Published in final edited form as:

Female Pelvic Med Reconstr Surg. 2015 ; 21(2): 111–115. doi:10.1097/SPV.0000000000000126.

The Association of Urinary Nerve Growth Factor Levels With Bladder Outlet Obstruction in Women

Robert Chan, MD^{*}, Alvaro Munoz, PhD^{*,†}, Evan P. Wenker[‡], Julie Stewart, MD^{*}, Timothy Boone, MD, PhD^{*,†,‡}, and Rose Khavari, MD^{*}

^{*}Department of Urology, Houston Methodist Hospital, Houston, TX

[†]Houston Methodist Research Institute, Houston, TX

[‡]Baylor College of Medicine, Houston, TX

Abstract

Objectives—Nerve growth factor (NGF) has been proposed as a urinary biomarker and previously shown to be elevated in male patients with bladder outlet obstruction (BOO) and other lower urinary tract symptoms. No published studies have examined NGF as a potential urinary biomarker in women with BOO. The aims of this prospective study were to evaluate NGF levels in urine from women with anatomic BOO resulting from pelvic organ prolapse (POP) and/or previous incontinence surgery and to measure the effect associated with surgical or conservative management.

Methods—From January to September 2012, all female patients referred for evaluation and management of BOO from POP or previous incontinence surgery were screened for enrollment. Inclusion criteria included elevated postvoid residual, valsalva voiding on urodynamics, or urinary peak flow (Q_{max}) of 12 mL/s or less. A control group of 10 asymptomatic age-matched female volunteers was also recruited. In all subjects, urinary NGF and creatinine (Cr) levels were measured and normalized to the urinary Cr concentrations (NGF/Cr). Urinary NGF levels were measured at 1 month and 3 months after either surgical correction or initiation of clean intermittent catheterization.

Results—A total of 10 female patients with anatomic BOO (mean [SD] age of 66.2 [3.88] years) and 10 female control subjects (mean [SD] age of 62 [7] years) were recruited. Nine patients had POP. Six patients had undergone a previous anti-incontinence procedure. Five patients had both POP and undergone a previous anti-incontinence procedure. The urinary NGF/Cr levels in the study patients with BOO (mean [SE] 20.8 [4.31] pg/mg) were significantly higher ($P = 0.0001$) than the levels in the age-matched control group (5.6 [0.65] pg/mg). After treatment, the urinary NGF/Cr level significantly decreased to 6.50 (0.57) pg/mg ($P = 0.01$).

Conclusions—In this study, female patients with anatomic BOO resulting from POP and/or previous incontinence surgery had significantly higher urinary NGF/Cr levels when compared with age-matched controls. After treatment, the urinary NGF/Cr levels significantly decreased.

Keywords

bladder outlet obstruction; urinary nerve growth factor; pelvic organ prolapse

Bladder outlet obstruction (BOO) in women can often be a diagnostic challenge that escapes simple identification. The prevalence of BOO in women had not been well studied but ranges in the literature from 2.7% to 23%.¹ The etiology of BOO in women varies widely and can include a combination of functional, neurological, and anatomic causes for obstruction.¹ Most anatomic causes for BOO in women include previous anti-incontinence surgery, pelvic organ prolapse (POP), prolapse surgery, urethral stricture, or urethral diverticulum. Many of these anatomic causes are reversible and can be treated with surgery such as urethrolysis or conservative management with the use of a pessary or intermittent catheterization.

Currently, there are no biologic markers for BOO to use in parallel with nomograms, urodynamics (UD), validated questionnaires, or quality-of-life surveys. Having a biomarker that can be easily obtained through a urine sample will be an invaluable tool to evaluate and counsel patients with lower urinary tract symptoms (LUTS) and possible BOO. In this regard, nerve growth factor (NGF) has been suggested as a diagnostic biomarker for various forms of overactive bladder and interstitial cystitis/painful bladder syndrome.² Previous studies support that bladder NGF plays an important role in bladder overactivity, irritative voiding symptoms, and afferent pathway plasticity after BOO such as in benign prostatic hypertrophy.^{3,4} The increased level of NGF can reduce the threshold or increase excitability in the afferent bladder fibers leading to increased bladder sensation or detrusor overactivity.⁵

There is only 1 study in the literature evaluating NGF in men with BOO.⁶ In this study, Liu and Kuo⁶ examined 153 male patients with BOO. They concluded that NGF levels are increased in men with BOO and overactive bladder symptoms. They also showed that these levels were reduced after medical or surgical treatment of the obstruction. Nevertheless, the etiology of BOO in women differs significantly from that of men. With more women presenting with obstruction associated with POP or after incontinence surgery obstruction, there is a need for a biomarker to help facilitate the diagnosis, treatment, and prognosis of BOO in women. The aims of this prospective, Institutional Review Board approved study were to evaluate NGF levels in women with anatomic BOO resulting from POP or previous incontinence surgery and to measure the effects of surgical correction or initiation of clean intermittent catheterization (CIC).

MATERIALS AND METHODS

From January to September 2012, all female patients with LUTS referred for evaluation and management of BOO from POP or previous anti-incontinence surgery were prospectively screened for enrollment. Inclusion criteria included women older than 18 with the following voiding parameters: elevated postvoid residual (PVR) of greater than 150 mL, valsalva

voiding on UD, or urinary peak flow (Qmax) of 12 mL/s or less. A control group of 10 asymptomatic age-matched female volunteers was also recruited. All women were free from urinary tract infection at the time of enrollment. The institutional review board and ethics committee of Houston Methodist Hospital approved this study. All study participants provided informed consent before the collection of urine samples. The Institutional Review Board number of our study was 0212-0029.

Urinary NGF levels were measured in 10 women with anatomic BOO and 10 asymptomatic female volunteers. At baseline, urinary NGF, urine creatinine (Cr), NGF/Cr ratio, baseline questionnaires including urinary distress inventory-6 (UDI-6) and pelvic organ prolapse distress inventory-6 (POPDI-6) uroflow, PVR, and urodynamic evaluation were evaluated. Moreover, 1 and 3 months after either surgical intervention or conservative management, urinary NGF, urine Cr, NGF/Cr ratio, uroflow, and PVR were reevaluated.

Urinary NGF-Cr Evaluation

A midstream second morning urine or random urine during the day was collected as recommended for urinary proteome analysis.⁷ First morning urine collection was avoided. Urine samples were immediately placed on ice and centrifuged at 3000 rpm for 10 minutes. At least 4 aliquots (1.5 mL) of the supernatant were preserved at -80°C . One of the aliquots was used to measure the urine Cr level.⁷ Urinary NGF was measured in triplicate from a second vial with an enzyme-linked immunosorbent assay using the NGF Emax ImmunoAssay System (Promega; Madison, WI). This assay is sensitive enough to detect picogram levels of NGF per milliliter of urine. The amount of NGF in each sample was calculated from the NGF standard curve. Each urinary NGF value was normalized to its corresponding Cr level, and results were determined after averaging the group values.

Statistical Analysis

A power analysis was performed with a power of 0.80 and a value of α of 0.05 based on results of a previous study by Liu and Kuo⁶ looking at urinary NGF/Cr levels in men with BOO. Sample size based on this calculation was 5 patients. The NGF levels and other variables in women with BOO, before and after surgical correction or initiation of CIC, and in the control group were compared using Student *t* tests for continuous variables. Figures and statistical analyses were performed using Minitab software (Minitab, State College, PA) and represent mean (SEM).

RESULTS

A screening of 17 women yielded a total of 10 female patients with anatomic BOO (mean age of 66.2 [3.88] years) who were then paired with 10 healthy female control subjects (mean age of 62 [7] years). There was no statistical difference in age ($P = 0.364$) between the 2 groups. Nine patients had POP. Six patients had undergone a previous anti-incontinence procedure. Five patients had both POP and undergone a previous anti-incontinence procedure. Table 1 details the cause of BOO in each patient along with the subsequent treatment. Seven patients completed a preoperative UDI-6 survey with a mean

score of 16.3 (1.8), and 6 patients completed a preoperative POPDI-6 survey with a mean score of 10.2 (2.6). The POPDI-6 and UDI-6 subsection scores are reported in Table 2.

The UD was obtained in 9 of the 10 patients, all with evidence of valsalva voiding. Two patients were unable to urinate. The mean max urinary flow (Q_{max}) for the other 7 patients was 6.3 (1.9) mL/s with P_{det} at Q_{max} of 7.5 (4.1) cm H₂O. Five of the 7 patients had Q_{max} less than 12 mL/s. The 1 patient who did not undergo UD had frank urinary retention with a PVR of 1200 mL. The mean PVR was 232 (101.23) mL.

Surgical interventions included colpocleisis in 3 patients, robotic sacrocolpopexy in 2 patients, uterosacral ligament suspension in 1 patient, sling excision and replacement in 1 patient, suprameatal urethrolisis in 1 patient, and robotic burch suspension suture takedown in 1 patient (Table 1). Conservative management with CIC was initiated in 1 patient. After treatment, mean PVR was reduced to 125.86 (95.94) mL ($P = 0.459$). The UDI-6 scores were significantly reduced from 16.3 (1.78) to 3.33 (2.4) after treatment ($P = 0.01$). Posttreatment Q_{max} was significantly increased to 12.4 (3.08) mL/s from 6.3 (1.86) mL/s ($P = 0.031$).

The urinary NGF/Cr levels in the study patients with BOO (20.8 [4.31] pg/mg) were significantly higher ($P = 0.006$) than the levels in the control group (5.6 [0.65] pg/mg), as seen in Figure 1. In addition, the level of urinary NGF showed a weak positive correlation ($r^2 = 0.16$) with patient-reported UDI-6 scores, as seen in Figure 2. After treatment with release of previous anti-incontinence procedure or POP repair, the urinary NGF/Cr level significantly decreased ($P = 0.009$) to 6.50 (0.57) pg/mg (Fig. 1). In 2 patients with 3 months post-intervention follow-up, the urine NGF/Cr levels continued to be significantly decreased ($P = 0.008$) at the 3 months postoperatively (4.95 [1.85] pg/mg) compared with before intervention. Figure 3 shows a scatterplot detailing the (1) urinary NGF and (2) urinary NGF/Cr levels for each individual patient.

DISCUSSION

The results of this study showed that female patients with anatomic BOO resulting from POP and/or previous incontinence surgery had significantly higher urinary NGF/Cr levels when compared with age-matched controls. Upon correction of the obstruction, either through surgical intervention or implementation of a CIC regimen, the urinary NGF/Cr levels significantly decreased. Furthermore, the decreases in NGF/Cr levels after treatment correlated with subjective improvement in the symptoms of patients as demonstrated by decreased UDI-6 survey scores and objective improvement as demonstrated by increased flow rates. These results suggest that urinary NGF/Cr levels could potentially serve as a biomarker for the diagnosis of BOO as well as an objective assessment of the therapeutic effects of surgical or medical intervention in women.

The NGF is produced by bladder smooth muscle cells, urothelial cells, and sensory afferent neurons that innervate the urinary bladder.⁸ Under normal conditions, NGF levels in the urine are low. However, increasing levels of NGF are known to be associated with bladder enlargement, sympathetic and sensory hyperinnervation, and bladder hyperactivity.⁹ In

addition, chemical or physical irritation, detrusor overactivity, and BOO have been shown to increase bladder inflammation, with a subsequent rise in urinary NGF levels.¹⁰ Indeed, NGF expression has been shown to increase in an in vitro model mimicking BOO. It has been hypothesized that, through mechanical stretching, NGF expression in the bladder wall may increase leading to a reduced sensory threshold resulting in urgency or a reduced threshold for mediating detrusor hyperactivity.¹¹

A previous work by Liu and Kuo¹² demonstrated that urinary NGF levels were increased in men with BOO compared with control subjects and that successful long-term medical management of BOO led to decreased urinary NGF levels. However, this previous study only assessed the use of NGF as an indicator of BOO in male patients. The current study has demonstrated that these results are generalizable to women as well. We have shown that women with BOO presented elevated urinary NGF/Cr levels when compared with normal controls and that these levels significantly decrease with appropriate surgical treatment.

A recent study by Chai et al¹³ looked at various urinary biomarkers before and after midurethral sling surgery for stress urinary incontinence. The urinary markers after continence surgery trial studied urinary NGF values in 150 women with stress urinary incontinence before and 1 year after midurethral sling surgery. The mean nonnormalized NGF level of 10.95 pg/mL at baseline is consistent with our study where our control patients (urinary incontinence-free healthy volunteers) had a nonnormalized NGF level of 8.2 pg/mL. Kuo et al¹⁴ noted a mean nonnormalized NGF level of 1.52 pg/mL in their control population. When NGF levels were normalized for Cr, the Chai study had a mean urinary NGF/Cr level of 24.23 pg/mg, which is higher than our study control levels of 5.6 pg/mg. This discrepancy could be explained by variation in different baseline levels when the assay is run by different laboratories. The important thing is that the urinary NGF/Cr values for the baseline serve as a “self” control when assessing the impact of an intervention. Furthermore, Chai found that the urinary NGF/Cr levels increased after placement of a midurethral sling, although it was not statistically significant. This suggests that midurethral slings may be obstructive, which are consistent with our results.

Considering these results, it seems likely that NGF/Cr levels could serve as a useful biomarker in patients with signs of BOO. Although elevated NGF/Cr levels in a patient complaining of obstructive bladder symptoms would likely supplement rather than replace formal urodynamic assessment, NGF/Cr levels may replace the need for postoperative urodynamic studies, saving money while decreasing invasive testing and potential harm to patients. However, additional studies need to be performed before the establishment of NGF/Cr levels as a BOO biomarker and a routine testing modality. Specifically, it should be determined whether there is a linear relationship between the severity of bladder inflammation and obstruction and the quantitative NGF levels. Despite the current limitations, however, urinary NGF/Cr levels seem to be a promising future biomarker for use in diagnosing and evaluating BOO.

The low number of patients involved in this study was the primary limitation. Although the results of this report are consistent and significant, further information regarding the use of NGF/Cr level testing could have been gathered if the study had enrolled additional patients.

Moreover, although the current investigation demonstrated elevated levels of urinary NGF/Cr in patients with BOO, with a larger patient population, the relationship between the degree of bladder obstruction severity and NGF/Cr levels could have been further elucidated. In addition, UD and survey data on controls would have allowed for further comparison. However, the data provided justify the development of a larger, more robust study in the future.

CONCLUSIONS

In this study, female patients with anatomic BOO resulting from POP and/or previous incontinence surgery had significantly higher urinary NGF/Cr levels when compared with control volunteers. After surgical correction, the urinary NGF/Cr levels significantly decreased. Therefore, urinary biomarkers are attractive as a possible adjunct to the evaluation and monitoring of various LUTS. Our future investigations will expand the observed correlation in larger groups of women with BOO.

Acknowledgments

Supported by the Houston Methodist Foundation.

REFERENCES

1. Blaivas JG, Groutz A. Bladder outlet obstruction nomogram for women with lower urinary tract symptomatology. *Neurourol Urodyn*. 2000; 19(5):553–564. [PubMed: 11002298]
2. Ochodnický P, Cruz CD, Yoshimura N, et al. Nerve growth factor in bladder dysfunction: contributing factor, biomarker, and therapeutic target. *Neurourol Urodyn*. 2011; 30(7):1227–1241. [PubMed: 21520250]
3. Steers WD, Kolbeck S, Creedon D, et al. Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. *J Clin Invest*. 1991; 88(5):1709–1715. [PubMed: 1939656]
4. Steers WD, Clemow DB, Persson K, et al. The spontaneously hypertensive rat: insight into the pathogenesis of irritative symptoms in benign prostatic hyperplasia and young anxious males. *Exp Physiol*. 1999; 84(1):137–147. [PubMed: 10081714]
5. Lamb K, Gebhart GF, Bielefeldt K. Increased nerve growth factor expression triggers bladder overactivity. *J Pain*. 2004; 5(3):150–156. [PubMed: 15106127]
6. Liu HT, Kuo HC. Urinary nerve growth factor level could be a potential biomarker for diagnosis of overactive bladder. *J Urol*. 2008; 179(6):2270–2274. [PubMed: 18423678]
7. Thongboonkerd V. Practical points in urinary proteomics. *J Proteome Res*. 2007; 6(10):3881–3890. [PubMed: 17824635]
8. Kuo HC, Liu HT, et al. Can urinary growth factor be a biomarker for overactive bladder? *Rev Urol*. 2010; 12(2–3):e69–e77. [PubMed: 20811555]
9. Liu HT, Chen CY, Kuo HC. Urinary nerve growth factor levels in overactive bladder syndrome and lower urinary tract disorders. *J Formos Med Assoc*. 2010; 109(12):862–878. [PubMed: 21195884]
10. Cruz CD. Neurotrophins in bladder function: what do we know and where do we go from here? *Neurourol Urodyn*. 2014; 33:39–45. [PubMed: 23775873]
11. Yamaguchi O. Response of bladder smooth muscle cells to obstruction: signal transduction and the role of mechanoreceptors. *Urology*. 2004; 63(suppl 3A):11–16. [PubMed: 15013647]
12. Liu HT, Kuo HC. Urinary nerve growth factor levels are increased in patients with bladder outlet obstruction with overactive bladder symptoms and reduced after successful medical treatment. *J Urol*. 2008; 72(1):104–108.

13. Chai TC, Richter HE, Moalli P, et al. Inflammatory and tissue remodeling urinary biomarkers before and after mid urethral sling surgery for stress urinary incontinence. *J Urol*. 2014; 191:703–709. [PubMed: 24140551]
14. Kuo HC, Liu HT, Chancellor MB. Urinary nerve growth factor is a better biomarker than detrusor wall thickness for the assessment of overactive bladder with incontinence. *Neurourol Urodyn*. 2010; 29(3):482–487. [PubMed: 19367641]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

FIGURE 1.

Urinary NGF/Cr (pg/mg) levels in control subjects and women with BOO before intervention (preoperative) and 1 month after intervention.

FIGURE 2.

Scatterplot showing correlation between UDI-6 total score and urinary NGF/Cr levels (pg/mg).

FIGURE 3.

Scatterplot showing (A) urinary NGF level (pg/mL) before and after interventions and (B) urinary NGF/Cr (pg/mg) level before and after interventions.

TABLE 1
Etiology of BOO With Subsequent Interventions and Corresponding Urinary NGF Levels

Patient	Prolapse	Previous Incontinence Procedure	Etiology of BOO	Intervention	Preintervention NGF/Cr, pg/mg	Postintervention NGF/Cr, pg/mg
1	None	Marshall-Marchetti-Krantz (MMK) bladder suspension	MMK	Suprameatal urethrolisis with martsius flap and autologous rectus fascial pubovaginal sling	22.4	7.9
2	Stage 4 prolapse	Retropubic midurethral sling	Prolapse	Colpocleisis and SPARC midurethral sling	13.2	6.3
3	None	Cadaveric pubovaginal sling	Sling	CIC	51.1	6.5
4	Stage 2 uterovaginal prolapse	None	Uterine prolapse	Le Fort colpocleisis/SPARC midurethral sling	11.5	4.2
5	Stage 2 cystocele	Transobuturator midurethral sling	Prolapse	Cystocele repair/bilateral uterosacral ligament suspension	30.6	10.3
6	Stage 3 vaginal vault, stage 2 cystocele, stage 2 rectocele	Retropubic midurethral sling	Prolapse	Colpocleisis with perineorrhaphy	10.1	8.5
7	Stage 2 vaginal vault, stage 2 cystocele	Retropubic midurethral sling	Prolapse	Robotic-assisted laparoscopic sacrocolpopexy	17.4	2.7
8	None	Transobuturator midurethral sling	Sling	Replacement of MONARC TOT with SPARC midurethral sling	5.5	10.1
9	Stage 3 vaginal vault, stage 2 cystocele, stage 2 rectocele	Transobuturator midurethral sling	Prolapse and sling	Robotic-assisted laparoscopic sacral colpopexy, cystocele/rectocele repair, perineorrhaphy, MONARC excision	14.6	5.3
10	None	Burch urethropexy	Urethropexy	Robotic burch urethropexy suture takedown	31.3	6.5

TOT indicates transobuturator tape.

TABLE 2

Preintervention POPDI-6 and UDI-6 Scores

n = 7	Pretreatment UDI-6 Total Score	Frequent Urination	Leakage Related to Feeling of Urgency	Leakage Related to Physical Activity, Coughing, or Sneezing	Small Amounts of Leakage (drops)	Difficulty Emptying the Bladder	Pain or Discomfort in the Lower Abdominal or Genital Area
Mean	16.3	2.6	2.9	2.3	2.7	3.6	2.3
SEM	1.8	0.6	0.6	0.7	0.6	0.4	0.6
n = 6	Pretreatment POPDI-6 Score	Pressure in the Lower Abdomen	Heaviness or Dullness in the Pelvic Area	Bulge or Something Falling Out That You Can See or Feel in Your Vaginal Area	Push on the Vagina or Around the Rectum to Have or Complete a Bowel Movement	Feeling of Incomplete Bladder Emptying?	Push up on a Bulge in the Vaginal Area With Your Fingers to Start or Complete Urination?
Mean	10.2	1.7	1.7	2	1.2	3.3	0.3
SEM	2.6	0.7	0.7	0.9	0.7	0.5	0.2

Values reported are derived from the following score system: 0, no; 1, not at all; 2, somewhat; 3, moderately; 4, quite a bit.