Novel Urinary Biomarkers in the Diagnosis and Assessment of Overactive Bladder

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INTRODUCTION

According to the 2002 guidelines of the International Continence Society (ICS), overactive bladder (OAB), also called urge syndrome or urgency-frequency syndrome, is a symptom syndrome consisting of urinary urgency with or without urgency incontinence, and is usually accompanied by daytime frequency and nocturia in the absence of proven infection or other obvious pathology [1] .Urgency is the core symptom of OAB [2]. Urgency is defined as "a sudden compelling desire to pass urine that is difficult to defer" and the degree is measured subjectively using an urgency severity scale. However, the method of measuring urgency leads to wide variation in different grades of severity, and there is no objective method to diagnose and define the severity of OAB. Urodynamics is a well-established method for diagnosing detrusor overactivity (DO). Although most patients with urgency-frequency symptoms have DO, patients with OAB symptoms do not necessarily have DO or incontinence [3]. A better way to diagnose OAB or DO in patients who present with storage lower urinary tract symptoms is needed.

PAPHOPHYSIOLOGY OF OVERACTICE BLADDER

OAB is a clinical and symptomatic diagnosis and DO is a urodynamic diagnosis. However the precise etiologies of OAB and DO have not yet been discovered. It is thought that bladder sensation originates from afferent nerve endings in the urothelium and suburothelium. Sensory stimuli associated with bladder fullness ascend to the spinal cord and brain centers via myelinated *Aδ*-afferent axons. Although the majority of suburothelial afferents are unmyelinated C-fibres, their receptors have a high mechanical threshold and they are of a nociceptive nature, responding only to irritant stimuli (changes in pH, temperature, chemical irritation).

Novel studies have used models that mimic the OAB associated with bladder instability, lower urinary tract obstruction, neuropathic disorders, diabetes, and interstitial cystitis. These models share the common features of increased connectivity and excitability of both detrusor smooth muscle and nerves. Increased excitability and connectivity of nerves involved in micturition rely on growth factors that orchestrate neural plasticity. Receptors (Transient Receptor Potential Vanilloid 1, Purinergic receptor $P2X_3$), Neurotransmitters (Adenosine Triphosphate, Nitro Oxide, Acetylcholine, tachykinins), prostaglandins, and nerve growth factor, provide mechanisms for bidirectional communication between muscle or urothelium and nerve, leading to OAB and DO [4,5].

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NERVE GROWTH FACTOR

Nerve growth factor (NGF) is characterized as a signaling protein that promotes the survival of dorsal root ganglia and sympathetic neurons during embryonic and perinatal life [6,7]. NGF is also involved in the ongoing regulation of neural function, as well as in inflammation and pain [8]. Evidence has shown that visceral epithelia are a major source of NGF production and that NGF may regulate the function of adult visceral sensory and motor neurons [9]. NGF is a signaling protein secreted by NGF producing cells which interacts with specific receptors in autocrine, paracrine and endocrine modes. In the urinary tract, NGF is produced by bladder smooth muscle and urothelium [10]. Although NGF can affect a variety of cell types in the bladder, the preponderance of data relates to the effects of increased or decreased NGF on bladder afferent fibers. Human and animal data reveal that the bladder can increase production of NGF in response to a wide array of conditions including spinal cord injury, denervation, inflammation, distension and hypertrophy [10,11]. NGF has been shown to act on the Trk-A receptor, which activates a signaling pathway and increases membrane expression of the TRPV1 heat-gated ion channel [12]. In OAB or bladder inflammation, NGF production might be increased and induce a lowering of the volume threshold for voiding as well as unstable detrusor contractions.

Clinical and experimental data indicate a direct link between increased levels of NGF in the bladder tissue and urine and painful inflammatory conditions in the lower urinary tract, such as interstitial cystitis and chronic prostatitis [10,11]. Intravesical instillation of NGF has been shown to induce bladder hyperactivity in rats [13]. Increased levels of NGF have also been reported in the bladder tissue and urine of patients with sensory urgency and DO [14-16]. Increased levels of NGF in urine could increase bladder sensation or cause DO through some undetermined pathways [17].

NGF in OAB

Kim et al compared the changes in urinary NGF in men and women with overactive bladder syndrome using the Emax ImmunoAssay System (Promega, Madison, WI, USA) to assess the NGF level in the urine. Data showed the urinary levels of NGF were significantly increased in patients with OAB compared with healthy controls [18,19]. It is rational to hypothesize that NGF produced in the urothelium and suburothelium can be secreted into the bladder lumen. Stretching of the urothelium

might induce production of NGF in the bladder tissue and secretion into the urine. Liu and Kuo et al found that patients with OAB dry (0.39 pg/mL ± 0.08) and OAB wet (1.7 pg/mL ± 0.26) had significantly higher urinary NGF levels than controls and patients with increased bladder sensation [20]. The sensitivity of a urinary NGF/Cr level >0.05 in the diagnosis of OAB dry or OAB wet was 67.9% and the specificity was 93.8%. The urinary NGF level seems to be a potential biomarker for diagnosis of OAB.

NGF in BOO/OAB

Patients with bladder outlet obstruction (BOO) could have coexistent storage symptoms such as OAB. Symptoms of OAB can resolve after relief of BOO but about 50% of patients have persistent OAB symptoms after surgical intervention for benign prostatic hyperplasia (BPH), suggesting OAB may occur directly or may not be related to BOO [21]. Therefore Liu and Kuo compared the urinary NGF levels in patients with BOO who had different types of bladder dysfunction [22]. The results showed urinary NGF levels were very low in the control group (0.005±0.003) and in patients with BOO/non-OAB (0.017±0.009), and significantly higher in patients with BOO/OAB (0.81±0.31) and BOO/DO (0.80±0.13). In addition, the urinary NGF levels returned to normal levels (0.059±0.021) after relief of OAB symptoms by medical treatment, suggesting that urinary NGF might be a potential biomarker for BOO with symptoms of OAB.

NGF in mixed SUI

OAB and stress urinary incontinence (SUI) are intimately associated and most urgency symptoms occur in combination with SUI [23]. Clinically, it might not be that easy to separate urge incontinence (UI) and SUI, especially when the bladder is extremely full. Previous studies have also found that anti-incontinence surgery in women with mixed SUI and DO can result in a higher failure rate and persistent UI after the operation [24,25]. Liu et al reported that urinary NGF levels were low both in controls (0.06±0.004) and in women with pure urodynamic stress incontinence (USI) (0.056 ± 0.037) (p=0.108) but were significantly higher in women with mixed USI and DO (1.00±0.244) than in controls (p=0.000) and in pure USI patients (p=0.006), but were similar to the levels in women with pure DO (0.58 ± 0.17, p=0.058) [26]. The urinary NGF level seems to be a potential biomarker of DO in women with mixed urinary incontinence.

NGF with therapeutic effect

Currently antimuscarinics are the most commonly used treatment for OAB. Antimuscarinic treatment has been considered to lower detrusor contractility during bladder filling in treating urgency and urge incontinence in OAB patients [27]. However, OAB is a condition related to bladder filling, during which period the sacral parasympathetic outflow is absent [28]. The urgency sensation or detrusor overactivity contractility might not be controlled through motor actions. Detrusor injection of botulinum toxin A (BoNT-A) has been demonstrated to provide therapeutic improvement for patients with OAB, intractable idiopathic detrusor overactivity (IDO) and neurogenic detrusor overactivity (NDO) [29-32]. Liu et al found that the urinary NGF levels decreased at 2 weeks and remained persistently low at 3 months after antimuscarinic treatment [33]. For patients with failed antimuscarinic therapy for OAB, the urinary NGF levels were also reduced but still higher than that in the controls. Furthermore Liu et al reported that patients who responded to BoNT-A treatment had significantly reduced urinary NGF/Cr levels in both the IDO (0.07 ± 0.12, p=0.025) and NDO (0.096 ± 0.17, p=0.033) groups compared to baseline levels [34]. However, the NGF levels remained significantly higher at 3 months in 7 IDO (1.01±1.25) and 5 NDO (1.64 ± 2.39) patients who failed BoNT-A treatment. The urinary NGF level could be used as a tool to evaluate therapeutic results of antimuscarinic therapy for OAB and the therapeutic effect of detrusor BoNT-A injection.

NGF with volume effect

In the above studies of urinary NGF, the urine samples were collected at varying bladder volumes. It is rational to postulate that only a small amount of NGF is secreted into the urine in diseased bladders if the urine samples are collected when the bladder is not full. Once the bladder is distended to a critical volume, the NGF secretion into urine might be highly elevated. NGF levels should be correlated with bladder volume or bladder sensation. To achieve a higher sensitivity rate for diagnosis of OAB, Liu and Kuo designed a study to measure the urinary NGF levels at different bladder volumes in patients with OAB and healthy controls [35]. Data showed that urinary NGF/Cr levels in healthy controls were very low at the first sensation of bladder filling (FSF) (0.011 ± 0.008) and were significantly higher at urge sensation (US) (0.086 ± 0.022, p=0.005). Patients with OAB had significantly higher urinary NGF/Cr levels at both FSF (0.45 ± 0.13, p=0.001) and US (1.00 ± 0.32, p=0.004) compared to controls. The difference in urinary NGF/Cr levels between FSF and US in OAB patients, however, was not significant (p=0.064). The urinary NGF/Cr level was well correlated with bladder volume at US in the controls but not significantly associated with bladder volume increase in patients with OAB. The results of this study suggest urinary NGF increases physiologically in healthy subjects at urge to void but is pathologically elevated in OAB patients at small bladder volumes and does not significantly increase at urgency sensation.

Summary

From the results of these studies, urinary NGF is found specifically higher in patients with OAB or DO but is much lower in healthy controls or patients with pure BOO or SUI. Urodynamic study is necessary to accurately diagnose DO. Although urodynamic study can not be replaced by measurement of urinary NGF, measurement of urinary NGF levels is a simple method of evaluating DO. It has been found to have good sensitivity and specificity for patients with OAB and could be an adjunct to the use of urodynamic study in the differential diagnosis of female incontinence and BOO. As with tumor markers, urinary NGF could be used as a biomarker of therapeutic results or severity of disease.

PROSTAGLANDIN E2

Prostaglandin E2 (PGE2) is produced directly in the tissue by cyclooxygenase (COX) from arachidonic acid, and it is produced on urothelium or bladder muscle [36,37]. PGE2 has been suggested to play a physiological role in contributing to the basal tone of the detrusor and modulating the activity of bladder nerves [38]. Intravesical instillation of PGE2 induces detrusor contraction while topical application of PGE2 to the urethra causes urethral relaxation in rats [39]. Prostaglangin EP3 receptors exert an excitatory effect on urinary blad-

der function via modulation of bladder afferent pathways [40]. An increased PGE2 level in the bladder is likely to be associated with long-standing storage dysfunction [41]. Kim et al found that in patients with OAB, the urinary PGE2 level significantly increased and the PGE2 levels were negatively correlated with the maximum cystometric capacity [19]. However Liu et al measured urinary PGE2 levels among patients with OAB-wet, OAB-dry, interstitial cystitis/painful bladder syndrome (IC/PBS) and controls [42]. They found that urinary PGE2/Cr levels were not significantly different among subgroups. Urinary PGE2 levels have no clinical implication in the differential diagnosis between OAB and IC/PBS. The role of urinary PGE2 in the diagnosis of OAB seems controversial and needs further clarification.

FUTURE STUDY

Recent studies have noted signs of inflammation in the bladder biopsy specimens of OAB patients [43,44]. It is hypothesized that in the absence of urinary tract infection, an inflammatory process in the bladder may be responsible for the pathophysiology of OAB. The urinary proteome is a potential easily accessible source of biomarkers for inflammatory bladder disorders, including IC/PBS and OAB. Analysis of multiple urinary proteins is a convenient approach to monitor the activation of inflammatory cells in bladder tissue [45]. A pilot study from Tyagi et al showed significant elevation of 11 proteins (MIP-1β, IL-17, IL-12p70/p41, MCP-1 etc) in the urine in OAB patients relative to controls [46]. In addition, the higher urine cytokine levels in OAB wet relative to OAB dry suggest a relationship between symptom severity and bladder inflammation. Elevated urinary cytokines could be found in inflammatory bladder disease, such as UTI, IC/PCS, OAB or tumors. So it is still difficult to justify and differentiate disease categories by using analysis of multiple urinary proteins. If we can find a cluster of urinary proteins which exhibit higher levels in IC/PBS than OAB, we might discover a different inflammatory pathway between these two conditions.

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