Tzu Chi Medical Journal 25 (2013) 13–18

Contents lists available at SciVerse ScienceDirect

Tzu Chi Medical Journal

journal homepage: www.tzuchimedjnl.com

Review Article

Potential urine and serum biomarkers for patients with overactive bladder and interstitial cystitis/bladder pain syndrome

Hann-Chorng Kuo [a,*], Hsin-Tzu Liu [a,b], Jia-Heng Shie [a]

[a] Department of Urology, Buddhist Tzu Chi General Hospital and Tzu Chi University, Hualien, Taiwan
[b] Ph.D. Program in Pharmacology and Toxicology, School of Medicine, Tzu Chi University, Hualien, Taiwan

ARTICLE INFO

Article history:
Received 1 October 2012
Received in revised form
19 October 2012
Accepted 24 October 2012

Keywords:
Biomarker
Chemokines
Interstitial cystitis
Overactive bladder
Proteomics

ABSTRACT

There is a lack of consensus on the pathophysiology of overactive bladder (OAB) and interstitial cystitis/bladder pain syndrome (IC/BPS). The chronic pain symptoms in IC/BPS and OAB refractory to anti-muscarinic agents may be due to central nervous system sensitization and persisting abnormalities in the bladder wall that activate the afferent sensory system. Evidence also indicates that IC/BPS is a heterogeneous syndrome and that the two subtypes, the ulcer type (classic) and nonulcer disease, represent different disease entities. There is a need for noninvasive markers for the differential diagnosis of the subtypes of IC/BPS and OAB. Increased levels of nerve growth factor (NGF) have been reported in the bladder tissue and urine of patients with OAB and IC/PBS. Recent studies have also revealed that serum NGF and C-reactive protein (CRP) are elevated in these two diseases. IC/BPS, but not OAB, involves an aberrant differentiation program in the bladder urothelium that leads to altered synthesis of several proteoglycans, cell adhesion and tight junction proteins, and bacterial defense molecules. These findings have led to the rationale for identifying urinary biomarkers to detect IC/BPS in patients with frequency urgency syndrome such as OAB-dry and OAB-wet. Recently, the markers that have been the focus of the most research are antiproliferative factor, epidermal growth factor, heparin-binding epidermal growth factor, glycosaminoglycans, and bladder nitric oxide. In addition to these urothelial defense molecules, inflammatory proteins in the urine and serum have been found to possess important roles in the pathogenesis of IC/BPS and OAB. The urinary proteome is a potential easily accessible source of biomarkers for differentiation between inflammatory bladder disorders. Analysis of multiple urinary proteins and serum cytokines is a convenient approach to monitoring the activation of inflammatory cells in the bladder tissue. Differences in urinary proteins and serum cytokines might provide a diagnostic basis for IC/BPS and could be a tool for the differential diagnosis between IC/BPS and OAB.

1. Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic sterile inflammatory disease of the bladder of unknown etiology characterized by urinary frequency, nocturia, and suprapubic pain when the bladder is full. Although there are many theories, the etiology of this condition remains obscure. There is a lack of consensus on the pathophysiology of IC/BPS. The chronic pain symptomatology in IC/BPS may be due to central nervous system sensitization and persisting abnormalities in the urinary bladder that activate the afferent sensory system [1]. Recent findings have proposed several pathophysiologic mechanisms including epithelial dysfunction, activation of mast cells, neurogenic inflammation, autoimmunity, and occult infection. Evidence also indicates that IC/BPS is a heterogeneous syndrome and that the two subtypes, the ulcer type and nonulcer type, represent different disease entities [2]. The diagnosis of IC/BPS is made by clinical and cystoscopic hydrodistention and exclusion of other bladder disorders [3]. There is a need for a noninvasive marker for diagnosing the subtypes of IC/BPS.

2. Chronic inflammation in overactive bladder and interstitial cystitis/bladder pain syndrome

The clinical presentations of overactive bladder (OAB) and nonulcer-type IC/BPS are very similar. OAB is considered when

* Corresponding author. Department of Urology, Buddhist Tzu Chi General Hospital, 707, Section 3, Chung-Yang Road, Hualien, Taiwan. Tel.: +886 3 8561825x2117; fax: +886 3 8560794.
E-mail address: hck@tzuchi.com.tw (H.-C. Kuo).

1016-3190/$ – see front matter
http://dx.doi.org/10.1016/j.tcmj.2012.10.005

patients have symptoms of urgency with or without urgency incontinence, and it is usually associated with frequency and nocturia [4]. Recently, some authors proposed that urothelial dysfunction, abnormal expression of sensory receptors, abnormal function of suburothelial interstitial cells, and increased excitability of detrusor muscles could be the etiologies of OAB [5–7]. IC/BPS, however, is a chronic inflammatory disorder of the urinary bladder manifested by frequency urgency with or without bladder pain. Histologically, bladders affected by IC/BPS show infiltration of mast cells, which suggests that the disease is mediated by an abnormality of the immune system [8,9]. Although the International Continence Society has a clear definition of urgency, most patients cannot clearly differentiate urgency from the urge to void. Overlap and confusion usually exist in the clinical presentations of OAB-dry and IC/BPS.

3. Potential urine biomarkers for OAB and IC/BPS

Several previous studies have linked OAB and IC/BPS to chronic inflammation of the urinary bladder. Nerve growth factor (NGF) in urine and bladder tissue, serum cytokines, and serum C-reactive protein (CRP) have been found to increase in OAB as well as IC/BPS [10–12]. Previous studies also showed that mast cells are multifunctional effectors of the immune system, and play an important role in the pathophysiology of IC/BPS [9]. Because there are similarities in inflammatory protein expression between OAB and IC/BPS, it is possible that there is an inflammatory reaction in OAB as well as in IC/BPS.

4. Urinary NGF in OAB and IC/BPS

Recent studies suggest that the lamina propria of the bladder plays an important role in transmitting the sensation of bladder fullness and in the response of the bladder to chemical stimuli and inflammation [5,13,14]. In the urinary tract, NGF is produced by bladder smooth muscle and urothelium [15]. Previous studies indicate that NGF is involved in the ongoing regulation of neural function in conditions such as spinal cord injury and denervation, as well as in inflammation and pain [1,15,16]. Increased levels of NGF have also been reported in the bladder tissue and urine of patients with sensory urgency and IC/BPS [10,17]. However, urine NGF is also increased in several lower urinary tract conditions such as urinary tract infection, bladder outlet obstruction, and urinary tract stones [18].

Bladder inflammation caused by intravesical instillation of cyclophosphamide or NGF in rats can induce inflammation and lead to acute afferent nerve activity and long-term plasticity that lowers the threshold for nociceptive and mechanoceptive afferent fibers [19–21]. Chronic sensitization of the afferent fibers might involve both peripheral and central mechanisms. An increase in bladder NGF in the muscle or urothelium initiates signals that have been found to transport along the afferent nerves from the bladder to the dorsal root ganglion or spinal cord [22,23]. A previous study has shown that intravesical injections of botulinum toxin A (BoNT-A) can reduce bladder pain in patients with refractory IC/BPS [24]. NGF levels in the bladder tissue are significantly increased in patients with IC/BPS and decreased to the normal range after BoNT-A treatment [25].

The clinical symptoms of IC/BPS and OAB are similar, but bladder pain is typically found in IC/BPS and urgency/urgency incontinence in OAB. However, patients with IC/BPS usually cannot tolerate a full bladder and are urged to void to avoid bladder pain. Therefore, patients with IC/BPS might not characteristically present with bladder pain but merely have frequency and nocturia, which are similar to the symptoms of OAB-dry. Although using urinary NGF as a biomarker can be a sensitive molecular diagnostic tool for OAB, recent research on urinary biomarkers revealed that urinary NGF levels also increase in patients with IC/BPS [11]. The urinary NGF levels are closely related to the visual analog scale score for pain and response to conventional treatment for IC/BPS. Differentiation of IC/BPS and OAB based on the urinary NGF level is difficult and does not have a high sensitivity. We need a more detailed analysis of urinary biomarkers as a noninvasive diagnostic tool for the differential diagnosis of IC/BPS and OAB. Investigation has shown that patients with IC/BPS and those with OAB have increased urinary NGF levels compared with those in control patients (Table 1). Patients with OAB and IC/BPS who respond to treatment have decreased urinary NGF levels, whereas those who do not respond have no change, suggesting both IC/BPS and OAB have similar pathways related to NGF production.

Histologic investigations of the bladder urothelium and suburothelium have found that chronic inflammation is present in 60% of baseline biopsies of patients with OAB [26]. OAB could be a subtype of neurogenic inflammation characterized by a series of vascular and nonvascular inflammatory responses, triggered by the activation of primary sensory neurons and the subsequent release of inflammatory neuropeptides, including substance P and calcitonin gene-related peptide [27]. These inflammatory responses also induce overexpression of transient receptor potential vanilloid receptors subfamily type 1 in the suburothelium as well as c-fos protein in the dorsal root ganglia, which have been demonstrated in rat models of OAB and in human bladder biopsies [5,14,22].

Chronic inflammation has been implicated in the development of OAB and IC/BPS. Elevated levels of CRP have been associated with chronic inflammation and lower urinary tract symptoms. Serum CRP levels were significantly higher in patients with OAB and IC/PBS than in control patients. No significant difference in CRP levels was noted between patients with OAB and IC/BPS. In a subgroup analysis, patients with OAB-wet had higher serum CRP levels than those with OAB-dry; however, the difference did not reach statistical significance [12]. In one study, the serum CRP level was significantly associated with residual urgency in patients with benign prostatic hyperplasia after medical treatment. The mean serum CRP level was 0.24 mg/dL (range 0.01–2.84), and residual urgency was identified in 90 patients (43.9%). Patients with residual urgency were older and had significantly higher serum CRP levels than those without urgency. Patients with serum CRP levels $\geq$ 0.3 mg/dL had more urgency (82.1%) than those with serum CRP levels $<$ 0.3 mg/dL (34.9%) [28]. High serum CRP levels

Table 1
Differences in urinary nerve growth factor levels in patients with interstitial cystitis/bladder pain syndrome, overactive bladder, and control patients [11,52].

Bladder dysfunction	Urine samples	Urinary total NGF (pg/mL)	Urinary NGF/Cr	Statistics
Normal control	38	0.46 ± 0.35	0.005 ± 0.003	
OAB-responder				
Baseline	50	33.3 ± 6.13	1.10 ± 0.26	$p < 0.0001$[a]
3 mo	50	17.8 ± 4.31	0.41 ± 0.09	$p = 0.008$[b]
OAB-nonresponder				
Baseline	20	56.9 ± 19.4	1.38 ± 0.54	$p = 0.0001$[a]
3 mo	20	70.57 ± 25.0	1.30 ± 0.46	$p = 0.879$[b]
IC/BPS not treated	58	50.1 ± 11.8	0.94 ± 0.23	$p < 0.0001$[a]
IC/BPS responders	36	21.3 ± 8.51	0.33 ± 0.09	$p = 0.050$[c]
IC/BPS nonresponders	78	46.8 ± 8.92	0.92 ± 018	$p = 0.957$[c]

Data are expressed as mean ± standard error.
Cr = creatinine; IC/BPS = interstitial cystitis/bladder pain syndrome; NGF = nerve growth factor.
[a] Comparison of urinary NGF/Cr level between control and different bladder dysfunction.
[b] Comparison of urinary NGF/Cr level between baseline and 3 mo.
[c] Comparison between IC/BPS not treated and posttreatment.

were also found in women with OAB-wet, and they were related to lower maximum urinary flow rates and higher body mass indices in nonstress urinary incontinence lower urinary tract dysfunction. Median CRP levels were significantly higher in women with OAB-wet than in women with bladder oversensitivity and the normal group. Further analysis revealed that body mass index and maximum flow rate were two independent factors that affected CRP levels in women [29].

Urinary inflammatory biomarkers might also be elevated in patients with OAB as well as in those with IC/BPS. Recent investigation of urinary chemokines in patients with OAB also showed increases in monocyte chemotactic protein-1 (MCP-1) and some proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), granulocyte colony-stimulating factor, and epidermal growth factor (EGF). In addition, IC/BPS involves an aberrant differentiation program in the bladder urothelium that leads to altered synthesis of several proteoglycans, cell adhesion and tight junction proteins, and bacterial defense molecules such as GP51. These findings led to the rationale for searching for potential clusters of urinary biomarkers to detect IC/BPS in patients with OAB and frequency urgency syndrome [30]. The biomarkers that have been the focus of the most research are antiproliferative factor, EGF, heparin-binding epidermal growth factor, glycosaminoglycans, and bladder nitric oxide [31].

5. Urinary proteomics in the differential diagnosis between OAB and IC/BPS

In addition to these urothelial defense molecules, inflammatory proteins in the urine have been found to have important roles in the pathogenesis of IC/BPS. A pilot study of urinary cytokines revealed elevated levels of chemokines relevant for chemotaxis of eosinophils and monocytes, and activation of mast cells in the urine of patients with IC/BPS. Chemokines are chemotactic cytokines that belong to a family of small secreted glycoproteins with a molecular weight of 7–10 kDa with more than 50 ligands that interact with multiple receptors. Chemokines exert their effects through interaction with seven transmembrane-spanning G protein-coupled receptors and are present on glycosaminoglycans linked to endothelial cell layers [32]. The most important chemokines causing chemotactic migration of macrophages, eosinophils, and mast cells belong to the CC family of chemokines distinguished by adjacent positions of the first two cysteines [33]. The chemokines of this family are MCP-1 (CCL2), macrophage inflammatory protein (MIP-1; CCL4), and eotaxin (CCL11). MCP-1 provokes mast cell activation and has chemotactic activity for monocytes that mature into macrophages at the site of inflammation [33]. MIP-1 released from mast cells and macrophages can bind heparin and has inflammatory and neutrophil chemokinetic properties [34]. Eotaxin possesses selective chemotactic activity for eosinophils [35,36]. Chemokines not only induce chemotaxis, but also activation of these target cells in the bladder and thereby contribute to the inflammatory-induced changes in rheumatoid arthritis, Crohn disease, and IC/BPS [37].

Analysis of urine from a single void using commercially available multiplex immunoassays based on Luminex xMAP technology from Millipore (Billerica, MA, USA) revealed 10-fold to 100-fold elevation in the levels of MCP-1, MIP-1, and eotaxin in patients with varying severities of IC/BPS compared with control patients [38]. The urinary proteome is a potential easily accessible source of biomarkers for inflammatory bladder disorders, including IC/BPS and OAB. Analysis of multiple urinary proteins is a convenient approach to monitoring the activation of inflammatory cells in the bladder tissue [39]. Simultaneous analysis of the chemokines MCP-1, MIP-1, and eotaxin in urine can show a disease-specific pattern of IC/BPS with improved sensitivity and specificity. In addition to these inflammatory proteins, we can also detect other urothelial defense proteins that have been found to have a close relationship with the pathogenesis of IC/BPS but are not found in OABs [31].

The urine levels of selected chemokines have been analyzed based on immunoassays using the specificity of antibodies [39]. Proteomics based on immunoassays provide specific detection of protein at much lower concentrations and are more suited to quantification than two-dimensional gel or other generic proteomic approaches. Conventional immunoassays of each chemokine for individual measurements using enzyme-linked immunosorbent assay (ELISA) can incur considerable time, cost, and sample volume, limiting the systematic examination of multiple biomarkers. The xMAP technology allows multiplexing of analytes in solution with flow cytometry, and studies show that there is good correlation of cytokine levels measured by ELISA and multiplex assay [40]. The increased dynamic movement during antibody–antigen reactions that occurs in solution may also increase its assay efficiency [41]. Theoretically, the Luminex platform can provide a wider dynamic range than conventional ELISA methods because of the greater linear range of fluorescence intensity compared with absorbance [42].

In a recent study, mean urine cytokine/chemokine levels were higher in OAB-wet than OAB-dry, suggesting a linear relationship between symptom severity and cytokine levels. This analysis revealed significant elevation of seven key proteins in the urine of patients with OAB relative to control patients. A greater than 10-fold elevation in the levels of MCP-1 and the soluble fraction of the CD40 ligand was measured in patients with OAB, relative to control patients. At least fivefold elevations were detected in the levels of MIP-1β, interleukin (IL)-12,p70/p40, IL-5, EGF, and growth-related oncogene-α compared with control patients. Significant threefold elevation was also noted in the urine levels of soluble IL-2Rα, and IL-10 in the OAB group [38].

In a cross-sectional study testing the hypothesis that select chemokines are increased in the urine of patients with IC/BPS, midstream urinary specimens were collected from 10 patients with ulcerative and nonulcerative IC/BPS, and from 10 asymptomatic control patients. Urinary levels of most chemokines/cytokines were 10-fold to 100-fold lower in asymptomatic control patients versus patients with ulcerative and nonulcerative IC/BPS. Univariate comparison of eight tested proteins in the ulcerative versus nonulcerative groups revealed a significant fivefold to 20-fold increase in C-X-C motif chemokine-10 and -1, IL-6, and NGF [43]. Because elevated urinary chemokines/cytokines have been noted in patients with OAB and those with IC/BPS, it will be interesting to investigate the differences in these levels between these two conditions.

IC/BPS is a difficult disease to diagnose and treat. Objective information on the disease is only available in patients with end-organ disease using expensive and invasive procedures such as cystoscopy and tissue biopsy [44]. Development of biomarkers for the diagnosis and prediction of IC/BPS progression is therefore a high priority. Urinary biomarker identification using proteome-wide analysis may provide an unbiased noninvasive and expeditious diagnosis of IC/BPS. Histologic studies done on tissue biopsies from IC/BPS patients document infiltration of mast cells, macrophages, and eosinophils as a consistent finding [45–48].

The general function of chemokines is inflammatory cell adhesion and infiltration of leukocytes into inflammatory foci. Chemokines measured in previous studies could originate in the urine from both resident and infiltrating cells, including leukocytes, epithelial cells, and detrusor smooth muscle cells [49]. Bladder biopsies of patients with IC/BPS reported previously have confirmed the involvement and presence of eosinophils and macrophages in the urothelium and mast cells in the detrusor.

Table 2
Comparison of urine nerve growth factor and serum nerve growth factor between patients with interstitial cystitis/bladder pain syndrome and control patients [53].

	Control ($n = 28$)	IC/BPS ($n = 30$)	p
Age (range)	32.6 ± 1.56 (22–55)	51.3 ± 1.87 (22–86)	< 0.001
Sex	F:17 M:11	F:26 M:4	
Urinary NGF (pg/mL)	1.40 ± 0.63 (0.00–13.6)	26.3 ± 11.2 (0.00–270.4)	0.014
Urinary NGF/Cr (pg/mg)	0.02 ± 0.01 (0.00–0.22)	0.69 ± 0.38 (0.00–-9.52)	0.011
Serum NGF (pg/mL)	1.90 ± 0.38 (0.00–5.85)	3.48 ± 0.55 (0.00–18.0)	0.015

Data are expressed as mean ± standard error.
Cr = creatinine; IC/BPS = interstitial cystitis/bladder pain syndrome; NGF = nerve growth factor.

Involvement of eosinophils was also supported by urine cytology results showing increased urinary eosinophil cationic protein in the urine of patients with IC/BPS [10,50,51]. Mast cells have been considered crucial effector cells for the immune response implicated in the pathogenesis of IC/BPS [9].

6. Serum biomarkers for OAB and IC/PBS

Previous study of serum NGF levels in patients with OAB and IC/BPS revealed that serum NGF levels were significantly elevated in patients with OAB compared with control patients. Urinary NGF/creatinine (Cr) levels were significantly elevated in patients with OAB compared with control patients. Serum NGF levels were significantly correlated with urinary NGF and NGF/Cr levels in patients with OAB. There was no significant difference in serum NGF levels between OAB-dry and OAB-wet. Increased serum and urinary NGF levels in patients with OAB refractory to anti-muscarinic treatment suggest these bladder disorders might be caused by chronic inflammation [52]. Interestingly, we also found that serum NGF was elevated in patients with IC/BPS. The mean serum NGF level was higher in patients with IC/BPS (3.48 ± 0.55 pg/mL) than in control patients (1.90 ± 0.38 pg/mL; $p = 0.015$). No significant correlation was found between the serum and urinary NGF levels in patients with IC/BPS. However, the clinical characteristics and medical comorbidities did not show significant differences between patients with IC/BPS with high and low serum NGF levels [53] (Table 2). These results suggest that although chronic inflammation is involved in both OAB and IC/BPS, the underlying pathophysiology for the phenotype presentation might be different. Investigation of serum chemokine/cytokine levels might provide a differentiation between these two bladder disorders.

Recent investigations have linked OAB with neurogenic inflammation. Neurotrophic factors have been implicated in the pathophysiologic mechanisms underlying the sensitization of bladder afferent nerves. NGF, initially described as a prototypical trophic factor in the development of sensory and sympathetic innervation, has emerged as a complex regulator of neural plasticity along the micturition pathways. Hepatocyte growth factor (HGF), a novel neurotrophic factor, also can promote neuronal survival and growth. In our previous studies, serum and urinary NGF levels were demonstrated to increase in patients with OAB. Therefore, the expression of serum HGF and NGF, and suburothelial neuron marker PGP9.5 was investigated in patients with OAB. The results of serum adipokine assay showed both serum HGF and serum NGF levels were significantly higher in patients with OAB than in control patients [54] (Table 3). There was a significant correlation between serum HGF and serum NGF levels. Although the patients with OAB were older then the control patients, neither serum HGF nor NGF correlated with age. Patients with OAB also had higher expression of the mucosal neuron marker PGP9.5 than control patients.

HGF is a strong neurotrophic factor that promotes cell survival and proliferation. Some studies have reported that HGF cooperates with NGF to enhance the growth of neurons *in vitro*. In our study, both serum HGF and NGF levels were high and were significantly correlated with each other in patients with OAB. The PGP9.5 suburothelial neuron marker expression in all of the patients with OAB was significantly higher than in the control patients, suggesting nerve fibers were increased in the bladder mucosa of patients with OAB. Our recent studies have also demonstrated that patients with OAB have higher expression of serum and urinary NGF than control patients, indicating that HGF has neurotrophic properties. Elevation of serum HGF and NGF in patients with OAB is associated with an increase in PGP9.5 expression, which suggests systemic neurotrophic factor elevation induces increases in nerve fibers in the suburothelium of the bladder, which might play a role in urgency and frequency sensory disorder in patients with OAB.

Cytokines and chemokines play crucial roles in the pathogenesis of several chronic inflammatory diseases. The upregulated profile of serum IL-1β, IL-6, TNF-α, and IL-8 levels in patients with IC/BPS might potentially have a prognostic role and/or serve as a tool in choosing a proper therapeutic agent for treatment. One recent study revealed that serum proinflammatory cytokines (IL-1β, IL-6, TNF-α) and chemokine (IL-8) levels were significantly higher in the serum of patients with IC/BPS than control patients [55] (Table 4). Although the patients with IC/BPS were older than the control patients, these serum cytokine/chemokine levels were not correlated with age. A significant correlation was found between IL-1β and IL-8, IL-6 and CRP, IL-6 and IL-8, and IL-6 and TNF-α in IC/BPS serum samples. Increased expression of the proinflammatory cytokines (IL-1β, IL-6, TNF-α) and chemokine (IL-8) in the serum of patients with IC/BPS patients that not only mast cell activation but also some other inflammatory mediators play important roles in the pathogenesis of IC/BPS.

Table 3
Serum cytokine levels between patients with overactive bladder and control patients [54].

	Control ($n = 26$)	OAB ($n = 30$)	p
Age	32.4 ± 1.56 (22–55)	61.0 ± 1.59 (39–73)	<0.0001
Sex	F:16 M:10	F: 17 M: 13	
HGF (pg/mL)	104.5 ± 11.39 (4.24–267.39)	193.6 ± 17.72 (50.11–430.57)	<0.0001
NGF (pg/mL)	2.78 ± 0.22 (1.0–5.85)	3.88 ± 0.60 (1.70–17.54)	0.04
PGP9.5	0.37 ± 0.059 ($n = 5$)	0.54 ± 0.06 ($n = 10$)	0.03

Mean ± standard error (range).
HGF = hepatocyte growth factor; NGF = nerve growth factor; OAB = overactive bladder.

Table 4
Expression of serum interleukin-1β, interleukin-6, tumor necrosis factor-α, and interleukin-8 in patients with interstitial cystitis/bladder pain syndrome and control patients [55].

	Controls ($n = 26$)	IC/BPS ($n = 30$)	p
Sex	F:16 M:10	F:26 M:4	
Age	32.4 ± 1.56 (22–55)	50.6 ± 2.68 (24–86)	<0.0001
IL-1β (pg/mL)	1.64 ± 0.47 (0.00–6.08)	6.45 ± 0.71 (2.77–23.96)	<0.0001
IL-6 (pg/mL)	0.79 ± 0.21 (0.00–3.67)	1.52 ± 0.24 (0.00–6.14)	<0.0001
TNF-α (pg/mL)	0.91 ± 0.17 (0.00–4.64)	2.63 ± 0.60 (0.62–13.70)	<0.0001
IL-8 (pg/mL)	1.45 ± 0.21 (0.00–4.09)	3.23 ± 0.48 (0.00–15.08)	<0.0001

Mean ± standard error (range).
IC/BPS = interstitial cystitis/bladder pain syndrome; IL = interleukin; TNF = tumor necrosis factor.

7. Conclusion

Analysis of multiple urinary proteins and serum cytokines is a convenient approach to monitoring the activation of inflammatory cells in the bladder tissue. Differences in urinary proteins and serum cytokines might provide a diagnostic basis for IC/BPS and could be a tool for the differential diagnosis between IC/BPS and OAB.

References

[1] Dupont MC, Spitsberegen JM, Kim KB, Tuttle JB, Steers WD. Histological and neurotrophic changes triggered by varying models of bladder inflammation. J Urol 2001;166:1111–8.
[2] Bouchelouche K, Nordling J. Recent developments in the management of interstitial cystitis. Curr Opin Urol 2003;13:309–13.
[3] Hanno PM, Sant GR. Clinical highlights of the National Institute of Diabetes and Digestive and Kidney Diseases/Interstitial Cystitis Association scientific conference on interstitial cystitis. Urology 2001;57:2–6.
[4] Abrams P, Kelleher CJ, Kerr LA, Rogers RG. Overactive bladder significantly affects quality of life. Am J Manag Care 2000;6:S580–90.
[5] Yiangou Y, Facer P, Ford A, Brady C, Wiseman O, Fowler CJ, et al. Capsaicin receptor VR1 and ATP-gated ion channel P2X3 in human urinary bladder. BJU Int 2001;87:774–9.
[6] Apostolidis A, Popat R, Yiangou Y, Cockayne D, Ford AP, Davis JB, et al. Decreased sensory receptors P2X3 and TRPV1 in suburothelial nerve fibers following intradetrusor injections of botulinum toxin for human detrusor overactivity. J Urol 2005;174:977–83.
[7] Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. II. Intestinal subepithelial myofibroblasts. Am J Physiol 1999;277: C183–201.
[8] Van De Merwe JP, Arendsen HJ. Interstitial cystitis: a review of immunological aspects of the aetiology and pathogenesis, with a hypothesis. BJU Int 2000;85: 995–9.
[9] Sant GR, Kempuraj D, Marchand JE, Theoharides TC. The mast cell in interstitial cystitis: role in pathophysiology and pathogenesis. Urology 2007;69: 34–40.
[10] Lowe EM, Anand P, Terenghi G, Williams-Chestnut RE, Sinicropi DV, Osborne JL. Increased nerve growth factor levels in the urinary bladder of women with idiopathic sensory urgency and interstitial cystitis. Br J Urol 1997;79:572–7.
[11] Liu HT, Tyagi P, Chancellor MB, Kuo HC. Urinary nerve growth factor level is increased in patients with interstitial cystitis/bladder pain syndrome and decreased in responders to treatment. BJU Int 2009;104:1476–81.
[12] Chung SD, Liu HT, Lin H, Kuo HC. Elevation of serum C-reactive protein in patients with OAB and IC/BPS implies chronic inflammation in the urinary bladder. Neurourol Urodyn 2011;30:417–20.
[13] Wiseman OJ, Fowler CJ, Landon DN. The role of the human bladder lamina propria myofibroblast. BJU Int 2003;91:89–93.
[14] Avelino A, Cruz C, Nagy I, Cruz F. Vanilloid receptor 1 expression in the rat urinary tract. Neuroscience 2002;109:787–98.
[15] Tuttle JB, Steers WD, Albo M, Nataluk E. Neural input regulates tissue NGF and growth of the adult urinary bladder. J Auton Nerv Syst 1994;49:147–58.
[16] Steers WD, Kolbeck S, Creedon D, Tuttle JB. Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. J Clin Invest 1991; 88:1709–15.
[17] Okragly AJ, Niles AL, Saban R, Schmidt D, Hoffman RL, Warner TF, et al. Elevated tryptase, nerve growth factor, neurotrophin-3 and glial cell line-derived neurotrophic factor levels in the urine of interstitial cystitis and bladder cancer patients. J Urol 1999;161:438–41.
[18] Kuo HC, Liu HT, Tyagi P, Chancellor MB. Urinary nerve growth factor levels in urinary tract diseases with or without frequency urgency symptoms. LUTS 2010;2:88–94.
[19] Murray E, Malley SE, Qiao LY, Hu VY, Vizzard MA. Cyclophosphamide induced cystitis alters neurotrophin and receptor tyrosine kinase expression in pelvic ganglia and bladder. J Urol 2004;172:2434–9.
[20] Oddiah D, Anand P, McMahon SB, Rattray M. Rapid increase of NGF, BDNF and NT-3 mRNAs in inflamed bladder. Neuroreport 1998;9:1455–8.
[21] Chung YC, Fraser MO, Yu Y, Chancellor MB, de Groat WC, Yoshimura N. The role of bladder afferent pathways in bladder hyperactivity induced by the intravesical administration of nerve growth factor. J Urol 2001;165: 975–9.
[22] Seki S, Sasaki K, Fraser MO, Igawa Y, Nishizawa O, Chancellor MB, et al. Immunoneutralization of nerve growth factor in lumbosacral spinal cord reduces bladder hyperreflexia in spinal cord injured rats. J Urol 2002;168: 2269–74.
[23] Lamb K, Gebhart GF, Bielefeldt K. Increased nerve growth factor expression triggers bladder overactivity. J Pain 2004;5:150–6.
[24] Kuo HC. Preliminary results of suburothelial injection of botulinum A toxin in the treatment of chronic interstitial cystitis. Urol Int 2005;75:170–4.
[25] Liu HT, Kuo HC. Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. Urology 2007;70:463–8.
[26] Apostolidis A, Jacques TS, Freeman A, Freeman A, Kalsi V, Popat R, et al. Histological changes in the urothelium and suburothelium of human overactive bladder following intradetrusor injections of botulinum neurotoxin type A for the treatment of neurogenic or idiopathic detrusor overactivity. Eur Urol 2008;53:1245–53.
[27] Geppetti P, Nassini R, Materazzi S, Benemei S. The concept of neurogenic inflammation. BJU Int 2008;101:2–6.
[28] Liao CH, Chung SD, Kuo HC. Serum C-reactive protein levels are associated with residual urgency symptoms in patients with benign prostatic hyperplasia after medical treatment. Urology 2011;78:1373–8.
[29] Hsiao SM, Lin HH, Kuo HC. The role of serum C-reactive protein in women with lower urinary tract symptoms. Int Urogynecol J 2012;23:935–40.
[30] Hurst RE, Moldwin RM, Mulholland SG. Bladder defense molecules, urothelial differentiation, urinary biomarkers, and interstitial cystitis. Urology 2007;69: 17–23.
[31] Wilkinson DR, Erickson AD. Urinary and serologic markers for interstitial cystitis: an update. Curr Urol Rep 2006;7:414–22.
[32] Mortier A, Van Damme J, Proost P. Regulation of chemokine activity by posttranslational modification. Pharmacol Ther 2008;120:197–217.
[33] Bouchelouche K, Alvarez S, Andersen L, Nordling J, Horn T, Bouchelouche P. Monocyte chemoattractant protein-1 production by human detrusor smooth muscle cells. J Urol 2004;171:462–6.
[34] Torrence AE, Brabb T, Viney JL, Bielefeldt-Ohmann H, Treuting P, Seamons A, et al. Serum biomarkers in a mouse model of bacterial-induced inflammatory bowel disease. Inflamm Bowel Dis 2008;14:480–90.
[35] Lintomen L, Franchi G, Nowill A, Condino-Neto A, de Nucci G, Zanesco A, et al. Human eosinophil adhesion and degranulation stimulated with eotaxin and RANTES in vitro: lack of interaction with nitric oxide. BMC Pulm Med 2008;8:13.
[36] Muessel MJ, Scott KS, Friedl P, Bradding P, Wardlaw AJ. CCL11 and GM-CSF differentially use the Rho GTPase pathway to regulate motility of human eosinophils in a three-dimensional microenvironment. J Immunol 2008;180: 8354–60.
[37] Segerer S, Nelson PJ. Chemokines in renal diseases. Scientific World J 2005;5: 835–44.
[38] Tyagi P, Barclay D, Zamora R, Yoshimura N, Peters K, Vodovotz Y, et al. Urine cytokines suggest an inflammatory response in the overactive bladder: a pilot study. Int Urol Nephrol 2010;42:629–35.
[39] Smaldone MC, Vodovotz Y, Tyagi V, Barclay D, Philips BJ, Yoshimura N, et al. Multiplex analysis of urinary cytokine levels in rat model of cyclophosphamide-induced cystitis. Urology 2009;73:421–6.
[40] Kofoed K, Schneider UV, Scheel T, Andersen O, Eugen-Olsen J. Development and validation of a multiplex add-on assay for sepsis biomarkers using xMAP technology. Clin Chem 2006;52:1284–93.
[41] Buttram SD, Wisniewski SR, Jackson EK, Adelson PD, Feldman K, Bayor H, et al. Multiplex assessment of cytokine and chemokine levels in cerebrospinal fluid following severe pediatric traumatic brain injury: effects of moderate hypothermia. J Neurotrauma 2007;24:1707–17.
[42] Prabhakar U, Eirikis E, Davis HM. Simultaneous quantification of proinflammatory cytokines in human plasma using the LabMAP assay. J Immunol Methods 2002;260:207–18.
[43] Tyagi P, Killinger K, Tyagi V, Nirmal J, Chancellor M, Peters KM. Urinary chemokines as noninvasive predictors of ulcerative interstitial cystitis. J Urol 2012;187:2243–8.
[44] Erickson DR, Tomaszewski JE, Kunselman AR, Bentley CM, Peters KM, Rovner ES, et al. Do the National Institute of Diabetes and Digestive and Kidney Diseases cystoscopic criteria associate with other clinical and objective features of interstitial cystitis? J Urol 2005;173:93–7.
[45] Koskela LR, Thiel T, Ehren I, De Verdier PJ, Wiklund NP. Localization and expression of inducible nitric oxide synthase in biopsies from patients with interstitial cystitis. J Urol 2008;180:737–41.
[46] Erickson DR, Belchis DA, Dabbs DJ. Inflammatory cell types and clinical features of interstitial cystitis. J Urol 1997;158:790–3.
[47] Christmas TJ, Bottazzo GF. Abnormal urothelial HLA-DR expression in interstitial cystitis. Clin Exp Immunol 1992;87:450–4.

[48] Christmas TJ, Rode J. Characteristics of mast cells in normal bladder, bacterial cystitis and interstitial cystitis. Br J Urol 1991;68:473–8.
[49] Bouchelouche K, Andresen L, Alvarez S, Nordling J, Nielsen OH, Bouchelouche P. Interleukin-4 and 13 induce the expression and release of monocyte chemoattractant protein 1, interleukin-6 and stem cell factor from human detrusor smooth muscle cells: synergy with interleukin-1beta and tumor necrosis factor-alpha. J Urol 2006;175:760–5.
[50] Dodd LG, Tello J. Cytologic examination of urine from patients with interstitial cystitis. Acta Cytol 1998;42:923–7.
[51] Bouchelouche K, Kristensen B, Nordling J, Horn T, Bouchelouche P. Increased urinary leukotriene E4 and eosinophil protein X excretion in patients with interstitial cystitis. J Urol 2001;166:2121–5.
[52] Liu HT, Lin H, Kuo HC. Increased serum nerve growth factor levels in patients with overactive bladder syndrome refractory to antimuscarinic therapy. Neurourol Urodyn 2011;30:1525–9.
[53] Liu HT, Kuo HC. Increased urine and serum nerve growth factor levels in interstitial cystitis suggest chronic inflammation is involved in the pathogenesis of disease. PLos One 2012;7:e44687.
[54] Liu HT, Kuo HC. Elevation of serum hepatic growth factor and nerve growth factor in patients with overactive bladder implies nerve fibers increasing in urinary bladder. Neurourol Urodyn 2012;31:806.
[55] Liu HT, Kuo HC. Increased pro-inflammatory cytokine and chemokine expressions in serum of patients with interstitial cystitis/bladder pain syndrome. ICS Abstract 480; 2012.