Urine and Serum C-Reactive Protein Levels as Potential Biomarkers of Lower Urinary Tract Symptoms

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Objective: The association of elevated serum C-reactive protein (CRP) with lower urinary tract symptoms (LUTS) suggests a possible role of inflammation. We investigated whether measurement of urine CRP levels can complement the deficit in the specificity of serum CRP levels as potential biomarkers of lower urinary tract inflammation.

Patients and Methods: Serum CRP levels were measured in 97 patients with LUTS; urinary CRP was additionally measured in 20 of the patients. Bladder expression of CRP was quantified by real-time polymerase chain reaction on human bladder tissue obtained from 15 organ donors.

Results: Significantly higher serum CRP levels were noted in overactive bladder (OAB) wet (n=18; 2.96±0.47 mg/L), chronic prostatitis (n=5; 3.00±1.05 mg/L), benign prostatic hyperplasia (n=20; 1.13±0.17 mg/L), and acute febrile bacterial infection (n=5; 97.71±36.28 mg/L) compared to in asymptomatic controls (n=20; 0.93±0.27 mg/L). Serum CRP level was higher in OAB wet than in OAB dry (n=20).

Urinary CRP level was higher than 0.15 mg/L in only one man with bacterial prostatitis and sepsis. The mRNA expression of CRP was very modest and several fold lower than the expression of housekeeping genes in the detrusor or urothelium.

Conclusion: Serum CRP was elevated with different disease entities in patients with LUTS. The synthesis of CRP is unlikely in the bladder and the protein is not a normal urine constituent. This pilot study has led us to infer that urinary CRP is unlikely to serve as a biomarker of LUTS. Sensitive but nonspecific elevation of serum CRP level suggests an inflammatory mechanism with LUTS.

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1. Introduction

The causes of lower urinary tract symptoms (LUTS) can be traced to a number of pathologic conditions, including benign prostatic hyperplasia (BPH), overactive bladder (OAB), interstitial cystitis (IC), chronic prostatitis, and urinary tract infection. No matter what the cause, inflammation has been linked to the development of LUTS.¹ ² Inflammation is often present in prostate biopsy specimens; this has led to the hypothesized association...
Urine and serum CRP

of chronic inflammation with BPH. Abnormal sensory function of the prostate plays an important role in the symptoms of chronic prostatitis or protatodynia.

Urine, which is easy to collect, is an ideal biologic sample for the discovery of noninvasive biomarkers of human diseases. Previous studies have suggested the association of inflammation with OAB symptoms by the significant elevation of nerve growth factor (NGF) and prostaglandin E2 (PGE2) levels in the urine of OAB patients. In addition, IC patients have also been reported to have elevated NGF levels in bladder tissue. Objective measurement of these inflammatory markers in the urine highlight the association of LUTS with lower urinary tract inflammation.

One widely studied general marker of inflammation and infection is C-reactive protein (CRP). Its serum levels have been reported to rise dramatically during inflammatory processes occurring in the body. Higher than normal serum CRP levels are well accepted as a proxy for heart disease risk, and serum CRP level is used to determine disease progression or treatment effectiveness. However, unlike other inflammatory biomarkers detected in human and animal urine, CRP levels in the bladder tissue and urine of LUTS patients has not been previously reported. Therefore, this study was undertaken to examine CRP expression in bladder tissue, and serum and urine levels of CRP in patients with LUTS.

2. Materials and Methods

2.1. Collection of urine and serum

A total of 97 patients with a mean age of 60.7 ± 2.0 years were enrolled in the study after they gave informed consent; 77 patients had LUTS (frequency, urgency with or without incontinence, nocturia, and voiding symptoms) and 20 did not (they served as the control group). After physical examination and record review, clinical characteristics were documented. Nine patients had IC, five had chronic prostatitis, 38 had OAB (OAB wet—with at least one episode of urgency incontinence daily, n = 18; OAB dry—without urgency incontinence, n = 20), 20 had BPH, and five had acute febrile bacterial infection related to the urinary tract. We did not have complete prostate fluid study in the patients with chronic prostatitis.

Serum was collected from all 97 patients, and urine was additionally collected from the 20 control patients (Table 1). Serum and urine samples were obtained during a single clinic visit. Collected urine samples were immediately placed on ice in the clinic. Samples were centrifuged at 2400 g for 10 minutes. The supernatant was separated into 1.5 mL aliquots and preserved in a −80°C freezer.

Table 1 Characteristics of patients with lower urinary tract symptoms and serum C-reactive protein levels

<table>
<thead>
<tr>
<th>Disease/serum</th>
<th>Control (n=20)</th>
<th>OAB dry (n=20)</th>
<th>OAB wet (n=18)</th>
<th>IC/PBS (n=9)</th>
<th>CP/CPPS (n=5)</th>
<th>BPH (n=20)</th>
<th>Acute febrile bacterial infection (n=5)</th>
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<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>0.56 0.15 1.72 0.15 2.76 3.20 177.00</td>
<td>0.18 0.69 3.70 1.57 13.50 0.62 190.00</td>
<td>0.31 0.26 4.16 5.01 1.08 1.32 38.40</td>
<td>0.34 0.46 4.30 1.12 1.83 0.77 70.80</td>
<td>1.33 0.84 1.27 0.52 2.12 0.82 12.33</td>
<td>0.00 0.61 1.55 0.52 0.16</td>
<td>0.18 0.69 3.70 1.57 13.50 0.62 190.00</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.93±0.27 1.06±0.16 2.96±0.47 2.30±1.06 4.26±2.33 1.13±0.17 97.71±36.28</td>
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*p compared with control

One way ANOVA with Kruskal-Wallis and Dunn’s post hoc test: control vs. OAB wet, p < 0.001; control vs. acute febrile UTI, p < 0.001; OAB dry vs. OAB wet, p < 0.05; OAB dry vs. acute febrile UTI, p < 0.01; BPH vs. acute febrile UTI, p < 0.01. CRP = C-reactive protein; OAB = overactive bladder; IC = interstitial cystitis; PBS = painful bladder syndrome; CP = chronic prostatitis; CPFS = chronic pelvic pain syndrome; BPH = benign prostatic hyperplasia; SE = standard error; UTI = urinary tract infection.
2.2. High-sensitivity CRP assay

Measurement of CRP level in venous blood samples and urine samples was done by the hospital's clinical pathology laboratory using a standard assay.

2.3. Real-time polymerase chain reaction assay for CRP

Real-time polymerase chain reaction (PCR) was done to quantify CRP expression in human bladder tissue samples obtained from 15 organ donors with unknown disease status. Separated tissue pieces of human detrusor (7) and urothelium (8) were stored in RNA later (Ambion Inc., Austin, TX, USA) at 4°C until RNA isolation. RNA samples were isolated from bladder tissues using RNeasy kits (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's protocol. RNA was quantified by a spectrophotometer (Eppendorf AG, Hamburg, Germany). Real-time quantitative PCR reactions were performed according to the manufacturer’s (SABiosciences, A QIAGEN Company, Frederick, MD, USA) protocol. Briefly, cDNA was prepared from 1 μg total RNA using an RT2 PCR array first strand kit (SABiosciences, A QIAGEN Company).

Quantitative PCR reactions were done in a 25 μL mixture, which included 12.5 μL RT2 real-time SYBR green/ROX PCR master mix (SABiosciences), 10.5 μL nuclease-free H2O, 1 μL specific human primers, including 10 μM each of CRP (SABiosciences) and beta-actin (forward: 5’ GAGCCTGTTACAGGAAG 3’; reverse: 5’ CATTACATAATTACGAAAGC 3’), and 1 μL template cDNA. Quantitative PCR amplification was done with an initial 10-minute step at 95°C, followed by 40 cycles at 95°C for 30 seconds, 55°C for 1 minute and 72°C for 30 seconds, followed by dissociation curve analysis. Real-time quantitation was performed using the Mx3000P QPCR System (Stratagene Corp., Santa Clara, CA, USA) with fluorescence threshold values calculated using Mx3000P System software.

2.4. Statistical analysis

Data were analyzed using GraphPad Prism version 4.0 (GraphPad Software Inc., La Jolla, CA, USA). Descriptive statistics were denoted as mean ± standard error. Kruskal-Wallis with Dunn’s post hoc test was used for continuous measures of non-normally distributed data. A p value less than 0.05 was considered to be statistically significant.

3. Results

Serum CRP levels were found to vary in patients with LUTS (Table 1). CRP levels were categorized into two groups: ≤ 1 mg/L and > 1 mg/L. Four of the 20 control patients (20%), 8 of the 20 OAB dry patients (40%), 10 of the 20 BPH patients (50%), 5 of the 9 IC patients (55.6%), and 5 of the 5 chronic prostatitis patients (100%), 16 of the 18 OAB wet patients (88.9%), and 5 of the 5 patients with acute febrile bacterial infection (100%) had CRP level > 1 mg/L. These results indicate that different disease entities of LUTS may have different proportions of patients with inflammation; among them, the highest proportion was noted in the OAB wet group (Figure 1).

OAB wet, chronic prostatitis, BPH, and acute febrile bacterial infection groups had significantly higher serum CRP levels than control (2.96 ± 0.47, 97.71 ± 36.28 mg/L, respectively, versus 0.93 ± 0.27 mg/L; p = 0.0002, 0.0109, 0.0515 and 0.0008, respectively). Furthermore, OAB wet (2.96 ± 0.47 mg/L) patients had higher serum CRP levels than OAB dry patients (1.06 ± 0.16 mg/L). Patients with acute febrile bacterial infection had higher serum CRP levels than patients with OAB dry and BPH. These results indicate that different disease entities of LUTS may have different degrees of inflammation.

Urinary CRP level was below 0.15 mg/L except for one man with bacterial prostatitis and sepsis, who had a urinary CRP level of 0.86 mg/L. The mRNA expression of CRP was very modest and several fold lower than the expression of housekeeping genes in the detrusor or urothelium (data not shown). These results indicate that CRP synthesis is unlikely in the bladder and the protein is unlikely to be a normal urine constituent.
4. Discussion

This study showed that serum CRP was elevated to various degrees in patients with LUTS. OAB wet patients had higher serum CRP than normal control and OAB dry patients. In patients with acute febrile infection, CRP levels were extraordinarily high. Serum CRP level in patients with LUTS may not specifically reflect the condition of the lower urinary tract, as its levels are more likely to be influenced by the systemic inflammatory condition. Our pilot study suggests that urinary CRP is unlikely to serve as a biomarker of local bladder inflammation.

LUTS comprises various lower urinary tract conditions and can also be attributed to systemic factors. The evaluation of LUTS is largely dependent on different kinds of symptom scores and questionnaires. However, objective parameters that can detect disease status and monitor disease progression or therapeutic effects are lacking. Recent studies from bladder or prostate biopsies have suggested that OAB, IC and BPH are related to tissue inflammation. Investigation of inflammation-associated proteins in the bio-fluid of these patients is of widespread interest. Kupelian et al. demonstrated a dose-response relationship between increased CRP levels and increased odds ratio of LUTS in both men and women, which supports the hypothesized role of inflammatory processes in the etiology of LUTS.

Our current results of elevated CRP levels in different LUTS conditions also support the concept of inflammation related to LUTS and gives credence to serum CRP levels as a potential surrogate marker of LUTS. In an effort to improve the specificity of markers, various studies have looked at the serum derivative, urine, to try to account for disease-specific markers of the functional status of different organs, including the urinary tract. It is logical to expect superior reflection of bladder biochemistry in urine compared to serum. Kim et al. reported that urinary NGF and PGE2 levels were elevated in OAB patients compared to controls. A study conducted by Liu and Kuo showed that urine NGF levels were very low in normal controls, while patients with OAB had significantly higher urinary NGF levels. Furthermore, OAB wet patients had significantly higher urinary NGF levels than OAB dry patients, which concurs with our observation of higher serum CRP levels in OAB wet than OAB dry patients. Taken together, it may be argued that OAB wet is associated with a more severe degree of inflammation than OAB dry. Recently reported urinary chemokines profile in OAB patients also corroborates this inference of severe inflammation in such patients.

CRP is produced and secreted by the liver in response to inflammatory processes occurring in the body. The current study showed that urinary CRP was rarely detected and the mRNA expression of CRP was very modest and several fold lower than the expression of housekeeping genes of beta-actin in the detrusor or urothelium. These observations led us to infer that urinary CRP levels are much lower than serum CRP levels, and the current available methods for detecting CRP might not be sensitive enough to develop a urinary assay. Therefore, urinary CRP cannot complement the deficit in the specificity of serum CRP level as a potential biomarker of local inflammation in the urinary tract.

The current study showed that the proportion of patients with LUTS and elevated CRP level (>1 mg/L) was higher than in controls and lower than in patients with acute febrile infection. Therefore, in assessing CRP elevation, the presence of other inflammatory disease and infection should be considered because CRP is a nonspecific inflammatory marker. However, assays for serum CRP are widely available and simple. Therefore, serum CRP might be useful as a surrogate marker for monitoring disease conditions and response to therapeutic interventions in patients with LUTS. Serum CRP may also be useful in conjunction with urinary inflammatory chemokines as a multivariant biomarker of LUTS. We suggest that checking serum CRP in patients who do not respond to conventional therapy for OAB or LUTS might guide specific anti-inflammatory treatment for those with elevated serum CRP.

Our study suggests that urinary CRP is unlikely to serve as a biomarker of local bladder inflammation. Serum CRP is elevated with different disease entities in patients with LUTS. Sensitive but nonspecific elevation of serum CRP suggests an inflammatory mechanism with LUTS.

References


