Can Urinary Nerve Growth Factor Be a Biomarker for Overactive Bladder?

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The clinical diagnosis of overactive bladder (OAB) greatly varies and is based on subjective symptoms. A more objective method to diagnose and assess therapeutic outcome in OAB patients, especially for health care providers not trained in urology, needs to be found. Evidence has shown that urinary proteins such as nerve growth factor (NGF) and prostaglandin E2 levels increase in patients with OAB, bladder outlet obstruction, and detrusor overactivity. Urinary NGF level increases physiologically in normal subjects at urge to void, but increases pathologically in OAB patients at a small bladder volume and with a sensation of urgency. Recent studies have shown that patients with OAB dry and OAB wet have significantly higher urinary NGF levels compared with control groups and patients with increased bladder sensation. Urinary NGF levels decrease after antimuscarinic therapy and further decrease after detrusor botulinum toxin injections in refractory OAB. Urinary NGF level could be a potential biomarker for diagnosis of OAB and assessment of the therapeutic effect of antimuscarinic therapy. The latest medical advances in this field are reviewed herein.


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Urgency-frequency symptoms may be due to psychologic factors, increased urine production, uninhibited urge to void due to central nervous system (CNS) lesions, and detrusor overactivity (DO). Patients with increased bladder sensation are often misdiagnosed as having OAB if they mistakenly report frequency as a strong desire to void. The increased bladder sensation may be caused by an increased alertness to bladder fullness or polyuria. If patients are requested to hold urine, they may have an adequate voided volume without the sensation of urgency. Clinically, it can be difficult to identify patients with increased bladder sensation from patients with OAB.

The pathophysiology of OAB has not been fully delineated. Recent studies have postulated that urothelial dysfunction, abnormal expression of sensory receptors, increased excitability of the detrusor muscles, and CNS sensitization may contribute to the development of OAB. Hashim and Abrams found that 69% of men and 44% of women with urgency (OAB dry) had DO, whereas 90% of men and 58% of women with urgency and urge incontinence (OAB wet) had DO. Although urodynamics is a well-established method for diagnosing the presence of DO, a simpler and more cost-effective method to diagnose OAB and assess therapeutic outcome in patients with OAB needs to be found.

Nerve Growth Factor in the Bladder Tissue and Urine

Nerve growth factor (NGF) is a small secreted protein that induces the differentiation and survival of particular target neurons. It is the prototypical growth factor, in that it is one of the first to be described. Stanley Cohen and Rita Levi-Montalcini won the 1986 Nobel Prize in Physiology or Medicine for their discovery of NGF and other growth factors. NGF has been implicated as a chemical mediator of pathology-induced changes in C-fiber afferent nerve excitability and reflex bladder activity. Levels of neurotrophic factors, including NGF, increase in the bladder after spinal cord injury (SCI), and increased levels of NGF have been detected in the lumbosacral spinal cord and dorsal root ganglia of rats after SCI. It has been demonstrated that chronic administration of NGF into the spinal cord or chronic administration of NGF into the bladder of rats induces bladder hyperactivity and increases the firing frequency of dissociated bladder afferent neurons.

NGF antibodies produce effects similar to the effect of desensitizing C-fiber afferents with capsaicin or resiniferatoxin. Intrathecal administration of NGF antibodies also block autonomic dysreflexia induced by bladder or distal bowel distension in SCI rats. Thus, NGF and its receptors in the bladder and/or the spinal cord are potential targets for new therapies to reduce voiding dysfunction after SCI.

NGF has also attracted considerable attention as a key player in the link between inflammation and altered pain signaling. NGF is expressed widely in various cells, including urothelial cells, smooth muscle cells, and mast cells, and can activate mast cells to degranulate and proliferate. In patients with painful bladder syndrome/interstitial cystitis (PBS/IC), neurotrophins, including NGF, neurotrophin-3, and glial cell line–derived neurotrophic factor, have been detected in the urine. Increased expression of NGF is also present in bladder biopsies from patients with PBS/IC.

**Figure 1.** Nerve growth factor (NGF) is released from target cells under irritation due to inflammation, obstruction, or denervation. NGF sensitizes afferent nerves, enhances synaptic transmission, and produces pain sensation as well as increased urinary frequency.

1. Irritation: Inflammation, obstruction, denervation
2. NGF↑
3. Sensitization of afferents and/or increased transport of neurotrophic factors
4. C-fiber phenotype change: Cell size↑, excitability↑, K⁺ channel↓
5. Enhanced synaptic transmission
6. Pain sensation
7. Increased urinary frequency
women with PBS/IC. Thus, target organ-neural interactions mediated by an increase of neurotrophins in the bladder and increased transport of neurotrophins to the neuronal cell bodies in afferent pathways may contribute to the emergence of bladder pain in PBS/IC. Patients with PBS/IC who responded to intravesical botulinum toxin injection have been found to have reduced bladder tissue NGF expression (Figure 2).

In the urinary tract, NGF is produced by urothelium and smooth muscle. Clinical and experimental data indicate a direct link between increased levels of NGF in bladder tissue and urine and painful inflammatory conditions in the lower urinary tract, such as bladder outlet obstruction, overactive bladder (OAB), painful bladder syndrome/interstitial cystitis, and chronic prostatitis.

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A recent study measuring NGF concentration using enzyme-linked immunosorbent assay (ELISA) in superficial bladder biopsies from 12 women with DO and 15 without urodynamic DO did not show a significant correlation with tissue NGF level. It is impossible to standardize the quantity of epithelium; suburothelium and muscle with a bladder biopsy and this study confirm our experience that urine NGF measurement is a simple, safe, and more accurate assay, and one that can be standardized. 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.

The level of NGF in urinary tract, such as bladder outlet obstruction (BOO), OAB, PBS/IC, and chronic prostatitis. Increased levels of NGF have also been reported in the bladder tissue and urine of patients with sensory urgency and DO.

Studies on NGF in OAB or DO usually measure the bladder tissue level. Urine could increase bladder sensation or cause DO through some undetermined pathway. If the urinary NGF level differs among normal controls and patients with increased bladder sensation, OAB dry, or OAB wet, then urinary NGF level could be a biomarker for diagnosing OAB or assessing therapeutic outcome.

Kim and colleagues found that urinary NGF levels increase in men and women with OAB syndrome. Yokoyama and associates evaluated urine NGF in 51 OAB patients that included men and women with DO, OAB without DO, BOO, and neurogenic DO. As there was a relatively small number in each group, the general conclusion was that urinary NGF level is elevated in neurogenic DO in response to BOO, spinal disease, and sensory urgency. However, urinary NGF was not found to elevate in idiopathic DO.

It is reasonable to hypothesize that NGF produced in the urothelium and suburothelium can be secreted into the bladder lumen. Stretching the urothelium might induce production of NGF in bladder tissue and its secretion into urine. Although the levels of NGF in bladder tissue and urine might not correlate well, an interaction between urinary NGF and sensory fibers, as well as an effect on detrusor hyperactivity, is likely. Detrusor injection of botulinum neurotoxin type A (BoNT/A) may have an effect on the inhibition of NGF production in the urothelium and suburothelium, and therefore may decrease urinary NGF levels as well as reduce urgency sensation during bladder filling. Measurement of NGF in the urine is likely to be a more relevant and sensitive biomarker for OAB than bladder tissue NGF level.

**Urinary NGF Level Measurement Technique**

Measurement of urinary NGF level is typically done by the ELISA method. In our studies, we use undiluted voiding urine that is placed on ice immediately and centrifuged at 3000 g at 4°C for 10 minutes. The supernatant is separated into aliquots in 1.5-mL tubes and preserved in a −80°C freezer. At the same time, 3 mL of
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Urinary NGF level

Urinary NGF levels were measured in patients with increased bladder sensation, OAB dry, and OAB wet, and in a group of control subjects without lower urinary tract symptoms (LUTS).29 Urinary NGF/Cr levels were very low in normal controls (0.041 ± 0.026) and patients with increased bladder sensation (0.033 ± 0.02). Patients with OAB dry (0.39 ± 0.08) and OAB wet (1.7 ± 0.26) had significantly higher urinary NGF levels compared with the control group and patients with increased bladder sensation.

This study concluded that patients with OAB dry and OAB wet had significantly higher urinary NGF levels compared with the control group and patients with increased bladder sensation, suggesting that elevated urinary NGF level plays an important role in mediating the sensation of urgency in OAB. This study also showed that patients with OAB wet had significantly higher urinary NGF levels than those with OAB dry. The possible reason for the difference in NGF levels between OAB dry and OAB wet is the higher percentage of DO in patients with OAB wet.

Urinary NGF Level in Patients With Bladder Outlet Obstruction

A previous study has shown that NGF may regulate the neural function of adult visceral sensory and motor neurons.25 The increased level of NGF could trigger changes in bladder afferent fibers, leading to a reduced threshold or increased excitability. Chronic BOO, such as benign prostatic hyperplasia (BPH), could result in stretching of the urothelium and smooth muscle, stimulate NGF production, and alter the afferent nerve pathway. Furthermore, chronic sensitization of afferent nerves could alter the conductance of dorsal nerve ganglia, causing increased excitability and enhanced spinal reflex.30 Incomplete reversibility of neural plasticity might be responsible for continuing urge symptoms following surgical intervention for BOO.31

BOO is associated with LUTS, major storage symptoms of urgency, and nocturia. OAB is frequently associated with BOO in men with BPH and has a high correlation with urodynamic DO. OAB symptoms can resolve after relief of BOO, but approximately 50% of patients have persistent OAB symptoms after surgical intervention for BPH, suggesting OAB may occur directly and may not be related to...
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Urodynamic study is a commonly used tool to diagnose DO in patients with BOO. However, not all patients with BOO and OAB have urodynamically proven DO, and not all patients with urodynamic DO have clinical OAB symptoms.34

In a recent study of urinary NGF/Cr levels in men with BOO, urinary NGF levels were very low in the control group and in patients with BOO/non-OAB, and were significantly elevated in patients with BOO/OAB and BOO/detrusor overactivity. Cr, creatinine; IDO, idiopathic detrusor overactivity; NGF, nerve growth factor; OAB, overactive bladder.

Urinary NGF/creatinine levels returned to normal after successful relief of OAB symptoms by medical treatment.35 These results suggest that urinary NGF might be a potential biomarker for BOO with symptoms of OAB (Figure 4).

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**Figure 4.** Urinary nerve growth factor levels were very low in the control group and in patients with bladder outlet obstruction (BOO)/non-overactive bladder, and were significantly elevated in patients with BOO/OAB and BOO/detrusor overactivity. Cr, creatinine; IDO, idiopathic detrusor overactivity; NGF, nerve growth factor; OAB, overactive bladder.

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**Urinary nerve growth factor (NGF) levels in patients with detrusor overactivity.** Detection of BoNT-A injection into the detrusor decreases NGF bladder tissue levels in patients with NDO.41

The mechanism responsible for the effectiveness of BoNT/A on refractory OAB is believed to occur by inhibition of acetylcholine release from the presynaptic nerve terminals of the neuromuscular junction.42,43 However, more evidence has shown that OAB and DO might be a cause of sensory nerve-mediated hypersensitivity or hyperactivity in addition to myogenic excitability.44 Detrusor injection of BoNT/A reduces urgency and decreases P2X3 and TRPV1 receptor expression in suburothelium.28 Urgency may be mediated by overproduction of some undefined sensory proteins such as NGF, prostaglandin E2, or calcitonine gene–related peptide or overexpression of receptors on suburothelial sensory fibers. NGF could play an important role in the connection between suburothelial sensory fibers and detrusor muscle excitability.44 NGF is believed to be an important mediator in the modulation of urothelial response to inflammation and the sensory threshold of urgency.18
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A cross-sectional study was performed in 143 patients with idiopathic DO and 100 patients with neurogenic DO who were untreated, well-treated, and failed-treated by antimuscarinics.\(^4\) Thirty-eight subjects without lower urinary tract symptoms served as controls. Detrusor injection of BoNT/A (100 U for IDO, 200 U for NDO) was given to 24 patients with IDO and 19 with NDO who had failed antimuscarinic treatment. The mean urinary NGF/Cr levels were significantly higher in 66 patients with untreated IDO (1.44 ± 2.66; \(P = .000\)) and 59 with untreated NDO (0.62 ± 1.22; \(P = .000\)) compared with the control group (0.005 ± 0.019). Patients with well-treated IDO or NDO had reduced NGF/Cr levels, whereas those with failed IDO or NDO treatment did not. Patients who responded to BoNT/A treatment had significantly reduced urinary NGF/Cr levels in both the IDO and NDO groups compared with baseline levels. However, the NGF levels remained significantly higher at 3 months in 7 IDO and 5 NDO patients who failed BoNT/A treatment (Figure 5).

**Urinary NGF Level in Women With Mixed Urinary Incontinence**

The presence of OAB, urodynamic DO, and urge incontinence (UI) can complicate the diagnosis and management of stress urinary incontinence (SUI) in women. Compared with women with SUI, women with UI and mixed urinary incontinence have reported not only significantly greater urinary urgency intensity and more episodes of incontinence, but also significantly worse health-related quality of life.\(^4\) There is a close association among SUI, OAB, and DO. It has been reported that 87% of women with OAB wet symptoms also had the symptoms of SUI, and that 58% of women with OAB wet had urodynamic DO.\(^5\) Although the presence of preoperative DO does not appear to significantly worsen the treatment outcome in women with SUI, OAB symptoms may only resolve in about 50% of patients with mixed urodynamic stress incontinence (USI) and DO after anti-incontinence surgery such as tension-free vaginal tape procedure.\(^4\)-\(^7\) However, persistent SUI, persistent OAB symptoms, or de novo DO may occur in women who have undergone a midurethral sling procedure or a bladder neck sling procedure, and especially in patients who have undergone an anti-incontinence procedure resulting in BOO.\(^4\)

Urinary NGF level was measured in 38 women with urodynamic SUI (UUSI) with OAB, in 26 women with urodynamic DO but not SUI, in 21 women with persistent USI after anti-incontinence surgery, in 15 women with de novo DO, and in 31 normal controls.\(^5\) All subjects underwent a videourodynamic study for differential diagnosis of the underlying causes of incontinence. Urinary NGF/Cr levels were low in controls and in women with pure USI (\(P = .108\)). The NGF/Cr levels were significantly higher in women with mixed USI and DO than in controls (\(P = .001\)) and in pure USI patients (\(P = .006\)), but were similar to the levels in women with pure DO (\(P = .058\)). NGF/Cr level was undetectable in women with persistent USI, but was significantly higher in those with de novo DO after anti-incontinence surgery compared with the controls and USI patients. A urinary NGF/Cr level higher than 0.05 was found in 9% of USI, 77% of DO, 81% of mixed USI and DO, and 80% of de novo DO patients (Figure 6). Analysis of clinical symptoms and urodynamic findings in this study also revealed that OAB symptoms in women with SUI are not reliable for the occurrence of DO. Although all of the women in this study had both SUI and OAB symptoms, urodynamic SUI was found in 45% and DO was detected in 55% of women. With the use of urinary NGF level as biomarker, the sensitivity of detecting urodynamic DO was higher than basing on the clinical symptoms alone.
Urinary NGF Level in OAB Patients After Antimuscarinic Therapy

NGF levels in urine were found to increase in patients with OAB. Effective antimuscarinic treatment of OAB might act mainly on the muscarinic receptors in sensory pathways and alter urinary NGF production, which in turn reduces urgency sensation during bladder filling. If urinary NGF can be demonstrated to reduce OAB, patients were treated with tolterodine 4 mg once daily. The urinary NGF/Cr levels and urgency severity scale (USS) were compared at baseline, 1, 2, and 3 months after antimuscarinics and 1 month after discontinuing treatment. Urinary NGF/Cr level was significantly reduced at 3 months in 50 responders (1.10 ± 0.26 before vs 0.41 ± 0.09 after, \( P = .008 \)), but not in 20 nonresponders (1.39 ± 0.54 before vs 1.30 ± 0.46 after).

If urinary NGF can be demonstrated to reduce OAB in patients with symptomatic improvement after antimuscarinic treatment, it would support the existence of a link between NGF production and muscarinic receptor activation in OAB.

Conclusions

Measurement of urinary NGF level in patients with OAB and other urinary conditions provides insight into the underlying pathophysiology of this sensory disorder. Patients with OAB had significantly higher urinary NGF levels compared with controls and patients with increased bladder sensation. BOO with OAB or DO correlates with elevated urinary NGF that returns to normal after medical treatment of BOO.
These results suggest that urinary NGF level is a promising biomarker for the diagnosis of OAB. Measurement of urine NGF level may serve as a useful objective test to evaluate IDO and NDO treatment outcome.

References


Main Points

• Before a validated overactive bladder (OAB) urine nerve growth factor (NGF) test can be considered, the rate of false-negative, low NGF levels in a number of patients with a diagnosis of detrusor overactivity (DO) must be flushed out.

• NGF might be a downstream protein produced in the face of several bladder dysfunction or systemic disorders. There could be several other pathways that mediate urgency sensation or development of DO in patients with OAB. Therefore, the sensitivity of the test may be better than its specificity.

• In patients with OAB who are well treated with antimuscarinics or botulinum toxin injection, urinary NGF levels have been shown to decrease significantly in association with reduction of urgency severity.

• It is possible that urinary NGF levels may be used as a surrogate biomarker for assessment of therapeutic outcome in patients with OAB or DO.

• As NGF correlates with OAB and decreases with successful OAB therapy, it would be logical to hypothesize that pharmacologically decreasing NGF levels in the urinary bladder may be a novel and rational therapy for the OAB.

• Systemic agents antagonistic to neurotrophic factors or local NGF antibody or antisense therapy may be considered.


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