Effect of Antibiotic Therapy on Interleukin-6 in Fresh Semen and Postmasturbation Urine Samples of Patients with Chronic Prostatitis/Chronic Pelvic Pain Syndrome

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OBJECTIVES To investigate the effect of antibiotic therapy on interleukin (IL)-6 in fresh semen and postmasturbation urine samples of patients with chronic prostatitis or chronic pelvic pain syndrome (CP/CPPS).

METHODS A total of 128 patients with CP/CPPS prospectively underwent the evaluation of IL-6 in fresh semen and postmasturbation urine samples. At 6 weeks after 4 weeks antibiotic therapy, the IL-6 levels were re-evaluated.

RESULTS Of the 128 patients, 109 (85.2%) were available for our analysis. Of the 109 patients, 72 (66.1%) met the criteria for National Institutes of Health (NIH) classification for inflammatory CP/CPPS (type IIIa) and 37 (33.9%) met the NIH criteria for noninflammatory CP/CPPS (type IIIb). Before antibiotic therapy, 86 patients (78.9%), irrespective of NIH classification, had an increased IL-6 level in fresh semen; 64 (88.9%) patients with CP/CPPS type IIIa and 22 (59.5%) with type IIIb CP/CPPS had increased IL-6 levels. After 4 weeks of therapy, a significant reduction was found in the IL-6 level, with only 44 (40.4%, $P = .009$) patients showing an increased IL-6 level: 34 patients with type IIIa (47.2%, $P = .0000$) and 10 with type IIIb (27.0%, $P = .0033$). An increased IL-6 level was found in the postmasturbation urine sample in 37 patients (33.9%), irrespective of NIH classification: 28 (38.9%) with type IIIa and 9 (24.3%) with type IIIb. At 6 weeks after therapy, only 3 patients (2.8%, $P = .000$) had an increased IL-6 level: 2 with type IIIa (2.8%, $P = .0000$) and 1 with type IIIb (2.7%, $P = .02$).

CONCLUSIONS The IL-6 levels had decreased significantly after antibiotic therapy in patients with CP/CPPS, suggesting a bacterial inflammatory character. The determination of IL-6 in seminal plasma and postmasturbation urine samples is useful as an addition to the diagnostic test for the patient with CP/CPPS and as an efficacy marker for therapy. UROLOGY 72: 336–339, 2008. © 2008 Elsevier Inc.

Chronic prostatitis (CP) or chronic pelvic pain syndrome (CPPS) is a disease of unknown origin, and its pathogenesis is poorly understood. The classic pelvic or genital pain, urinary symptoms, and/or sexual disorders are rarely associated with representative urinary tract infection. The urine and/or semen cultures often do not show any bacterial growth; therefore, new, reliable diagnostic tools are needed for the diagnosis of CP/CPPS. Recent publications have shown that the immune response plays one of the important roles in the pathogenesis of chronic prostatitis.[1,2]

Evidence is increasing for a role for cytokines as inflammation mediators in CP/CPPS. Pro-inflammatory, anti-inflammatory, and regulatory cytokines have been tested for their diagnostic usefulness, particularly, interleukin (IL)-6 and IL-8 as representatives of pro-inflammatory cytokines with greater levels in seminal plasma.[3] They were put forward as promising tools in the differentiation of CP/CPPS. We investigated the IL-6 level in the fresh semen and postmasturbation urine samples of men with CP/CPPS. The objective of this study was to evaluate the effect of antibiotic treatment on the IL-6 levels in the fresh semen and postmasturbation urine samples of men with CP/CPPS.

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MATERIAL AND METHODS

A total of 128 patients (all white men) presenting with chronic genital or pelvic pain of ≥3 months' duration were prospectively recruited from our outpatient unit. All the men underwent routine urologic investigations and screening for acute urinary tract infection (ie, urinary sediment, culture) and sexually transmitted diseases (ie, urethral swabs). Fresh semen was obtained by masturbation and immediately investigated. Moreover, postmasturbation urine samples was also used to determine the IL-6 level. Patients who presented with acute urinary infection or positive urethral swabs for *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, or *Candida* were excluded from additional analysis.

A lateral flow immunoassay for the semiquantitative measurement of human IL-6 (Milenia, QuickLine, MLQ6, Milenia Biotec, Germany) was used to determine the IL-6 level. A 100-μL sample of fresh semen (obtained by masturbation immediately before the investigation and subsequent to ejaculation abstinence for 5 days) was applied with a micropipette into the sample application of the test. After 15 minutes of incubation, 2 drops of chase buffer were added, and after incubating for an additional 5 minutes, the test was interpreted using the PicoScan (Milenia Biotec) analysis device. The Pico System densitometrically measures the color intensity of the test band and calculates the result according to a stored standard curve. The measurement range is from >50 to 10 000 pg/mL. An IL-6 level >100 pg/mL and leukocytes $\geq 1 \times 10^6$ was considered a pathologic finding. The 100-pg/mL level was chosen on the basis of findings from previous studies that reported IL-6 levels in seminal fluid from healthy patients.[4,5] All the patients were invited for a follow-up investigation 6 weeks after a 4-week antibiotic therapy course with standardized ciprofloxacin 500 mg or 1000 mg daily dosage depending on the patient's body weight. The patients were asked to complete the National Institutes of Health (NIH)-Chronic Prostatitis Symptom Index (CPSI), German version, questionnaire. All patients gave their written consent for the study.

Statistical Analysis

The McNemar χ^2 test was used to compare the results before and after the antibiotic therapy. $P < .05$ was considered statistically significant.

RESULTS

Of the 128 patients, 109 (85.2%) were available for our analysis. Of the 109 patients, 72 (66.1%), with a mean age of 39.5 years (range 23-46), presented with a positive leukocyte count in the seminal plasma and were considered to have CP/CPPS type IIIa. The remaining 37 patients (33.9%), with a mean age of 40.8 years (range 21-67), had negative leukocyte counts and were considered to have type IIIb according to the NIH classification.[6]

The measurement of IL-6 in fresh semen before antibiotic therapy showed an increased IL-6 level, irrespective of NIH classification, in 86 patients (78.9%) and a normal IL-6 level in 23 (21.1%). In the NIH-IIIa group, 64 (88.9%) had an increased and 8 (11.1%) a normal IL-6 level. In the NIH-IIIb group, 22 (59.5%) had an increased and 15 (40.5%) a normal IL-6 level in fresh semen.

Table 1. IL-6 levels in fresh semen before and after therapy with antibiotics

	IL-6		
NIH Classification	Normal	Increased	*P* Value
NIH-IIIa+IIIb			.009
Before therapy	23 (21.1)	86 (78.9)	
After therapy	65 (59.3)	44 (40.4)	
NIH-IIIa			.0000
Before therapy	8 (11.1)	64 (88.89)	
After therapy	38 (52.8)	34 (47.2)	
NIH-IIIb			.0033
Before therapy	15 (40.5)	22 (59.5)	
After therapy	27 (73.0)	10 (27.0)	

IL-6 = interleukin 6; NIH = National Institutes of Health; IIIa = inflammatory; IIIb = noninflammatory.

Data presented as numbers of patients, with percentages in parentheses.

Table 2. IL-6 levels in postmasturbation urine samples before and after therapy with antibiotics

	IL-6		
NIH Classification	Normal	Increased	*P* Value
NIH-IIIa+IIIb			.000
Before therapy	72 (66.1)	37 (33.9)	
After therapy	106 (97.2)	3 (2.8)	
NIH-IIIa			.0000
Before therapy	44 (61.1)	28 (38.9)	
After therapy	70 (97.2)	2 (2.8)	
NIH-IIIb			.02
Before therapy	28 (75.7)	9 (24.32)	
After therapy	36 (97.3)	1 (2.7)	

Abbreviations as in Table 1.

Data presented as numbers of patients, with percentages in parentheses.

The repeat evaluation of IL-6 after a 4-week course of antibiotic therapy showed a significant reduction in IL-6 in fresh semen, with only 44 patients (40.4%) still having an increased IL-6 level ($P = .009$). Of these 44 patients, 34 (47.2%) had type IIIa and 10 (27.0%) had type IIIb CP/CPPS ($P = .0000$ and $P = .0033$, respectively). The results are summarized in Table 1.

The analysis of IL-6 in the postmasturbation urine samples, irrespective of NIH classification, showed that 37 patients (33.9%) had an increased and 72 (66.1%) a normal IL-6 level. Of the patients with type IIIa, 28 (38.9%) had an increased and 44 (61.1%) a normal IL-6 level. Of the patients with type IIIb, 9 (24.3%) had an increased and 28 (75.7%) a normal IL-6 level in the postmasturbation urine samples.

In the re-evaluation, only 3 (2.8%) of the study group patients had an increased IL-6 level after antibiotic therapy ($P = .000$). Of the 3 patients, 2 (2.8%) had type IIIa and 1 (2.7%) had type IIIb ($P = .0000$ and $P = .02$, respectively). The results are summarized in Table 2.

However, we were unable to show the correlation between the IL-6 decrease and patient complaints because of the lack of completed NIH-CPSI questionnaires.

A high percentage of non-German native speakers (33%) might explain why only 37 completed NIH-CPSI questionnaires German version were returned. This also represents the weak point of our study.

COMMENT

The NIH classification of CP differentiates between inflammatory (type IIIa) and noninflammatory (type IIIb) for the description of 2 entities with very similar, if not identical, symptoms. The differentiation is determined by the leukocyte count in the seminal fluid, expressed prostatic secretions, or postprostatic massage semen or urine.[6] Additional information, such as a positive semen or urine culture, is helpful, but does not often provide reliable information. It was recently reported that only 8% of a sample of 488 patients with CP/CPPS presented with uropathogenic bacteria and 65.4% had nonuropathogenic bacteria in the semen, postprostatic massage semen or urine sample, or expressed prostatic secretions. However, no association with symptoms could be shown.[7]

To identify additional parameters of prostatic inflammation, cytokines and cytokine-related factors, in particular sets of interleukins, have been investigated.[3,8-10] Even though pro-inflammatory cytokines such as IL-6 and IL-8 are also increased in arthritis, pancreatitis, and gastritis,[11-13] and despite the unclear pro- or anti-inflammatory role of IL-6,[14] recent studies were able to show increased levels of IL-6 and IL-8 in the seminal fluid and correlation with the severity of the symptoms in patients with CP/CPPS.[10]

IL-6 is not specific to prostatitis only, but is also involved in other benign or malignant prostatic diseases. In benign prostatic hyperplasia (BPH), IL-6 production of stromal BPH cells is stimulated by IL-17 up to ninefold.[15] Moreover, IL-6 and IL-8 have been shown to be the favorite executors of stromal growth in BPH.[16] Penna et al.[10] analyzed the correlation between IL-8 and prostate-specific antigen levels in patients with CP/CPPS and BPH and found a significant correlation between them. Kramer et al.,[17] in their review published in 2007, suggested that BPH is an immune inflammatory disease.

The role of chronic inflammation in prostate cancer is also under intensive investigation. A study published in 2003 showed the involvement of IL-6 in patients with hormone-refractory prostate cancer.[18] The investigators suggested that IL-6 might be a surrogate marker of the androgen-independent prostate cancer phenotype.[19]

Investigations of prostatic inflammation have repeatedly demonstrated a significant correlation among IL-6, IL-8, patients' complaints, and NIH-CPSI score.[4,5,9] Thus, if the pro-inflammatory interleukins are involved in the other 2 major prostatic diseases (BPH and prostate cancer), it can be hypothesized that IL-6 and IL-8 have diagnostic value in the differentiation of CP/CPPS.

A previous investigation showed a difference in IL-6 levels between patients with NIH-IIIa and NIH-IIIb prostatitis compared with healthy individuals, with a threefold and twofold increase in IL-6 level; however, only IL-8 was significantly different between those with NIH-IIIa and those with NIH-IIIb.[4]

To the best of our knowledge, our study is the first to investigate whether any difference appears in the IL-6 level after antibiotic therapy. Our study results showed a significant difference before and after therapy (86 patients [78.9%] vs 44 patients [40.4%]). Although the IL-6 levels were increased in both subgroups at the primary diagnosis, patients with NIH-IIIb disease showed increased IL-6 levels nearly 2 times less often than did NIH-IIIa patients. This corresponds to similar patterns reported by Orhan et al.[4]

Our comparison of NIH-IIIa and NIH-IIIb subgroups before and after antibiotic therapy showed the positive effect of antibiotic therapy with a decrease in IL-6, suggesting inflammatory components for both subgroups, as mentioned by John et al.[20] Whether this is an antibiotic therapeutic effect on a bacterial pathogen in the prostatic fluid and subsequent decrease in IL-6 or whether it is a direct antiphlogistic/immunomodulatory effect on IL-6 remains unanswered. Recent investigations have suggested such an effect for azythromycin.[21] However, this was rejected by Bouwman et al.[22] in their investigation showing inhibition of IL-6, but not of fibrinogen production, in infected hepatocytes, as a result of an antimicrobial and not a direct anti-inflammatory effect of azithromycin.

Whether IL-6 or IL-8 is more representative for patients with CP/CPPS (bacterial and nonbacterial) needs additional evaluation with larger numbers of patients, ideally in a randomized setting.

CONCLUSIONS

The results of our study have demonstrated that IL-6 decreases significantly after antibiotic therapy in patients with CP/CPPS, suggesting the disease has a bacterial inflammatory character. We believe that the determination of interleukin-6 in fresh semen and in postmasturbation urine samples is fast and practical in everyday urologic practice. It is useful as an addition to the diagnostics for the patient with CP/CPPS and as an efficacy marker for therapy. Assessment with the NIH-CPSI should be performed for evaluation of patients' complaints and correlation with cytokine levels.

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