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Review Article

Urinary Nerve Growth Factor Levels in Overactive Bladder Syndrome and Lower Urinary Tract Disorders

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Overactive bladder (OAB) is a syndrome based on self-reported symptoms of urgency and frequency with or without urge incontinence. Although urgency is the core symptom of OAB, patients might have difficulty to distinguish urgency from the urge to void. Urodynamic study is a useful diagnostic tool to discover detrusor overactivity (DO) in patients with OAB; however, not all OAB patients have DO. Therefore, a more objective and non-invasive way to diagnose and assess OAB including DO is needed. Recent research has focused on urinary biomarkers in assessment of OAB. Urinary nerve growth factor (NGF) level increases in patients with OAB-wet, bladder outlet obstruction, mixed urinary incontinence and urodynamic DO. Urinary NGF levels are correlated with severity of OAB symptoms. In patients with OAB and DO who have been well treated with antimuscarinics or botulinum toxin injection, urinary NGF levels have been shown to decrease significantly in association with reduction of urgency severity. However, not all patients with OAB have an elevated urinary NGF level. It might also be increased in patients with interstitial cystitis/painful bladder syndrome, cerebrovascular accident and lower urinary tract diseases such as urinary tract stone, bacterial infection and urothelial tumor. It is possible to use urinary NGF levels as a biomarker for diagnosis of OAB as well as for the assessment of therapeutic outcome in patients with OAB or DO. Here, we review the latest medical advances in this field.

Key Words: biomarker, detrusor, nerve growth factor, overactive bladder, urodynamics

Overactive bladder (OAB) syndrome is a condition of urinary urgency and frequency with or without urge incontinence, and urgency is the core symptom for the presence of OAB.¹ Clinical assessment of OAB severity or grade is based on subjectively reported symptoms and patients might not be able to distinguish urgency from the urge to void.^{2,3} Patients with OAB might have detrusor

overactivity (DO) or sensory urgency. However, only 69% of men and 44% of women with urgency (OAB-dry) have DO, whereas 90% of men and 58% of women with urgency and urgency incontinence (OAB-wet) have DO.⁴ It is possible that many patients with increased bladder sensation without urgency are grouped into OAB-dry, which might cause a high non-response rate in

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clinical therapeutic trials.^{5,6} It is necessary to develop a better way to diagnose OAB and assess therapeutic outcome in patients with OAB.

Nerve Growth Factor in the Lower Urinary Tract

Nerve growth factor (NGF) is a small secreted protein that induces differentiation and survival of particular target neurons. NGF can be produced from the urinary bladder through protein-kinase-C- and protein-kinase-A-dependent intracellular pathways that play physiological and pathophysiological roles in the lower urinary tract.⁷ NGF can sensitize afferent nerves and induce bladder hyperactivity^{8,9} and is linked to mechanical stretch and reflex bladder activity.¹⁰ The levels of neurotrophic factors including NGF increase in rat bladders, as well as in the lumbosacral spinal cord and dorsal root ganglia (DRGs), after spinal cord injury (SCI) and cyclophosphamide-induced cystitis.^{8,11} Chronic administration of NGF into the spinal cord or bladder of rats induces bladder hyperactivity and increases the firing frequency of bladder afferent neurons.¹²⁻¹⁴ Intravesical instillation of NGF in rats reduces mechanical hypersensitivity that can be reversed by NGF antiserum.¹⁵ In long-term diabetic rats induced by streptozotocin, the mean NGF levels in bladder tissue and lumbosacral DRGs were significantly lower than in the controls, and after treatment of diabetes the reduced NGF levels returned to normal.^{16,17}

Increased levels of NGF have also been reported in the bladder urothelium and smooth muscle and urine of patients with interstitial cystitis/painful bladder syndrome (IC/PBS), sensory urgency, and DO¹⁸⁻²⁰ (Figure 1). In patients with neurogenic DO (NDO), NGF level in bladder tissue increases and decreases after detrusor botulinum neurotoxin A (BoNT-A) injection, in association with decreased detrusor contractility.²¹ This finding further provides evidence that NGF is involved in visceral afferent nerve transmission and can be modulated by treatment for NDO. These observations in animal models and humans suggest that

NGF has a potential role as a urinary biomarker for the diagnostic and therapeutic implications of lower urinary tract dysfunction.

NGF in the Urine

Clinical and experimental data have indicated 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 bladder outlet obstruction (BOO), OAB, IC/PBS, and chronic prostatitis.^{18,22,23} Bladder inflammation results in altered NGF levels in the bladder and morphological changes in sensory and motor neurons that innervate the bladder.²⁴ Denervation of rat bladder results in transient increases in bladder NGF level, which indicates that innervation influences tissue levels of NGF in the bladder.²⁵

Previous studies of NGF in OAB or DO usually have measured bladder tissue level, which are elevated in patients with idiopathic DO (IDO), men with benign prostatic hyperplasia (BPH) and women with IC/PBS.²⁶ A recent study that has measured NGF concentration using enzyme-linked immunosorbent assay (ELISA) in superficial bladder biopsies from 12 women with urodynamic DO and 15 without, did not show a significant correlation between bladder NGF level and DO.¹⁹ It is difficult to standardize the quantity of NGF in epithelium, suburothelium and muscle with a bladder biopsy, and a simpler and safer urine level of NGF measurement is in fact a better assay and one that can be standardized.

Visceral epithelia have been demonstrated as a major source of NGF production, and NGF might regulate the function of adult visceral sensory and motor neurons.²⁷ Using gene transfer techniques in rats, a transient increase in NGF expression without associated inflammation sensitizes visceral reflex pathways, which leads to bladder overactivity.¹² Exogenous overexpression of NGF in the urinary bladder produces DO and nociceptive Fos expression in the lumbosacral spinal cord.²⁸ Stretching of the urothelium during bladder distention might induce production of NGF in the

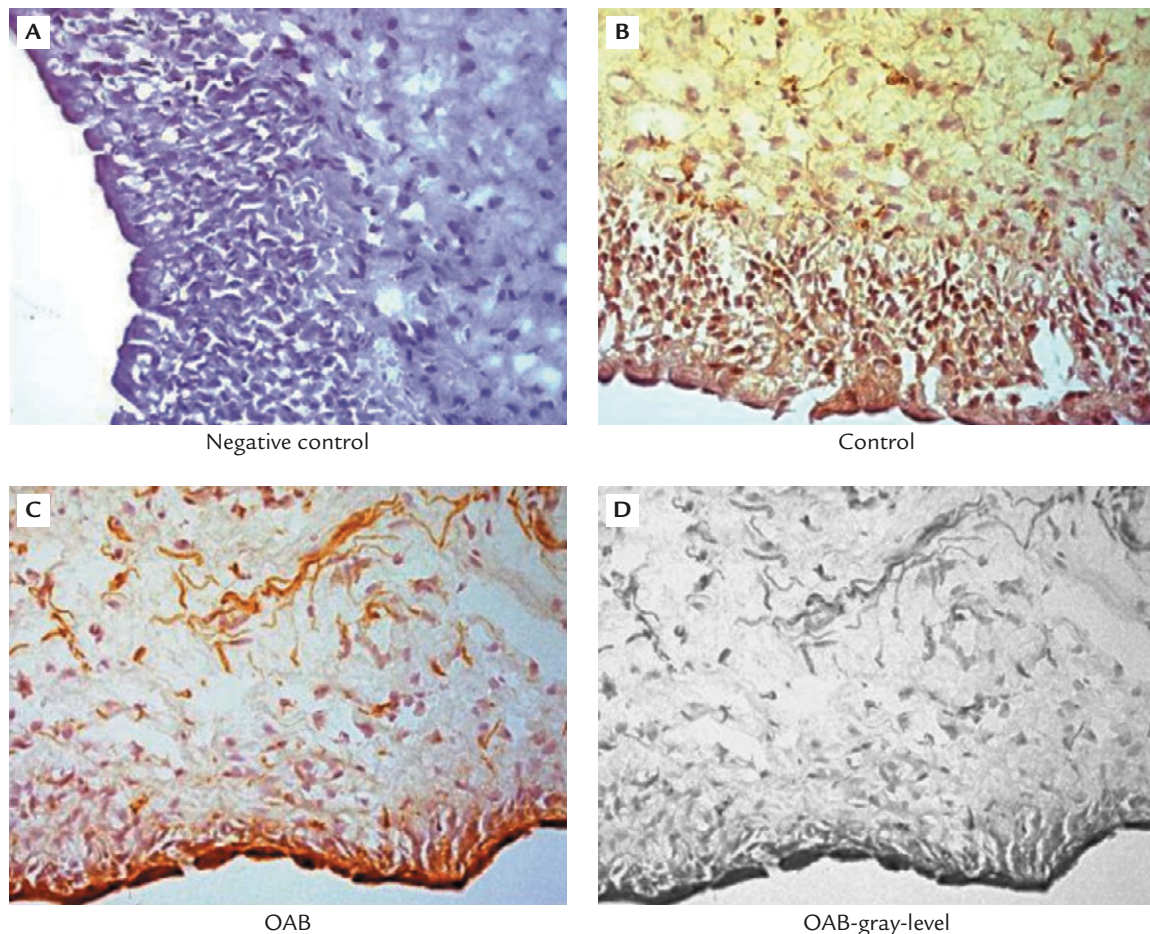


Figure 1. Increased nerve growth factor immunohistochemistry staining in the urothelium and suburothelium of overactive bladder.

bladder tissue and secretion into the urine. It is rational to hypothesize that NGF produced in the urothelium and suburothelium can be secreted into the bladder lumen.¹⁰ Kim and Park found that urinary NGF levels are increased in men and women with OAB syndrome.²⁹ Yokoyama evaluated urine NGF in patients with OAB, BOO, and NDO.³⁰ Although NGF levels in the 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 probable.

Detrusor injection of BoNT-A in patients with IDO might inhibit NGF production in the urothelium and suburothelium and therefore decrease the urinary NGF levels as well as reduce the urgency sensation.³¹ Measurement of NGF in the urine is likely to be a more relevant and sensitive biomarker for OAB than is bladder tissue

NGF levels. If the urinary NGF levels differ among normal controls and patients with increased bladder sensation, OAB-dry and OAB-wet, then urinary NGF levels could be a convenient biomarker for diagnosis or assessment of the therapeutic outcome of OAB.

Measurement of Urinary NGF Levels

Measurement of urinary NGF levels is performed by ELISA using non-diluted urine samples. Voided urine is put on ice immediately and centrifuged at 3,000 g for 10 minutes at 4°C. The supernatant is separated into aliquots in 1.5-mL tubes and preserved in a -80°C freezer. At the same time, 3 mL urine is taken to measure urinary creatinine level. Urinary NGF concentration is determined using the Emax ImmunoAssay System (Promega,

Madison, WI, USA) with a specific and highly sensitive ELISA kit, which has a minimum sensitivity of 7.8 pg/mL. The amount of NGF in urine samples that falls below the detection limits of the NGF assay is extracted from an NGF standard curve. We run samples in triplicate, and urinary NGF levels without a consistent value in three measures are repeated and the values averaged. When the urinary NGF concentration is higher than the upper detection limit (250 pg/mL), the urine samples are diluted to fit the detection limit. For urine samples with NGF concentrations lower than the detectable limit but above zero, a concentration method is performed using a column-protein concentration kit (Amicon Ultra-15; Millipore, Billerica, MA, USA) to measure the NGF value. Total urinary NGF levels are further normalized by the concentration of urinary creatinine (NGF/Cr level).

IC/PBS and Urinary NGF

NGF has attracted considerable attention as a key player in the link between inflammation and altered pain signaling.⁶ Intravesical instillation of NGF in Wistar rats has been shown to induce bladder hyperactivity.³² In an experimental cystitis rat model, systemic treatment with the NGF-sequestering molecule partially and significantly reverses established inflammatory changes.³³ Endogenous NGF produced in inflamed tissue is a crucial mediator of the sensory disorders that are associated with inflammation and some persistent pain states.³⁴ Sensitization of sensory pathways by bladder inflammation or NGF contributes to the development of hypersensitivity in referral sites, which suggests that NGF can undergo retrograde transport to major pelvic ganglia or DRGs.^{24,35,36} In patients with IC/PBS, neurotrophins [including NGF, neurotrophin-3 and glial-cell-derived neurotrophic factor (GDNF)] have been detected in the urine.²³ Increased expression of NGF is also present in bladder biopsies from women with IC/PBS.^{18,37} Patients with IC/PBS who respond to intravesical BoNT-A injection have been

found to have reduced bladder tissue NGF expression.³⁷

In a recent study of urinary NGF expression in women with IC/PBS, the urinary NGF/Cr levels were very low when the bladder was not distended and significantly elevated when the bladder was full.³⁸ However, urinary NGF/Cr levels were not correlated with visual analog score (VAS), cystometric bladder capacity at diagnosis, or maximal bladder capacity during hydrodistention. Patients who responded to treatment and had an improved VAS pain score of ≥ 2 had significantly decreased NGF/Cr levels compared with non-responders who had a VAS improvement of < 2 . A decrease in urinary NGF/Cr level was associated with greater pain reduction and successful response, which suggests that urinary NGF level can be used as a biomarker for detection of the severity of the bladder condition in IC/PBS.

During bladder inflammation, endogenous NGF is rapidly upregulated and released to mediate sensory and reflex changes.²⁸ A rise in bladder NGF in the muscle or urothelium initiates signals that are transported along the afferent nerves of the bladder to the DRGs or spinal cord.^{28,36} These findings suggest that neural up-regulation through activation of afferent nerves occurs peripherally and centrally in subjects with chronic cystitis. The increase in urinary NGF levels in patients with IC/PBS indicates a higher degree of bladder inflammation. Although treatment improves bladder pain in some patients, their urinary NGF levels do not return to those of normal controls.³⁸ This finding suggests that urinary NGF level can be monitored as a biomarker for IC/PBS severity and response to therapy. Long-term medication and anti-inflammatory management might be necessary in patients with symptomatic improvement but chronically elevated urinary NGF level.

OAB and Urinary NGF

Bladder and urine NGF levels have been found to be elevated in patients with idiopathic sensory

urgency and IC/PBS.^{18,23} A recent study has measured urinary NGF levels in patients with increased bladder sensation, OAB-dry and OAB-wet, and in a group of control subjects without lower urinary tract symptoms (LUTS).³⁹ Urinary NGF/Cr levels were very low in normal controls (mean \pm standard deviation, 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 (1.70 ± 0.26) had significantly higher urinary NGF/Cr levels compared with the controls and patients with increased bladder sensation (Figure 2). Patients with OAB-wet had significantly higher urinary NGF levels than those with OAB-dry.

These results suggest that elevated urinary NGF level plays an important role in mediating the sensation of urgency in OAB. Increased bladder sensation might be caused by increased alertness of bladder fullness or polyuria. Clinically, it is sometimes difficult to identify these patients from those with OAB-dry. The results of this study provide strong evidence for this hypothesis and suggest that urinary NGF levels can be used as a biomarker for differential diagnosis of OAB.

The possible reason for the difference in NGF levels between OAB-dry and OAB-wet might be the higher percentage of DO in patients with OAB-wet. Digesu et al investigated a group of 843 women classified with OAB, and 457 (54.2%) were found to have urodynamically proven DO.⁴⁰

Hyman et al also found a higher incidence of DO associated with urge incontinence (OAB-wet) compared with symptoms of urgency and frequency, nocturia, or difficult urination (75% vs. 36%) in men with LUTS.⁴¹ Taken together, these clinical observations suggest that urinary NGF level is strongly associated with the urgency symptom, and a higher urinary NGF level might have an impact on the occurrence of DO.

Cerebrovascular Accident and Urinary NGF

NGF is essential in nerve growth and regeneration. The level of urinary NGF increases in patients with IDO and incontinence. Cortical and brainstem areas are involved in the control of micturition.⁴² Cerebrovascular accident (CVA) results in transient detrusor underactivity in the acute stage, reduced bladder capacity and uninhibited detrusor contractions in the following period, and stabilization in the resolution stage.⁴³

In a recent study of urinary NGF levels in patients with CVA, the urinary NGF/Cr levels were found to be significantly higher in CVA patients than in normal subjects.⁴⁴ Urinary NGF/Cr levels correlated well with the severity of neurological impairment. Patients with no or minimal neurological impairment had low urinary NGF/Cr

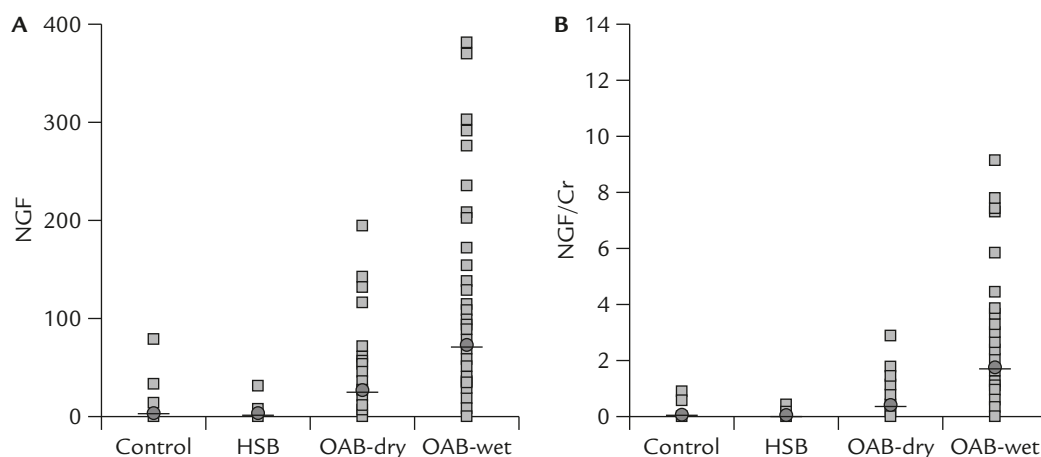


Figure 2. Patients with overactive bladder-dry and overactive bladder-wet had significantly higher urinary nerve growth factor and nerve growth factor/creatinine levels compared with the controls and patients with increased bladder sensation.³⁹ NGF = nerve growth factor; Cr = creatinine; HSB = hypersensitive bladder; OAB = overactive bladder.

levels similar to those of the controls. Patients with mild/moderate or severe impairment had significantly greater urinary NGF levels than those with no or minimal impairment. However, urinary NGF/Cr levels were not correlated with age, location of CVA, multiplicity of CVA, duration of CVA, urodynamic findings, or the presence of urge incontinence, which suggests that urinary NGF is a result of neurological lesions rather than a cause of bladder dysfunction in CVA.

NGF is involved in the development and maintenance of specific peripheral and central populations of neuronal cells. NGF might operate through multiple pathways ultimately to regulate physiological homeostasis and behavioral coping.⁴⁵ A previous study has shown that brain-derived neurotrophic factor mRNA is increased after stroke.⁴⁶ In stroke patients, serum neurotrophins are significantly associated with clinical and neuroradiological parameters of brain injury in the acute phase of stroke, which suggests that stroke modulates peripheral neurotrophin levels.⁴⁷ The plasticity of bladder afferents after CVA might be due to increased circulating NGF levels.⁴⁸ Increased serum NGF levels might reduce the excitatory threshold of bladder to DRGs, which results in increased mechanosensitivity of the bladder wall.¹⁵ Increased urinary truncated nerve growth factor receptor (NGF-Rt) levels have also been found in patients with mild Alzheimer's disease.⁴⁹ It seems rational to hypothesize that stroke patients with moderate to severe neurological impairment have increased circulating NGF, which causes an increase in urinary NGF level. Systemic neurological impairment might play a role in elevating NGF and causing OAB symptoms. Stress-related events might also result in increased plasma NGF levels and involvement of neuroendocrine functions.⁵⁰

BOO and Urinary NGF

The increased level of NGF could trigger changes in bladder afferent fibers, which leads to reduced threshold or increased excitability.²⁸

NGF expression has been shown to increase in an *in vitro* spontaneously hypertensive rat model with hyperactive voiding and mechanical stress that mimics BOO.¹⁰ Through mechanical stretching, NGF expression in the bladder wall might increase, which results in a reduced sensory threshold for the bladder sensation of urgency or a reduced threshold for mediation of detrusor hyperactivity.⁵¹ Incomplete reversibility of neural plasticity might be responsible for continuing urge symptoms after surgical intervention for BOO.⁵²

OAB is frequently associated with BOO in men with BPH and can resolve after relief of BOO, but about 50% of patients have persistent OAB symptoms after surgical intervention for BPH, which suggests that OAB occurs directly or independent of BOO.⁵³ In a recent study of urinary NGF/Cr levels in men with BOO, urinary NGF/Cr levels were low in those with BOO/non-OAB and significantly elevated in patients with BOO/OAB and BOO/DO. Urinary NGF/Cr levels were not significantly different between the BOO/OAB and BOO/DO groups. The urinary NGF/Cr levels returned to normal levels after successful relief of OAB symptoms by medical treatment.⁵⁴

Chronic BOO, such as in BPH, can result in stretching of the urothelium and smooth muscle, stimulation of NGF production, and alteration of the afferent nerve pathway. Furthermore, chronic sensitization of afferent nerves could alter the conductance of DRGs, which causes increased excitability and enhanced spinal reflexes. Immunity to NGF prevents afferent nerve plasticity and occurrence of OAB associated with BOO.⁵⁵ The results of this study suggest that elevated NGF level plays an important role in mediating the sensation of urgency and causing increased nerve fiber excitability in BOO. The lower urinary NGF levels in patients with relief of OAB after treatment further suggests that the pathophysiology of OAB and DO in patients with BOO involves NGF over-production in the bladder, which can return towards normal levels after adequate relief of BOO.

IDO and Urinary NGF

DO is a urodynamic diagnosis. OAB is a syndrome that is characterized by urgency and frequency with or without urge incontinence.¹ Sensory urgency and DO might be involved in the pathophysiology of this syndrome. Patients with OAB might not have urodynamic DO, whereas those with urodynamically proven DO might not present with OAB.⁴ Clinically, it is essential to differentiate DO from patients presenting with OAB symptoms.

The urothelial release of neurotransmitters such as acetylcholine and ATP and the expression of transient receptor potential vanilloid receptor subfamily 1 (TRPV1) and purinergic receptor P2X₃ strongly imply a role for the urothelium in human bladder mechanosensation.^{56–58} The suburothelial innervation expresses TRPV1, P2X₃ receptor, and the sensory neuropeptides substance P and calcitonin gene-related peptide in the pathophysiology of human DO.^{59–61} Intradetrusor injection of BoNT-A decreases immunoreactivity of P2X₃ and TRPV1 expression in suburothelial fibers, which correlates with improvement in OAB symptoms.⁶²

In rats, increased NGF expression with or without associated inflammation leads to bladder overactivity.^{12,28,32} The bladder mRNA expression of NGF and TRPV1 is higher in the unstable bladder following urethral obstruction in rats.⁶³ Inflammation increases NGF and glial-cell-derived neurotrophic factor which facilitates TRPV1 expression and induces thermal hyperalgesia.⁶⁴ These findings suggest that NGF is involved in sensory pathway neuromodulation in DO. Urinary NGF is highly expressed in patients with OAB-wet; therefore, we hypothesize that determination of urinary NGF level could serve as a valuable biomarker for the diagnosis of DO and monitoring of disease progression of lower urinary tract dysfunction.³⁹ Moreover, if urinary NGF levels can be reduced after successful therapy, such as BoNT-A treatment for DO, measurement of urinary NGF could be a useful objective tool for assessing the therapeutic outcome of DO.

A cross-sectional study has been performed in 143 patients with IDO who had no treatment, or successful or failed treatment with antimuscarinic agents. The mean urinary NGF/Cr levels were significantly higher in 66 patients with untreated IDO compared with the controls. Patients with successfully treated IDO had reduced NGF/Cr levels, whereas those with failed treatment did not. Detrusor injection of 100 U BoNT-A was given to 24 patients who had failed antimuscarinic treatment. Patients who responded to BoNT-A treatment had significantly reduced urinary NGF/Cr levels compared with baseline levels; however, the NGF levels remained significantly higher at 3 months in patients who failed BoNT-A treatment.⁶⁵

Detrusor injection of BoNT-A has been demonstrated to provide therapeutic improvement for patients with OAB, intractable IDO, and NDO.^{66–70} One recent study has found that BoNT-A injections into the detrusor decrease NGF bladder tissue levels in patients with NDO.²¹ The mechanism responsible for the effectiveness of BoNT-A on refractory DO is believed to occur by inhibition of acetylcholine release from the presynaptic nerve terminals of the neuromuscular junction.^{71,72} Detrusor injection of BoNT-A reduces urgency and decreases P2X₃ and TRPV1 receptor expression in the suburothelium.⁶² NGF could play an important role in the connection between suburothelial sensory fibers and detrusor muscle excitability.⁷³

Antimuscarinic Therapeutic Effect on Urinary NGF

NGF levels in urine increase in patients with OAB.^{29,30,39} Improvement of OAB symptoms after antimuscarinic therapy reduces urinary NGF levels.³⁹ Effective antimuscarinic treatment of OAB might act mainly on the muscarinic receptors in sensory pathways and alter urinary NGF production, which in turn reduces the urgency sensation during bladder filling. The reduction of urinary NGF levels in OAB patients

with symptomatic improvement after antimuscarinic treatment supports the existence of a link between NGF production and muscarinic receptor activation in OAB.⁷⁴ Urinary NGF level could therefore be used as an objective tool to assess the therapeutic outcome of antimuscarinic treatment for OAB.

In a recent study of urinary NGF levels and urgency severity score (USS) in patients with OAB after antimuscarinic therapy and after discontinuing treatment, urinary NGF/Cr levels were significantly reduced at 3 months in 50 responders who had a reduction of USS by ≥ 2 but not in 20 non-responders. After discontinuation of antimuscarinic treatment for 1 month, however, urinary NGF/Cr level was elevated in 23 responders and in five non-responders. The USS significantly changed with the change of urinary NGF/Cr levels in responders at different time points. The change of urinary NGF levels is correlated with the change of USS after antimuscarinic treatment and discontinuation⁷⁵ (Figure 3).

It is possible that NGF is taken up by sensory nerves and transported through the central nervous system in a retrograde fashion. Therefore, NGF production could be a biomarker for neuroplasticity via some common pathway involved in the pathogenesis of OAB.⁷³ A lag response time between changes in USS and NGF has been noted in responders. The mechanism for this difference could be due to a time lag of decreased NGF

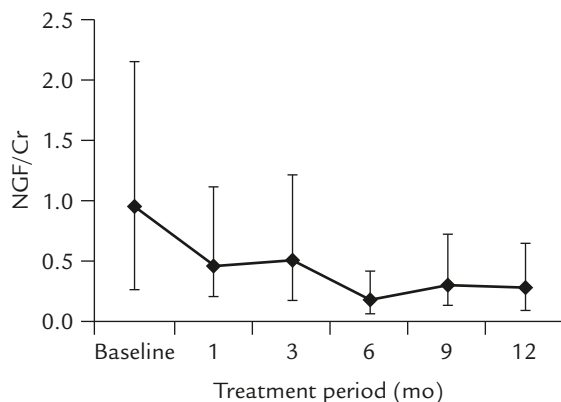


Figure 3. Changes in urinary nerve growth factor levels with time in patients with overactive bladder-wet who have been successfully treated with antimuscarinic agents.⁷⁶ NGF = nerve growth factor; Cr = creatinine.

production after antimuscarinic treatment. However, after antimuscarinic treatment for 3 months, the USS did not decrease to zero and urinary NGF levels remained significantly higher than those of the controls. Another recent study of the changes in urinary NGF in OAB patients after antimuscarinic therapy for 12 months has revealed that the decrease in urinary NGF levels did not reach a nadir.⁷⁶ Although OAB symptoms were relieved after long-term antimuscarinic treatment, urinary NGF/Cr levels decreased significantly from baseline to 6 months but remained stationary at 9 and 12 months. The urinary NGF levels at 12 months after antimuscarinic treatment were significantly lower than the baseline but were higher than the controls (Figure 3).

Inflammatory processes are likely to be involved in the etiology of OAB. Tyagi and Chancellor have proposed the hypothesis that local inflammation is a cause and plays a central role in the etiology of OAB. Multiplex analysis has revealed increased urinary cytokine levels in a rat model of cyclophosphamide-induced cystitis.⁷⁷ Pre-clinical studies have shown that increased urine levels of monocyte chemoattractant protein 1 and CXC chemokines CXCL1 are evidence of bladder inflammation.⁷⁸ The higher urine cytokine levels in OAB-wet relative to OAB-dry suggest a relationship between OAB symptom severity and bladder inflammation. Thus, the elevated urinary NGF levels after long-term antimuscarinic treatment might imply the existence of residual inflammation in the bladder or central nervous system. Continuation of antimuscarinic therapy might be necessary to achieve therapeutic effectiveness.

NDO and Urinary NGF

Increased expression of NGF and brain-derived neurotrophic factor has been noted in a rat SCI model.^{9,79} Increased NGF levels in the bladder, DRGs, and spinal cord contribute to the emergence of detrusor sphincter dyssynergia (DSD) that is partially mediated by C-fiber bladder afferents

after SCI.⁸⁰ The SCI rats with higher severity of autonomic dysreflexia (AD) are correlated with higher NGF expression in lumbosacral segments.⁷⁹ Immunoneutralization of NGF in the spinal cord suppresses NGF levels in the lumbosacral DRGs and DSD.⁸² In human bladders, increased NGF expression has been found in patients with SCI and NDO.²⁹ Detrusor injection of BoNT-A can reduce DO as well as the expression of NGF.²¹ It is possible to monitor the changes in urinary NGF levels as biomarkers for the degree of DSD and AD in patients with SCI and NDO.

Urinary NGF levels have been measured in patients with NDO due to SCI at baseline, after antimuscarinic treatment, and after BoNT-A injections.⁶⁵ The urinary NGF/Cr levels were significantly elevated compared with those in the controls. Patients with DSD and AD had a higher NGF/Cr level compared with those without AD. After BoNT-A injections, a successful result was achieved in 14 (74%) patients with NDO. Analysis of the therapeutic outcome has revealed a significant decrease in urinary NGF/Cr level in BoNT-A responders with NDO but no significant decrease in non-responders of NDO at 3 months. Persistent low urinary NGF/Cr levels compared with baseline were also detected at 6–12 months in patients with NDO who had a long-lasting BoNT-A effect for > 6 months.

Mixed Urinary Incontinence and Urinary NGF

The presence of OAB, urodynamic DO, and urge incontinence can complicate the diagnosis and management of stress urinary incontinence (SUI) in women. Compared with women with SUI, women with urge incontinence 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.⁸³ There is a close association between SUI, OAB, and DO. OAB symptoms might only resolve in about 50% of patients with mixed urodynamic stress incontinence (USI)

and DO after anti-incontinence surgery such as the tension-free vaginal tape procedure.^{84–86} However, persistent SUI, persistent OAB symptoms, or *de novo* DO might occur in women who have undergone an anti-incontinence procedure which results in BOO.⁸⁶ A recent study has also revealed that in patients with urge syndrome after a pubo-vaginal sling procedure, 85.9% of patients had a chronic condition, and 83.3% of those with *de novo* DO had preoperative urge syndrome.⁸⁷ It would be important if we could detect DO before undertaking anti-incontinence surgery in patients with mixed urinary incontinence.

Among a cohort of women with urinary incontinence, urinary NGF/Cr levels were low in the controls and in women with pure USI. The NGF/Cr levels were significantly higher in women with mixed USI and DO than in controls and in those with pure USI but were similar to the levels in women with pure DO. The NGF/Cr levels were undetectable in women with persistent USI but were significantly higher in those with *de novo* DO after anti-incontinence surgery, compared with the controls and USI patients.⁸⁸

Effect of Bladder Volume on Urinary NGF

Patients with OAB usually have a smaller bladder capacity and hypersensitivity, whereas non-OAB patients can tolerate a large bladder capacity when they have the urge to void.⁸⁹ Stretch of bladder smooth muscle cells stimulates increased NGF production which is associated with hyperactive voiding in spontaneously hypertensive rats.¹⁰ The difference of bladder sensitivity to volume increase might be due to abnormal secretion of sensory proteins such as NGF or overexpression of sensory receptors in the bladder wall with a small bladder volume or during bladder distention.

Urinary NGF levels have been measured in 39 patients with OAB and in 35 control subjects without LUTSs. Urine samples were collected at the first sensation of bladder filling (FSF) and at urge sensation (US), respectively, in both groups. Urinary NGF/Cr levels were very low at FSF and

were significantly increased at US in normal controls. Patients with OAB had significantly higher urinary NGF/Cr levels at FSF and US compared with those in the controls. The urinary NGF/Cr levels showed a non-significant change between FSF and US in OAB patients, but the NGF/Cr levels of OAB patients at FSF were significantly higher than those in the controls at US.⁹⁰ The results of this study suggest that urinary NGF increases physiologically in normal subjects at the urge to void but increases pathologically in OAB patients with a small bladder volume and at urgency sensation.

Bladder distention can stretch the urothelium, and evidence has shown that ATP released from the urothelium during bladder distention can provoke a sensation of bladder filling.⁹¹ Physiologically, the release of urinary NGF increases with increased bladder volume, but NGF production might be limited until a threshold level. When the bladder is fully distended, a cascade of neuromediators might be released from the urothelium, which act on receptors in the suburothelial afferents, which might induce the sensation of urge to void.⁹² However, in patients with bladder dysfunction such as hypersensitivity, IC/PBS, or OAB, sensitization of bladder afferents or lowering the threshold of sensory firing by NGF might occur, which induces urgency or urge incontinence during bladder filling at a sub-threshold volume.⁹³

Urinary NGF Levels in Natural or Catheter Bladder Filling

Previous investigations have shown that urinary NGF levels are elevated in patients with OAB-dry, and are even higher in patients with OAB-wet compared with normal controls and patients with increased bladder sensation.^{29,39} Whether the urinary NGF is produced by bladder wall distention or from other sites of the urinary tract has not yet been elucidated. Urinary urgency occurs when the bladder is full, and rapid distention during urodynamic studies can induce urgency sensation or DO. Could these different conditions have similar levels of NGF production?

In a recent study, urinary NGF/Cr levels in natural-filling (drinking water) were significantly greater than those with catheter-filling (infused by normal saline) in OAB-wet or urodynamic DO subgroups.⁹⁴ Urinary NGF/Cr levels were also significantly elevated in patients with OAB-wet or urodynamic DO (Table 1). This study provides results of urinary NGF in patients with OAB-wet or DO that were similar to those in previous studies. One particularly interesting finding was that urinary NGF levels in OAB-wet increased only in natural-filling but not in catheter-filling. The NGF levels in OAB-dry were also slightly increased in natural-filling compared with catheter-filling; however, no difference was noted between natural and catheter-filling in the controls. This suggests that urinary NGF could be produced secondary to bladder dysfunction, whereas the severity of bladder dysfunction results in different OAB conditions. A longer duration of bladder distention might affect the production of urinary NGF in natural-filling.

Comparison of Urinary NGF Between OAB and IC/PBS

The clinical symptoms of IC/PBS and OAB are similar except that bladder pain typically presents in IC/PBS, and urgency or urge incontinence presents in OAB-dry and OAB-wet. Although urinary NGF as a biomarker can be a sensitive molecular diagnostic tool for OAB,^{29,39} recent research has revealed that urinary NGF levels are also increased in patients with IC/PBS.⁶⁵ Thus, a more detailed analysis of urinary biomarkers is needed to establish their role as a non-invasive diagnostic tool for the differential diagnosis of IC/PBS and OAB.

In a recent study that has compared urinary NGF levels among IC/PBS, OAB-dry, and OAB-wet, increased urinary NGF levels have been found in women with IC/PBS and OAB-wet or DO but not in controls and women with OAB-dry or hypersensitive bladder.⁹⁵ Urinary NGF/Cr levels did not differ significantly between patients with

Table 1. Total urinary nerve growth factor and nerve growth factor/creatinine levels in symptomatic and urodynamic subgroups at natural filling and catheter filling

Subgroups	Natural-filling Total NGF	Natural-filling NGF/Cr	Catheter-filling Total NGF	Catheter-filling NGF/Cr
Symptomatic				
Control (<i>n</i> = 28)	1.52 ± 4.05	0.085 ± 0.219	1.06 ± 3.37	0.395 ± 0.955
OAB-dry (<i>n</i> = 28)	6.92 ± 15.4	0.296 ± 0.556	1.81 ± 6.32	0.243 ± 1.20
OAB-wet (<i>n</i> = 25)	49.3 ± 97.9	1.66 ± 3.30	3.17 ± 13.3	0.388 ± 1.84
ANOVA	Control vs. OAB-wet, <i>p</i> = 0.013 OAB-dry vs. OAB-wet, <i>p</i> = 0.031	Control vs. OAB-wet, <i>p</i> = 0.015 OAB-dry vs. OAB-wet, <i>p</i> = 0.040	All NS	All NS
Urodynamic				
Normal (<i>n</i> = 27)	1.76 ± 4.18	0.090 ± 0.217	1.02 ± 3.43	0.242 ± 0.775
HSB (<i>n</i> = 30)	6.21 ± 14.4	0.252 ± 0.522	1.69 ± 6.11	0.309 ± 1.15
DO (<i>n</i> = 22)	55.8 ± 103.0	1.88 ± 3.47	3.60 ± 14.1	0.441 ± 1.96
ANOVA	Normal vs. DO, <i>p</i> = 0.009 HSB vs. DO, <i>p</i> = 0.013	Normal vs. DO, <i>p</i> = 0.010 HSB vs. DO, <i>p</i> = 0.016	All NS	All NS

NGF = nerve growth factor; Cr = creatinine; OAB = overactive bladder; ANOVA = analysis of variance; NS = not significant; HSB = hypersensitive bladder; DO = detrusor overactivity.

IC/PBS and OAB-wet or DO. However, urinary NGF levels were significantly higher in women with IC/PBS than in those with OAB-dry or hypersensitive bladder (Figure 4). Using receiver operating characteristic curves to assess urinary NGF/Cr levels in patients with IC/PBS and OAB-dry, the diagnosis of IC/PBS could be achieved with a sensitivity and specificity of 75% and 56.6%, respectively, based on a urinary NGF/Cr cut-off value of 0.015.

The symptoms of IC/PBS and OAB-wet are distinct, and patients with IC/PBS have bladder pain and those with OAB-wet have urge incontinence. Thus, there is no need for a biomarker to make the differential diagnosis between IC/PBS and OAB-wet. There is potential for the use of a biomarker for non-invasive differential diagnosis between IC/PBS and OAB-dry or hypersensitive bladder. Diagnosis of IC/PBS by cystoscopic hydrodistention under general anesthesia could thus be avoided in most patients.

Previous histological studies on tissue biopsies from patients with IC/PBS have documented infiltration of mast cells, macrophages, and eosinophils

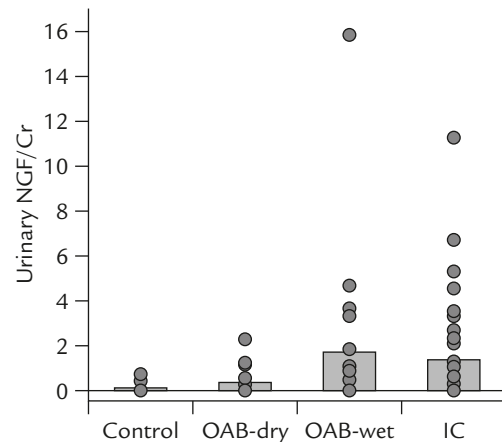


Figure 4. Urinary nerve growth factor/creatinine levels were not significantly different between patients with interstitial cystitis/painful bladder syndrome and overactive bladder-wet or detrusor overactivity. However, urinary nerve growth factor levels were significantly higher in women with interstitial cystitis/painful bladder syndrome than in those with overactive bladder-dry.⁹⁵ NGF = nerve growth factor; Cr = creatinine; OAB = overactive bladder; IC = interstitial cystitis.

as a consistent finding, and the significant association for T- and B-cell staining was similar to that for overall inflammation.⁹⁶ Therefore, an increased NGF level in the bladder or urine indicates the presence of chronic inflammation in

patients with IC/PBS. Recent biopsy studies on OAB patients have reported signs of mild to moderate inflammation in the bladder wall.⁹⁷ Although urinary NGF levels in IC/PBS and OAB-wet are similar, the underlying inflammatory protein expression responsible for bladder pain in IC/PBS and urge incontinence in OAB-wet needs clarification. 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,⁹⁸ and a detailed diagnosis by proteomic study using a urine sample might be feasible to differentiate these two bladder disorders.

Urinary NGF in Urinary Tract Diseases

Several urological diseases, including bacterial cystitis, lower ureteral stone, and urothelial cell carcinoma, might develop storage symptoms that mimic OAB or IC/PBS. It is essential to understand whether these disorders can also produce high levels of urinary NGF and whether the increased NGF production is related to the associated storage symptoms in these diseases⁷⁶ (Table 2).

Bacterial cystitis

Patients with acute bacterial cystitis with or without OAB symptoms have elevated urinary NGF/Cr levels compared with controls. The urinary NGF/Cr levels in patients with urinary tract infection

Table 2. Urinary nerve growth factor levels in patients with different lower urinary tract diseases

	<i>n</i>	NGF (pg/mL)	NGF/Cr	Statistics*†
Control	49	2.27±0.87	0.05±0.02	
OAB-dry	26	7.19±3.06	0.31±0.11	<i>p</i> =0.000*, <i>p</i> =0.033†
OAB-wet	22	54.3±22.1	1.83±0.74	<i>p</i> =0.012*
IC/PBS	60	69.7±14.0	1.73±0.44	<i>p</i> =0.000*, <i>p</i> =0.902†
OAB				
Natural filling	14	40.1±16.1	0.96±0.29	Natural vs. KCl, <i>p</i> =0.102
N/S filling	14	0.00	0.00	Natural vs. N/S, <i>p</i> =0.005
KCl test	14	3.18±1.71	0.46±0.21	KCl vs. N/S, <i>p</i> =0.045
IC/PBS				
Natural filling	9	75.47±44.64	1.13±0.50	Natural vs. KCl, <i>p</i> =0.115
N/S filling	9	1.25±1.25	0.15±0.15	Natural vs. N/S, <i>p</i> =0.052
KCl test	9	1.07±0.74	0.17±0.14	KCl vs. N/S, <i>p</i> =0.914
UTI				
Before treatment	40	99.0±19.2	2.97±0.86	<i>p</i> =0.000*, <i>p</i> =0.378†
After treatment	30	33.5±18.3	1.09±0.67	<i>p</i> =0.000*, <i>p</i> =0.467†
Renal stone				
No UTI	18	30.5±9.36	0.48±0.15	<i>p</i> =0.000*, <i>p</i> =0.130†
With UTI	7	232.9±64.9	4.68±1.72	<i>p</i> =0.000*, <i>p</i> =0.000†
Ureteral stone				
With OAB	32	61.2±20.7	0.81±0.19	<i>p</i> =0.000*, <i>p</i> =0.248†
Without OAB	19	40.3±14.9	0.56±0.17	<i>p</i> =0.000*, <i>p</i> =0.066†
Bladder tumor				
TCC	23	73.7±41.5	1.52±0.79	<i>p</i> =0.000*, <i>p</i> =0.737†
Benign tumor	2	0	0	

Results are presented as mean±standard error. *Comparison of NGF/Cr with the controls, †comparison of NGF/Cr with OAB-wet. NGF=nerve growth factor; Cr=creatinine; OAB=overactive bladder; IC/PBS=interstitial cystitis/painful bladder syndrome; N/S=normal saline; UTI=urinary tract infection; TCC=transitional cell carcinoma.

(UTI) is no different from that in OAB-wet or IC/PBS but is significantly greater than in OAB-dry. The urinary NGF/Cr levels in patients who have UTI-associated OAB and those without OAB at baseline do not differ significantly. The urinary NGF/Cr level decreases significantly after antibiotic treatment for 1 week but remains significantly higher than in the controls. The urinary NGF/Cr decreases significantly in patients without OAB after treatment but remains high in patients who have persistent OAB after treatment. Among patients who have baseline and post-treatment data, urinary NGF/Cr levels decrease significantly in those without OAB and remain high in those with OAB after UTI treatment (Figure 5).

Ureteral stone and renal stone

Urinary NGF/Cr levels in patients who have urinary tract stones without UTI are significantly higher than in controls or those with OAB-dry but are significantly lower than in patients with IC/PBS and OAB-wet. There is no significant difference

in urinary NGF/Cr levels between patients with renal or ureteral stones. Urinary NGF/Cr levels do not differ significantly in patients with renal stones without UTI compared with those with OAB-wet or IC/PBS but they are significantly lower in patients with ureteral stones compared with those with OAB-wet or IC/PBS. Patients with renal stones and UTI show a 10-fold significantly higher urinary NGF/Cr level than those without UTI. The urinary NGF/Cr level is not significantly different between patients who have ureteral stones associated with OAB and those without OAB.

Bladder urothelial carcinoma and benign tumor

Patients with urothelial cancer also have elevated urinary NGF/Cr levels compared with controls. However, the NGF level in patients with benign bladder tumor is not detectable. The urinary NGF levels in patients with urothelial transitional cell carcinoma (TCC) are not significantly different

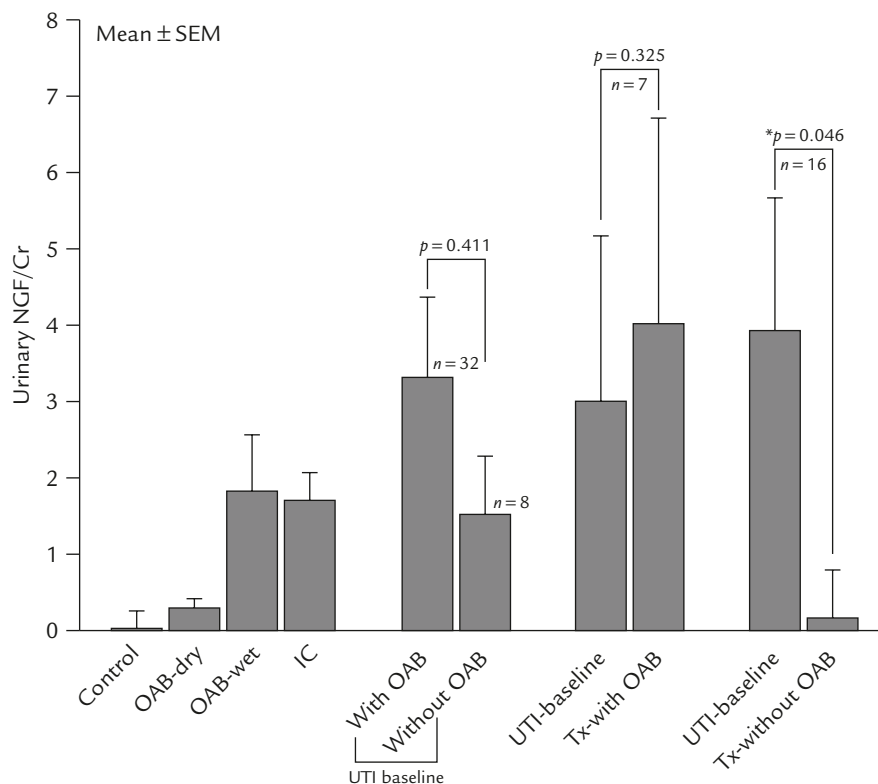


Figure 5. Urinary nerve growth factor/creatinine levels in patients with bacterial cystitis at baseline and after treatment.⁷⁶ SEM=standard error of the mean; NGF=nerve growth factor; Cr=creatinine; OAB=overactive bladder; IC=interstitial cystitis; UTI=urinary tract infection; Tx=treatment. * $p < 0.05$.

from those in patients with IC/PBS or OAB-wet but are significantly higher than in patients with OAB-dry. The urinary NGF/Cr level is significantly higher in high-grade than low-grade TCC. Patients with ureteral TCC and muscle-invasive TCC do not have a significantly higher urinary NGF/Cr level.

Other Considerations of Using Urinary NGF as a Biomarker for OAB

NGF is not the sole protein that is responsible for OAB; therefore, measurement of other inflammatory proteins in the urine, or comparison of urinary NGF levels at different bladder volumes and different severity of urgencies could clarify this question. In addition, collection of urine samples at different time points might have the effect of increased urothelial uptake, whereas delayed preparation of urine samples might result in proteolytic degradation of urinary NGF, and these factors might influence the levels of urinary NGF. Thus, standardization of urine sample collection and enrollment of larger groups of patients in further studies are necessary before we conclude that urinary NGF levels can be a biomarker of OAB.

Conclusions

Measurement of urinary NGF levels in patients with OAB and LUTSs provides insight into the underlying pathophysiology of these conditions. Urinary NGF levels could augment the use of urodynamic studies for differential diagnosis of OAB and urinary incontinence. The results of recent studies suggest that urinary NGF level is a promising biomarker for the differential diagnosis of OAB and could serve as a useful objective test to evaluate IDO and NDO treatment outcome. However, because urinary NGF level can also increase in many lower urinary tract diseases, such as stones, tumors, acute bacterial infection, and IC/PBS, it is important to exclude these diseases before a diagnosis of OAB is made.

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