



Determination of IL-10 levels in chronic pelvic pain syndrome patients

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Abstract

Chronic pelvic pain syndrome (CPPS) is very common pathology that is characterized by pain in the perineum, pelvis, supra pubic area, or the external genitalia, with variable degrees of voiding or ejaculatory disturbance. The etiology of disorder is controversial with groups postulating infections, autoimmune, inflammatory and neuromuscular mechanism. CPPS is the most common urologic diagnosis in men younger than 50 years and the third most common urologic diagnosis in men older than 50 years after benign prostate hyperplasia and prostate cancer. Although high incidence rate, the etiology and pathophysiology of the disease are still poor.

Key words: CPPS, cytokines, IL-10, prostatitis

Introduction

The National Institutes of Health (NIH) classification and definition of the four categories of prostatitis defines category IIIa as an inflammatory CPPS with white blood cells (WBCs) in semen, expressed prostatic secretions (EPSs), or voided bladder 3 (VB3) (Krieger *etal.*, 1999). Category IIIb is defined as

noninflammatory CPPS without WBCs in semen, EPSs, or VB3 (Orsilles and piante-Depaoli, 1998). Patients with CPPS often have leukocytes in EPS's, suggesting evidence of an inflammatory component in the pathogenesis of the disease (Alexander *etal.*, 1998). Cytokines represent an important group of regulatory proteins produced by leukocytes and other cells that control inflammation and tissue repair functions. They were first characterized as regulators of inflammation and the immune response but in the last decade it has been clear that they also have a critical role in tissue damage and repair (Moore *et a/.*, 2001). Cytokines and cytokine related factors can be divided into 3 broad groups or classes, namely proinflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor and interferon- α , anti-inflammatory cytokines such as IL-10, IL-1RA, IL-1s0lR and tumor necrosis factor-s0lR, and regulatory-growth factor cytokines such as IL-2, IL-3, IL-4, IL-5 and IL-7. Proinflammatory cytokines are involved in promoting inflammatory processes within tissue. Anti-inflammatory cytokines tend to suppress inflammation and promote tissue repair, regeneration and act as an antioxidant. Generally the balance of proinflammatory and anti-inflammatory cytokines determines the outcome of tissue trauma and inflammation.

These studies clearly indicate that in CPPS, an inflammatory component is critically involved in disease pathogenesis (Pontari and Ruggieri, 2004). Among the cytokines tested, although the effects were of low magnitude, the proinflammatory cytokines IL-1a, IL-1p, IL-6, and IL-12p70 were significantly elevated in CP/CPPS whereas the anti-inflammatory cytokine IL-10, was increased in patients with CP/CPPS IIIB compared to controls, as previously reported (Miller *et al.*, 2002). Interleukin-10 (IL-10) is an important immunoregulatory cytokine mainly produced by monocytes, macrophages and T cells (Nadler *et al.*, 2000). The human IL-10 gene is located on chromosome 1 and encodes 5 exons (5.1 kb) (Orhan *et al.*, 2001). The IL-10 promoter is highly polymorphic with two informative microsatellites, IL10.G and IL10.R, located 1.2 and 4 kb upstream of the transcription start

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site and three frequent point mutations 1082(G/A), 819(C/T), and 592(C/A) (Hochreiter *et al.*, 2000). The -1082(G), 592(C), and 819(C) alleles were associated with higher IL-10 production. Numerous studies have shown that IL-10 may be involved in the pathogenesis of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, myasthenia gravis, Grave's disease, psoriasis, asthma, inflammatory bowel disease and so forth (Nickel *et al.*, 1999).

The aim of the present study is to determine the serum IL-10 levels in patients with CPPS compare to healthy controls.

Material and methods

A 5ml sample of whole blood was collected from 72 chronic pelvic pain syndrome patients. Apart from these 72 patients, a further 12 were excluded from the study due to their incompatibility with the set criteria (Supplemented data 1). 40 healthy donors with normal semen characteristics as proven by various reports were recruited as controls. Donors with a history of genitourinary infection, symptoms, or instrumentation were excluded from the study. The following criteria was approved for research studies on chronic nonbacterial prostatitis/CPPS (Chronic prostatitis workshop, 1995). The Helsinki Declaration was strictly observed regarding the use of human samples. Also, all studies were undertaken with the approval and institutional oversight of the Institutional Review Board for Ethics of Human Subjects.

Semen analysis

Semen samples were collected by masturbation into sterile containers after at least 72 hours of sexual abstinence. After liquefaction, semen specimens were evaluated for semen volume, appearance, and viscosity. Semen characteristics (concentration, motility, and morphology) were examined in accordance with WHO criteria (WHO laboratory manual, 1999). Computer assisted semen analyzer were loaded for 5 ul for each semen sample and motile cells were examined.

White blood cells

The presence of white blood cells (WBCs) in semen specimens were assessed by the myeloperoxidase (Endtz) test (Shekarriz *et al.*, 1995). A 20 ul volume of liquified semen was placed in a 1.5 ml cryogenic vial, followed by 20 ul of phosphate buffer saline (pH 7.0) and 40 ul of benzidine solution. The sample was mixed,

allowed to sit for 5 min and examined for cells that had stained brown, indicating cells positive for peroxidase. Leukocytospermia was defined as 1×10^6 or more WBC/ml of semen.

IL-10 level determination in blood and semen specimens

Blood samples were centrifuged for 2 min at 1500 rpm at room temperature and plasma was separated into a clean tube. After liquefaction, semen samples were centrifuged at 1500 rpm for 5 min at 4°C. All plasma samples were separated into aliquots and frozen at -20°C for later measurement. 200 ul blood plasma or semen samples were analyzed each enzyme activity spectrophotometrically (Shimadzu, Japan). IL-10 levels in semen and blood plasma were determined according to manufacturer instructions using specific kit by the Quantikine Human Immunoassay (R&D Systems Inc., USA). Enzyme analysis were done with RANSOD kit and IL-10 were determined), which employs the quantitative sandwich enzyme immunoassay technique (Figure 1).

Statistical analysis

The laboratory researchers were unaware about case-control status. IL-10 levels were expressed as mean \pm standard error (SEM). All experiments were replicated in triplicate. Student t-test was used for comparison between NIH classified CPPS patients and control subjects. The 0.05 level was selected as point of the minimal statistical significance.

Results

The average age was 35 years (range 22 to 63 years) in CPPS patients and 32 years (range 21 to 58 years) in disease free control patients. According to NIH category classification, 40 patients were category 3a and 32 of them were category 3b. 47 of 72 (65 %) patients were smoker and 8 of 72 (11 %) gave up smoking in 5 years and the rest of them were never used. The case and control group were not used to take antioxidant pills regularly and they were eating vegetables and meat products daily (Table 1).

They had symptoms of CPPS for a mean of 8.9 years (median 3.9 years, range 3 months to 14 years). Mean NIH-CPSI component values were pain 9.2 urinary 3.6, quality of life 9.0, and total score 21.8. Symptomatic categories were typical for men with this illness since 97 % had pain, 58 % had irritative voiding symptoms, 56 % had obstructive voiding symptoms and

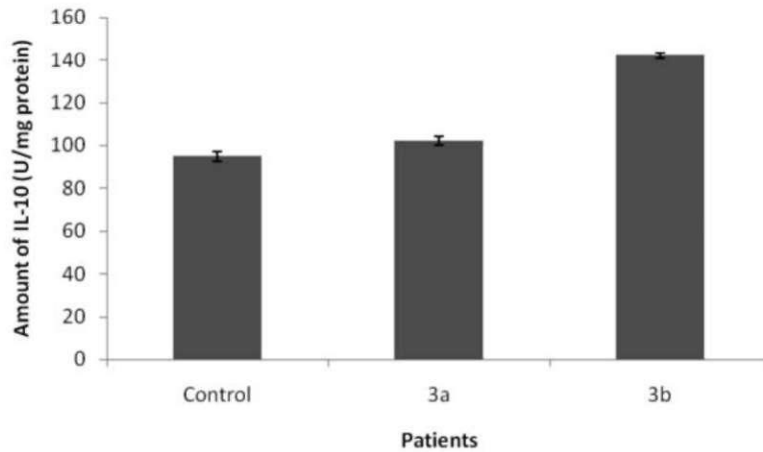


Figure 1. Determination of IL-10 amount in chronic pelvic pain syndrome patients.

Table 1. Patient and healthy group characteristics

	Type 3a	Type 3b	Control	Total
Number of patient	40	31	36	107
Age	33.7 (22-54)	36.35 (24-63)	32.44 (21-58)	34.04 (21-63)
Smoking				
Yes	28/40 (70 %)	18/31 (58.1 %)	20/36 (55.5 %)	66/107 (61.6 %)
No	12/40 (30 %)	13/31 (41.9 %)	16/36 (44.5 %)	41/107 (38.4 %)
Mean of PSA levels ng/dl (range)	1.043 (0.458-2.84)	1.048 (0.54- 2.94)	0.995 (0.324-2.58)	1.028 (0.324-2.94)
Other medical problems*	3 (7.5 %)	4 (12.5 %)	2 (5.5 %)	9 (8.4 %)

*Hepatitis, HIV, Hypertension, Cardiovascular disease

Table 2. Comparison of semen characteristics and measures of MnSOD between controls and patients with chronic pelvic pain prostaticitis with or without leukocytospermia.

Variable	Controls n= 40	CP (Endtz negative) n= 60	CP (Endtz positive) n= 12
Concentration (x 10 ⁶ /ml)	59.9 ± 1.2	37.6 ± 0.9	40.1 ± 2.3
Motility (%)	60.2 ± 4.3	51.0 ± 6.7	47.2 ± 5.0
WHO morphology (%)	63.9 ± 2.4	31.3 ± 2.7	29.6 ± 1.3

Data presented as mean ± SE

CP= Chronic prostatitis

39 % had erectile dysfunction. In these patients there was no urethral or prostatic bacterial infection. All groups were investigated for semen characteristics such as concentration, motility and WHO morphology (Table 2).

IL-10 concentration were determined in blood and semen (Figure 2). IL-10 concentration was 95 U/mg total protein and 102 U/mg total protein in control and category 3a specimens, respectively. However IL-10 concentration for category 3b patients were 142 U/mg total protein. Upregulation of IL-10 which is anti-inflammatory cytokine in category 3b patients were significantly higher than control and category 3a CPPS patients ($p=0,004$).

Discussion

The causes and pathophysiology of CPPS remain unknown, but the evidence is accumulating for a role of radical oxygen species and cytokines in the development of illness. Cytokines have pivotal role in the immune system response which allow inflammatory cells to communicate with each other. IL-10 is an anti-inflammatory and anti-oxidant cytokine that inhibits the production of pro-inflammatory cytokines such as IL-6 and tumor necrosis factor alpha TNF- α . In IL-10 gene-deficient mice, an overproduction of inflammatory cytokines and the development of chronic inflammatory diseases have been noted (Khadra *et al.*, 2006). Furthermore, IL-10 has been reported to have an anti-inflammatory role in many kind of tissues (Pease and Sabroe, 2002), and reduced production of IL-10 has been found in some CPPS patients (Alexander *et al.*, 2001). Therefore, IL-10 is generally correlated with autoimmune disorders. IL-10 promoter polymorphisms may be the molecular basis of the involvement of IL-10 in the genetic susceptibility to disease. Inter-individual variations in IL-10 production were genetically contributed to polymorphisms within IL-10 promoter region. It has been shown that A at the site -1082 in the promoter region of the IL-10 gene is associated with low and G with high production of IL-10. Like category 3b patients IL-10 amount, autoimmune suspected illness such as rheumatoid arthritis has elevated IL-10 concentration.

In conclusion, we found that IL-10 levels elevated in semen and blood plasma in category 3b patients. However the difference between control and category 3a specimens were not significant ($p>0.05$). This might be linked with other transcriptional factors are having role in synthesis of IL-10. In the light of these findings, it might be concluded that IL-10 expression level is not a predictive to understand difference between

prostatitis subtypes. In further studies, IL-10 polymorphic site determination in patients could be more informative to understand the role of IL-10 in pathogenesis of disease.

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