] Data has shown that about 50% of men suffer from prostatitis in a period of their lifetime.[4] It is not only brought kinds of discomfort to the patients, but also took significant harm to their mental health[5-8] and caused huge economic burden on public health.[9-12] Due to the complexity of its etiology and various symptoms, clinical diagnosis of the cause and therapy has been lack of effective programs and methods and the therapy effect is usually unsatisfactory. According to The National Institute of Health (NIH) classification method in 1995, type III prostatitis (chronic nonbacterial prostatitis) is the most common disease, accounting for approximately 95% of the urology outpatients.[13] It is also divided into two subtypes IIIA and IIIB according to whether there are white blood cells (WBCs) in expressed prostatic secretions (EPS). More evidences revealed that the therapy and treatment were significantly different between two subtypes of type III prostatitis.[14,15] However, in clinical treatment, IIIA and IIIB of chronic prostatitis has similar clinical symptoms and it is difficult and not effective to distinguish them by WBC count only.[16,17] Researchers tried to find other factors, in addition to leukocytes, which could effectively differentiate IIIA and IIIB prostatitis, for chronic prostatitis caused the prostate secretory abnormality on prostate secretory functions.[18-21]

In this study, we detected concentrations of the neutrophil elastase (NE) in EPS among IIIA, IIIB prostatitis patients and normal control group and compared the difference of NE concentrations in EPS among type IIIA, IIIB chronic prostatitis patients and normal control group to attempt to provide a reliable measure for distinguishing and diagnosing the type III chronic prostatitis.

MATERIALS AND METHODS

An observational prospective design was applied in this study. A total of 123 patients diagnosed with type III prostatitis were recruited to participate in the study between April
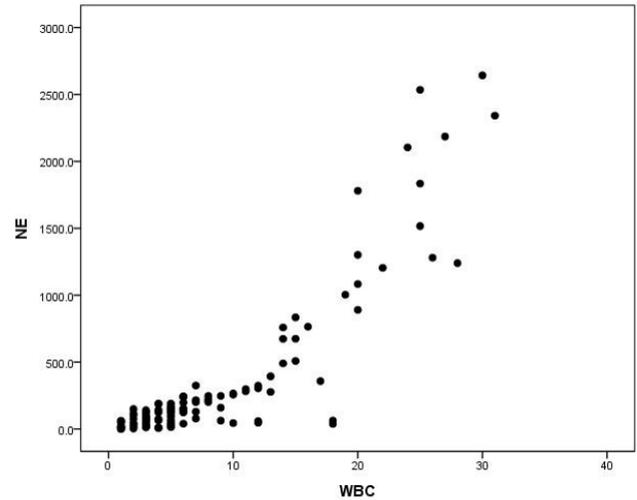
Figure . Correlation analysis of NE and WBC count between IIIA and B groups. NE concentrations was positively correlated with WBC count (Spearman's rank correlation coefficient, r = 0.596, P < .05). **Keys:** WBC, white blood cell; NE, neutrophil elastase.

2008 and July 2012. The exclusion criteria were the presence of cancer of the genitourinary tract; active urinary stone disease or herpes of the genitourinary system; perirectal inflammatory disorders; inflammatory bowel disease; a history of pelvic radiation or systemic chemotherapy; a history of intravesical chemotherapy; urethral stricture 12 French (F) or smaller; neurologic disease or disorder affecting the bladder; and prostate surgery within the past 3 months. The inclusion criteria were, patients between 18 to 50 years old to reduce the effect of age factor, the course of the disease lasted for a period of at least three months, patients receiving no antibiotic treatment for any reason for the last 4 weeks, patients whose prostatic fluid having no bacterial growth and patients having symptoms of discomfort or pain in the pelvic region. The diagnosis of patients was consistent with the NIH definition of the chronic prostatitis/pelvic pain syndrome.[22] Patients with type III prostatitis were classified as having subtype IIIA (36 cases) or IIIB (87 cases). Ejaculated samples from healthy men showed a good sperm density and progressive motility and morphology ($\geq$ 20%) and were considered normal ejaculates according to World Health Organization criteria.[23] Eighty-four normal volunteers who didn't show any signs of prostatic diseases were used as controls. They were recruited from the subjects undergoing complete history and physical examination. Informed consent was obtained from their parents. Study protocols were approved by

Table 1. Changes in IPSS and uroflowmetry parameters after treatment with tamsulosin.

Characteristics	Patients		Controls
	IIIA	IIIB	---
Number of subjects	36	87	84
Mean age, years	32	30	28.88
Course (months)			
3-6	22	52	------
> 6	14	35	
Bacterial infection			
Positive	0	0	0
Negative	36	87	84
CPPS symptom	36	87	-----
Associated symptom			
LUTS	28	48	
Non-LUTS	8	39	
CPPS treatment			
α-Blockers	28	65	
NSAID	25	48	

Keys: CPPS, chronic pelvic pain syndrome; NSAID, nonsteroidal anti-inflammatory drug; LUTS, lower urinary tract symptoms.

the Institutional Research Ethics Committee of the General Hospital of Tianjin Medical University.

A complete history and physical examination were performed, including laboratory analysis, which was microscopy and culture of the urine specimen before massage, and EPS and/or urine specimen after prostatic massage. For category IIIA the EPS and/or urine specimen after prostatic massage had to be sterile with no uropathogenic growth, and there had to be a documented inflammatory pattern on microscopy of EPS that was greater than 10 WBCs per high power field (hpf), and/or urine sediment after prostatic massage that was greater than 5 WBCs per hpf. To be classified as IIIB, the EPS and/or urine specimen after prostatic massage had to be sterile with no uropathogen growth, and there had to be no documented inflammatory pattern on microscopy of EPS that was less than 10 WBCs per hpf, and/or urine sediment after prostatic massage that was less than 5 WBCs per hpf. All patients had a complete medical history, a physical examination, and a 4-glass urinalysis, WBC counts in EPS, serum prostate specific antigen (PSA), NIH-CPSI score, and transrectal ultrasonography, according to Hochreiter and colleagues.[24]

Prostatic fluid samples were obtained at the hospital by prostatic massage, after a period of sexual abstinence of 3-5 days. The samples were collected in a sterile container and transferred to a cryovial, stored at –20°C. Standard microbial investigations (e.g., for aerobic and anaerobic bacterial infections, ureaplasma urealyticum and mycoplasma infections, chlamydia trachomatis, trichomonas vaginalis and candida infections) were performed for all prostatic fluid samples. Some of the fluid was transferred for storage at –80°C until it was used for the analysis of NE. The remaining fluid on the glass slide was placed under a coverslip and examined for WBC count in 5 fields at high power (400 ×). The average number of WBCs per hpf was recorded.

NE was determined by quantitative sandwich enzyme immunoassay [Human PMN Elastase ELISA (enzyme-linked immunosorbent assay), Ray Biotech., Inc., Minneapolis, MN, USA] according to the manufacturer's instructions. The intensity of the color was measured at 450 nm.

Statistical Analysis

Data were analyzed by using the statistical package for the social science (SPSS Inc, Chicago, Illinois, USA) version 19.0. Normally distributed continuous data are presented as means ± standard deviation (SD) and were compared using t tests. Non-normally distributed continuous data are presented as the median and range, and were compared using the rank test. Difference among 2 or more than 2 groups were

Table 2. Related data in the test groups and the control group.*

Groups	No.	WBC count	NIH-CPSI score	NE
IIIA	36	17.89 ± 6.18	24.50 ± 5.41	907.33 ± 769.70
IIIB	87	4.01 ± 2.21	21.80 ± 5.01	0.119 ± 0.009
Control	84	3.60 ± 2.21	1.72 ± 0.74	0.068 ± 0.015ᵃ

Keys: WBC, white blood cells; NIH-CPSI, National Institutes of Health-Chronic Prostatitis Symptom Index; NE, neutrophil elastase (ng/mL).
* Data are expressed as means ± SD.

assessed by using t tests, ANOVA or post hoc Dunnett's T3 tests, as required. Correlations of NE, NIH-CPSI score and WBC count were analyzed by Spearman's rank correlation coefficient. The P value < .05 was considered statistically significant.

RESULTS

Patients' Characteristics

A total of 207 participants in Tianjin Medical University General Hospital were studied. There were 36 cases of type IIIA prostatitis, 87 cases of type IIIB prostatitis and 84 cases of normal controls. The general characteristics of the recruited patients are shown in Table 1.

NE Concentrations Were Elevated in Prostatitis Patients as Compared with Controls

We summarized the WBC count, NIH-CPSI score and NE concentrations in prostatitis IIIA, IIIB and control groups, respectively (Table 2). NE concentration in patients with prostatitis IIIA displayed significantly higher as compared with that of prostatitis IIIB group (median 907.33 ng/mL, in controls vs. 94.92 ng/mL, respectively, P < .05; Table 3).

Association Analysis of NE Levels CPSI Score and WBC Count

As shown in Table 3 and Figure, the level of NE was positively correlated with WBC count (Spearman's rank correlation coefficient, $r = 0.596$, P < .05). Data revealed that there was significant differences on the values of CPSI between prostatitis IIIA and prostatitis IIIB ($P = .037$).

Test data of the experimental and control groups were also compared by SPSS 19.0. The NE in EPS of patients with prostatitis IIIA and IIIB was statistically different from that of control group (all P < .05). The CPSI score of patients with prostatitis IIIA and IIIB was also statistically different from that of control group (all P < .05). WBC count in patients with prostatitis IIIA displayed statistically significant difference as compared with that of control group (P < .05), but there was no statistical difference on WBC count between prostatitis IIIB group and control group ($P = .360$).

DISCCUSSION

It has been diagnosed as type III prostatitis that patients showing obvious symptoms of chronic bacterial prostatitis but microbiological culture results were negative.[25] However, with the improvement and optimization of culture methods, we observed the microbial growth in the EPS from patients that were traditionally diagnosed for type III prostatitis. For example, coagulase-negative cocci was difficult to grow in general medium, however, after obligate culture, it was demonstrated that coagulase negative cocci existed in the EPS of about 68% of patients with type III prostatitis, which was further to be confirmed by microscopic examination.[26] The traditional culture method had played an important role in the diagnosis and treatment of prostatitis, but it was a time-consuming and laborious process and susceptible to contaminate, especially, only a small number of microbial species were able to cultivate.[27,28] Thus, the traditional culture method played a limited role in the course of recognition about type III prostatitis related microorganisms.

Elastase is a kind of enzyme that can hydrolyze elastin in the body and named by the generated sites, such as neutrophils elastase (NE), which was present in neutrophils. Under physiological conditions, NE played an effective protection in host defense system, and its activity was strictly regulated by the inhibitors of endogenous protease. In early stage of inflammation, WBCs were the first line of defense when pathogen invaded into the body. The gathered at the site of inflammation by chemotaxis of kinds of chemokines and NE were released by neutrophils. NE can escape from the regulation

Table 3. Difference of related indicators among 2 or more than 2 groups.

Groups	IIIA/IIIB		IIIA/Control		IIIB/Control		III (A+B)/Control	
	MD (I-J)	*p*	MD (I-J)	*p*	MD (I-J)	*p*	t	*p*
WBC count	14.061	< .05	14.591	< .05	0.530	.360	6.438937	< .05
NIH-CPSI score	2.695	.037	23.762	< .05	21.067	< .05	44.99526	< .05
NE	812.4092	< .05	834.7583	< .05	22.34914	.097	5.125425	< .05

Keys: WBC, white blood cells; NIH-CPSI, National Institutes of Health- Chronic Prostatitis Symptom Index; NE, neutrophil elastase; MD, mean difference.

of multiple protease inhibitors at the inflammation site. The balance was broke out between NE and its endogenous protease inhibitors and NE maintained the activated state, resulting in the damage and dysfunction of the tissues and organs. Recent studies have found that infectious NE levels in EPS of prostatitis patients were significantly higher than that in patients with non-infectious prostatitis, which demonstrated that NE could distinguish infectious and non-infectious prostatitis to a certain extent.[29] Some researchers reported that compared with C3, the terminal complement complex and plasma ceruloplasmin, NE was the best indicator to diagnose of the acute and chronic urethritis/prostatitis.[30,31]

Simultaneously, NE also can be used as a detected indicator of clinical efficacy. NE concentration rapidly increased to more than 290 ng/mL from the normal level at the stage of acute inflammation until the inflammation was cured. Otherwise, the concentration of NE would maintain a high level.[32] Decreased NE levels showed that anti-inflammatory treatments were effective. If NE levels had not declined, the treatment plan should be redesigned, prompting doctors to find out the other chronic inflammatory lesions.[11]

In the study, we identified a NE cutoffs (246.4 ng/mL) for diagnosing type prostatitis patients, which is slightly lower than the revised cutoff of 280 ng/mL for diagnosing inflammatory disease as depicted in the literature.[30] This findings may be the results that NE secretion is different in various tissues and organs or affected by different individuals or populations. Besides, we measured the NE concentration in EPS of patients with type IIIA and IIIB prostatitis. We found that NE concentrations in EPS of prostatitis IIIA was significantly higher than that of prostatitis IIIB. NE concentrations in EPS of patients with type IIIA and IIIB prostatitis were both significantly higher than that in the normal control group. These results indicated that patients with type IIIA and type IIIB prostatitis were both infected with microorganisms. The infection in patients with type IIIA prostatitis was more serious than that with type IIIB prostatitis. When tissues were infectious, NE was released by neutrophils. Thus, we can make a conclusion that antibiotic treatment may be a possible method for the type III prostatitis, especially type IIIA chronic prostatitis. In addition, we compared the correlation between NE concentration and leukocytes in EPS of patients and found that NE concentration positively correlated with leucocyte amount, which indicated that NE can be used as a meaningful indicator in the diagnosis of type III prostatitis, and also demonstrated that the levels of NE concentration were able to reflect the severity of chronic prostatitis.

However, we also observed that higher NE concentration accompanied with few leucocyte amount or low CPSI score which was uncommon, but with some meaning. For example, the results that high NE concentration accompanied with few WBCs were observed and it was largely because prostate duct was blocked that the WBCs at inflammatory sites could not spread into EPS and were not calculated, but NE could spread

Table 4. Correlation analysis of WBC count, NIH-CPSI score and NE between IIIA and IIIB groups.

Parameters	WBC/NIH-CPSI score	WBC/NE	NIH-CPSI score/NE
r	0.402	0.596	0.382
P	< .05	< .05	< .05

Keys: WBC, white blood cells; NIH-CPSI, National Institutes of Health- Chronic Prostatitis Symptom Index; NE, neutrophil elastase; r, Pearson correlation coefficient.

into EPS. Currently, the main criteria of prostatitis in clinical diagnosis were that WBC count was more than 10 in EPS, but it was not accurate only relying on one diagnostic indicator. Diagnosis of prostatitis should be a systematic work and all of the treatment should be checked including the symptoms, pathological examination (EPS microscopy), physiological and biochemical test, bacteriological examination, ultrasound examination and urodynamic examination and etc. All of the diagnostic treatments should be a comprehensive, objective and scientific on prostatitis. In conclusion, NE concentration in EPS of patients can be used as a reference for diagnosis of type III A and B prostatitis. With the promotion of inspection techniques, such as protein chip, the detection of NE concentration would be more convenient and may become one of the diagnostic indicators of type III prostatitis.

CONCLUSION

The limitation of our study is the lack of a large group of patients which may create the risk of a type II statistical error, but despite that, the study has shown that the level of NE in EPS is a significant indicator in diagnosis of type IIIA and IIIB prostatitis. There was a positive correlation between NE concentration and the leukocyte mount, which demonstrated the levels of NE in EPS could reflect the severity of type III chronic prostatitis.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Krieger JN, Riley DE, Cheah PY, Liong ML, Yuen KH. Epidemiology of prostatitis. New evidence for a world-wide problem. World J Urol. 2003;21:70-4.

2. Rizzo M, Marchetti F, Travaglini F, Trinchieri A, Nickel JC. Prevalence, diagnosis and treatment of prostatitis in italy: A prospective urology outpatient practice study. BJU Int. 2003;92:955-9.

3. Izadyar F, Den Ouden K, Stout Ta, et al. Autologous and homologous transplantation of bovine spermatogonial stem cells. Reproduction. 2003;126:765-74.

4. Kulchavenya E, Azizoff A, Brizhatyuk E, et al. Improved diagnostics of chronic inflammatory prostatitis. Minerva Urol Nefrol. 2012;64:273-8.

5. Nickel JC, Tripp DA, Chuai S, et al. NIH-CPCRN Study Group. Psychosocial variables affect the quality of life of men diagnosed with chronic prostatitis/chronic pelvic pain syndrome. BJU Int. 2008;101:59-64.

6. Tripp DA, Nickel JC, Wang Y, et al. National Institutes of Health-Chronic Prostatitis Collaborative Research Network (NIH-CPCRN) Study Group. Catastrophizing and pain-contingent rest predict patient adjustment in men with chronic prostatitis/chronic pelvic pain syndrome. J Pain. 2006;7:697-708.

7. Clemens JQ, Brown SO, Calhoun EA. Mental health diagnoses in patients with interstitial cystitis/painful bladder syndrome and chronic prostatitis/chronic pelvic pain syndrome: A case/control study. J Urol. 2008;180:1378-82.

8. Lee KS, Choi JD. Chronic prostatitis: Approaches for best management. Korean J Urol. 2012;53:69-77.

9. Calhoun EA, McNaughton Collins M, Pontari MA, et al. Chronic Prostatitis Collaborative Research Network. The economic impact of chronic prostatitis. Arch Intern Med. 2004;164:1231-6.

10. Clemens JQ, Markossian T, Calhoun EA. Comparison of economic impact of chronic prostatitis/chronic pelvic pain syndrome and interstitial cystitis/painful bladder syndrome. Urology. 2009;73:743-6.

11. Schaeffer AJ. The economic impact of chronic prostatitis. J Urol. 2005;173:844.

12. Duloy AM, Calhoun EA, Clemens JQ: Economic impact of chronic prostatitis. Curr Urol Rep. 2007;8:336-9.

13. Shoskes DA. The challenge of erectile dysfunction in the man with chronic prostatitis/chronic pelvic pain syndrome. Curr Urol Rep. 2012;13:263-7.

14. Nickel JC, True LD, Krieger JN, Berger RE, Boag AH, Young ID. Consensus development of a histopathological classification system for chronic prostatic inflammation. BJU Int. 2001;87:797-805.

15. Kogan MI, Belousov, II, Shornikov PV. Neurophysiologic evaluation of patients with chronic prostatitis (iii b chronic pain syndrome). Urologiia. 2012:37-42.

16. Wise GJ. Prostatitis: Myths and realities. Urology. 1999;53:240-1.

17. Nickel JC. Prostatitis: Myths and realities. Urology. 1998;51:362-6.

18. Prando A, Billis A. Focal prostatic atrophy: Mimicry of prostatic cancer on trus and 3d-mrsi studies. Abdom Imaging. 2009;34:271-5.

19. Shukla-Dave A, Hricak H, Eberhardt SC, et al. Chronic prostatitis: MR imaging and 1h MR spectroscopic imaging findings--initial observations. Radiology. 2004;231:717-24.

20. Guo K, Qiu MX, Cai SL, et al. A multi-center clinical trial of qianlieantong tablets for chronic prostatitis. Zhonghua Nan Ke Xue. 2007;13:950-2.

21. Hu WL, Zhong SZ, He HX: Treatment of chronic bacterial prostatitis with amikacin through anal submucosal injection. Asian J Androl. 2002;4:163-7.

22. Krieger J N, Nyberg Jr L, Nickel J C. NIH consensus definition and classification of prostatitis. JAMA.1999;282:236-7.

23. WHO Laboratory Manual for the Examination of Human Semen and. Sperm-Cervical Mucus Interaction. 4th edn. Cambridge: Cambridge university Press, 1999.

24. Hochreiter WW, Nadler RB, Koch AE, et al. Evaluation of the cytokines interleukin 8 and epithelial neutrophil activating peptide 78 as indicators of inflammation in prostatic secretions. Urology. 2000;56:1025-9.

25. Magri V, Wagenlehner FM, Montanari E, et al. Semen analysis in chronic bacterial prostatitis: Diagnostic and therapeutic implications. Asian J Androl. 2009;11:461-77.

26. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev. 1995;59:143-69.

27. Bjerklund Johansen TE, Gruneberg RN, Guibert J, et al. The role of antibiotics in the treatment of chronic prostatitis: A consensus statement. Eur Urol. 1998;34:457-66.

28. Delavierre D, Rigaud J, Sibert L, Labat JJ. Symptomatic approach to chronic prostatitis/chronic pelvic pain syndrome. Prog Urol. 2010;20:940-53.

29. Zorn B, Virant-Klun I, Meden-Vrtovec H. Semen granulocyte elastase: Its relevance for the diagnosis and prognosis of silent genital tract inflammation. Hum Reprod. 2000;15:1978-84.

30. Ludwig M, Kummel C, Schroeder-Printzen I, Ringert RH, Weidner W. Evaluation of seminal plasma parameters in patients with chronic prostatitis or leukocytospermia. Andrologia. 1998;30(Suppl 1):41-7.

31. Zopfgen A, Priem F, Sudhoff F, et al. Relationship between semen quality and the seminal plasma components carnitine, alpha-glucosidase, fructose, citrate and granulocyte elastase in infertile men compared with a normal population. Hum Reprod. 2000;15:840-5.

32. Cumming JA, Dawes J, Hargreave TB. Granulocyte elastase levels do not correlate with anaerobic and aerobic bacterial growth in seminal plasma from infertile men. Int J Androl. 1990;13:273-7.