

Urinary Nerve Growth Factor Can Predict Therapeutic Efficacy in Children With Overactive Bladder



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OBJECTIVE	To assess urinary nerve growth factor (NGF) in children with overactive bladder (OAB) and to investigate the relationship between urinary NGF/creatinine (Cr) levels and OAB.
PATIENTS AND METHODS	Thirty-five children (27 boys and 8 girls) with OAB and 11 children (6 boys and 5 girls) without OAB or any other urinary symptoms, who served as controls, were included in this study. Urinary NGF levels were measured using enzyme-linked immunosorbent assay. The total urinary NGF levels were adjusted with the concentration of urinary creatinine (NGF/Cr level). Refractory OAB was defined as little improvement in OAB symptoms despite at least 3 months of urotherapy and anticholinergic agent treatment. Urinary NGF/Cr was compared between the children with OAB and the controls. The relationship between urinary NGF/Cr and treatment outcomes was also evaluated.
RESULTS	Urinary NGF/Cr was significantly higher in the children with OAB when compared with those in the control group (0.65 ± 0.82 vs 0.11 ± 0.09 , $P = .0007$). Improvement of OAB symptoms was observed in 26 out of 35 children (74%). The remaining 9 children showed refractory OAB symptoms (the refractory group). Urinary NGF/Cr was significantly higher in the refractory group than in the improved group (1.28 ± 1.34 vs 0.44 ± 0.39 , $P = .027$).
CONCLUSION	Urinary NGF/Cr was significantly higher in the children with OAB than in the controls, and was significantly higher in the refractory group than in the improved group. Urinary NGF/Cr could not only be a potential biomarker for children with OAB, but also a predictor of therapeutic efficacy in children with OAB. UROLOGY 103: 214–217, 2017. © 2017 Elsevier Inc.

Overactive bladder (OAB) is one of the most common voiding dysfunctions in children. Its diagnosis is mainly based on results of clinical examinations, including a frequency volume chart and uroflowmetry. These methods, however, are often inaccurate in children. Urinary nerve growth factor (NGF) is considered a biomarker for OAB in the adult population.¹ Only a few reports^{2,3} have focused on the association between urinary NGF levels and OAB in children.

In this study, we measured urinary NGF in children with OAB and investigated the relationship between urinary NGF/creatinine (Cr) levels and OAB. We also analyzed whether urinary NGF/Cr levels can predict therapeutic efficacy in children with OAB.

PATIENTS AND METHODS

The institutional review board at Nara Medical University approved this study. Among children treated at Nara Medical University Hospital between July 2013 and July 2014, urine samples were collected from 35 children with OAB (27 boys and 8 girls) immediately before treatment and 11 children (6 boys and 5 girls) without OAB or any other urinary symptoms as controls. Children with neurogenic bladder, a urinary tract infection in the previous 3 months, asthma, and allergic diseases were excluded from the study. Lower urinary tract function was evaluated according to dysfunctional voiding symptom score,⁴ frequency volume chart, and maximum voided volume (MVV) during the daytime in all children. Uroflowmetry and ultrasonography were also performed in all cases. Those who showed abnormal uroflowmetry patterns, such as a plateau or interrupted pattern, those who showed residual urine of more than 20 mL, or those who showed bladder wall thickness of greater than 3 mm before urination were excluded from the study. Expected bladder capacity was calculated as $(\text{age} + 2) \times 25$ mL, according to the formula described by Hamano et al.⁵

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Urine samples were centrifuged at 2500-3000 g for 10 minutes within 1 hour of collection at room temperature. The supernatants were frozen at -80°C promptly until analysis. Urinary NGF levels were measured with enzyme-linked immunosorbent assay using the Emax ImmunoAssay Systems (Promega, Madison, WI). The total urinary NGF levels were adjusted with the concentration of urinary creatinine (NGF/Cr level).

Children with OAB were first treated with common urotherapy, and then with an anticholinergic therapy. Urotherapy included timed voiding, bladder training, and bowel treatment. Oxybutynin (0.2-0.4 mg/kg/d) or solifenacin (2.5-5.0 mg/d) was administered as anticholinergic agent according to the physicians' choice. The treatment outcomes were assessed after at least 3 months of treatment. Outcomes were defined according to International Children's Continence Society classifications.⁶ Those who showed a complete response (100% reduction) and partial response (50%-99% reduction) were defined as being part of the "improved group," and those with no response (less than 50% reduction) were defined as the "refractory group."

Urinary NGF/Cr levels were compared between the children with OAB and the controls. The relationship between urinary NGF/Cr levels and treatment outcomes was also evaluated.

Mann-Whitney test and Fisher exact test were used to compare the groups. A *P* value of less than .05 was considered to be statistically significant.

RESULTS

The mean age of patients was 7.5 ± 1.9 years (range 5-12) in children with OAB and 9.9 ± 2.5 years (range 7-15) in the control group. There was a significant difference between the groups (*P* = .003).

According to uroflowmetry examination in children with OAB, a bell-shaped flow curve was observed in 29 (82.9%) patients and a tower-shaped flow curve was observed in 6 (17.1%) patients.

Urinary NGF/Cr levels were significantly higher in the children with OAB than in the controls (0.65 ± 0.82 vs $0.11 \pm .09$, respectively) (*P* = .0007) (Fig. 1). If we excluded the outlier case with extremely high urinary NGF/Cr levels (4.15), there were still significant differences in the urinary NGF/Cr levels between the children with OAB and the controls (*P* = .005). Urinary NGF/Cr levels, excluding the outlier case with extremely high urinary NGF/Cr levels, had a sensitivity of 67.7% and a specificity of 90.9% (cutoff 0.26; area under the curve 0.83; 95%) for diagnosing OAB.

After urotherapy and anticholinergic therapy, improvement in OAB symptoms was observed in 26 (74%) out of 35 children (the improved group; urotherapy alone in 11 children [31%]) and urotherapy with a subsequent anticholinergic agent in 15 [43%]). The remaining 9 children (26%) showed refractory OAB symptoms (the refractory group).

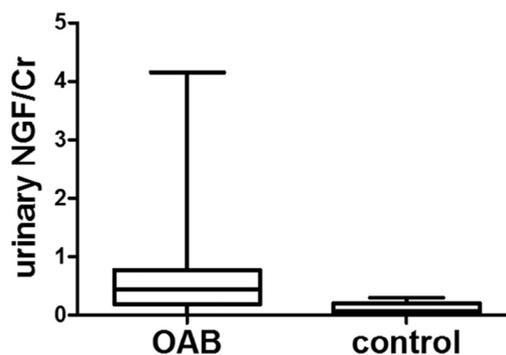


Figure 1. Urinary nerve growth factor/creatinine (NGF/Cr) levels were significantly higher in children with overactive bladder (OAB) than in children in the control group.

Characteristics according to the treatment outcomes are listed in Table 1. There were no significant differences between the groups according to gender, age, dysfunctional voiding symptom score, MVV during daytime, MVV/expected bladder capacity (%), post-void residual urine volume, the proportion with nocturnal enuresis, and the proportion with constipation. Urinary NGF/Cr levels were significantly higher in the refractory group than in the improved group (1.28 ± 1.34 vs 0.44 ± 0.39 , respectively, *P* = .027) (Fig. 2).

Among the patients in the refractory group, 4 children continued to take anticholinergic agents according to the patients' and parents' wishes. Others were subject to video-urodynamic studies. Urethral stenosis was detected in 2 boys (40%), detrusor sphincter dyssynergia (DSD) in 1 girl (20%), and detrusor overactivity in 1 boy (20%). One with urethral stenosis was treated with a transurethral incision (TUI) of the urethral stenosis, and his OAB symptoms improved. The others with urethral stenosis continued with the administration of an anticholinergic agent without TUI. One with DSD had been administered an alpha-blocker after the video-urodynamic study, and the OAB symptoms improved. The child with detrusor overactivity continued to use an anticholinergic agent, although the OAB symptoms persisted.

DISCUSSION

OAB is one of the most common voiding dysfunctions in children. Several studies have shown the significant correlation between NGF levels and OAB in the adult population.^{1,7,8} It is suggested that NGF is responsible for the growth and maintenance of sensory neurons, and NGF appears to have a role in neuroimmune interactions, in tissue inflammation, and in neuroplasticity for neuronal events.⁹ NGF is associated with the activation of sensory receptors TRPV1, which leads to OAB symptoms.¹⁰ There are, however, only a few reports on the association between urinary NGF levels and OAB in children.

In the present study, the results mirrored that of the adult population; there was a significant difference in urinary

Table 1. Characteristics according to the treatment outcomes

	Improved (n = 26)	Refractory (n = 9)	P Value
Gender (boy/girl)	21/5	6/3	n.s.
Age (y)	7.6 ± 1.9	7.6 ± 2.1	n.s.
DVSS	9.4 ± 3.6	8.0 ± 2.2	n.s.
Daytime incontinence(+)	20 (76.9%)	8 (88.9%)	n.s.
MVV (mL)	139.3 ± 66.5	150.0 ± 69.0	n.s.
MVV/EBC (%)	57.9 ± 27.6	61.2 ± 18.3	n.s.
PVR (mL)	3.6 ± 7.0	4.5 ± 8.5	n.s.
Nocturnal enuresis(+)	25 (96.2%)	8 (88.9%)	n.s.
Constipation(+)	3 (11.5%)	0 (0%)	n.s.
Urinary NGF/Cr	0.44 ± 0.39	1.28 ± 1.34	P = .027

DVSS, dysfunctional voiding scoring system; EBC, expected bladder capacity; MVV, maximum voided volume; PVR, post-void residual urine volume.

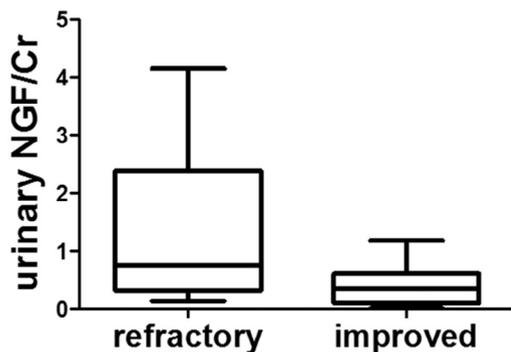


Figure 2. Urinary nerve growth factor/creatinine (NGF/Cr) levels were significantly higher in the refractory group than in the improved group in children with overactive bladder (OAB) who were treated with urotherapy and anticholinergic therapy.

NGF/Cr between the children with OAB and the control group. Urinary NGF/Cr levels were significantly higher in children with OAB. Furthermore, urinary NGF/Cr was significantly higher in children with OAB who refract to the urotherapy with a subsequent anticholinergic treatment than in the improved group.

Recent studies in adult patients with OAB have shown that serum NGF levels are significantly correlated with urinary NGF and urinary NGF/Cr levels, and that urinary NGF levels are correlated with the severity of OAB symptoms.¹¹ The significant correlation between serum NGF and OAB symptoms indicates that a systemic inflammation might exist in some patients with OAB. Liu and colleagues¹¹ reported that serum NGF and urinary NGF/Cr levels become high in adult patients with OAB who refract to anticholinergic therapy, and they remain high after subsequent other anticholinergic therapies. These results suggest that increased circulating NGF is associated with refractory OAB.

In our study, urinary NGF/Cr levels were significantly higher in children with OAB who refract to urotherapy and anticholinergic therapy. However, we measured only urinary NGF levels and did not measure serum NGF levels. Further studies are needed to evaluate if serum NGF levels

were increased in children with OAB who refract to the treatment compared with the improved group.

Liu and Kuo¹² observed that urinary NGF levels increased in adult population with bladder outlet obstruction. In our study, among those in the refractory group, 3 children showed urethral stenosis (2 boys) and DSD (1 girl), according to video-urodynamic studies. One boy with urethral stenosis underwent TUI, and 1 girl with DSD who was administered an alpha-blocker saw improved OAB symptoms. There is also some possibility of the presence of a bladder outlet obstruction in those with elevated urinary NGF/Cr levels before treatment and in those who refract to the treatment, although this is likely only a small number of cases. Further studies are needed to evaluate whether video-urodynamic studies should be considered to detect urethral stenosis or bladder outlet obstruction among those children with OAB who refract to the treatment and who showed increased levels of urinary NGF/Cr before treatment.

Guney and colleagues¹³ noted that the mean serum NGF levels of children with attention deficit hyperactivity disorder (ADHD) are significantly higher than in those of the controls. Niemczyk and colleagues¹⁴ also noticed that ADHD and daytime urinary incontinence are strongly associated with one another, and suggested that treatments for ADHD may be associated with positive effects on incontinence outcomes. There might be some possibility of ADHD among children who showed increased urinary NGF/Cr levels and who were resistant to the treatment.

One limitation of this study is that the children with OAB and the controls were not age-matched. However, urinary NGF/Cr levels had no correlation with increasing age in children with OAB and controls. Another limitation is the small number of cases with OAB and controls and the short duration of follow-up. Further large studies are needed to explore the possible underlying mechanisms for the resistance of anticholinergic therapy in children with OAB.

CONCLUSION

Urinary NGF/Cr was significantly higher in children with OAB than in the control group, and it was significantly

higher in the refractory group than in the improved group. Urinary NGF/Cr may not only be a potential biomarker for children with OAB, but also a predictor of therapeutic efficacy in children with OAB.

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