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## THE ROLE OF URINARY NEUROTROPHIC FACTORS IN OVERACTIVE BLADDER SYNDROME

### Hypothesis / aims of study

Most well known neurotrophic factors (NFs) include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF). In the last few years, NGF has been extensively studied and may constitute a useful biomarker of overactive bladder (OAB) syndrome. Less is known about BDNF, the most abundant neurotrophin in the human body, which expression may be regulated by NGF. Recently, BDNF was found in high concentration in the urine of patients with bladder pain syndrome (1). While NGF and BDNF are committed with a peptidergic subpopulation of primary afferents, GDNF is a potent neurotrophic factor, important to maintain the non-peptidergic population of primary afferents, both in central and peripheral nervous system. The recently identified cross-talk between GDNF and NGF downstream cascades, suggests an eventual role of GDNF in lower urinary tract function, along with the other NFs.

The aims of our study were: 1) to investigate urinary levels of NGF, BDNF and GDNF in OAB patients and in a control group of healthy volunteers, 2) to assess urinary levels of NGF and BDNF in OAB patients, after lifestyle counseling and pharmacological intervention and 3) to correlate urinary NGF/Cr and BDNF/Cr ratios with the severity of symptoms.

### Study design, materials and methods

Urine samples from 20 female healthy volunteers were collected and adequately stored until further processing. Urine samples from 21 female naïve OAB patients were collected at baseline. Eighteen patients were evaluated at 3 months, after lifestyle counseling and at 6 months, after 3 months of antimuscarinic treatment (oxybutynin). Urine samples were collected at these time points (3 and 6 months).

For both groups, baseline urine samples were processed for ELISA analysis of NGF, BDNF and GDNF. The urinary content of neurotrophin was normalized against creatinine (Cr) concentration.

For each OAB patient, the severity of symptoms was assessed using Indevus Urgency Severity Scale (IUSS), at the time of urine collection, and correlated with the respective urinary NGF/Cr and BDNF/Cr ratios (pg/mg).

### Results

At baseline, urinary NGF/Cr and BDNF/Cr ratios were significantly higher in OAB patients, compared to healthy female volunteers (NGF/Cr:  $430.0 \pm 477.9$  vs  $188.3 \pm 290.2$ ,  $p=0.023$ ; BDNF/Cr:  $898.8 \pm 1603.8$  vs  $110.4 \pm 159.5$ ,  $p<0.01$ ). Nine out of 21 patients had high levels of both urinary NGF and BDNF, 4 had only high levels of urinary NGF and 6 had only high levels of urinary BDNF. In 2 patients both urinary NFs were low. In contrast, urinary GDNF/Cr ratio showed no statistical significant differences between the control and OAB groups ( $2746.4 \pm 3233.8$  vs  $1691.0 \pm 2309.7$ ,  $p>0.05$ ).

Three months after lifestyle counseling, there was a decrease in urinary NGF/Cr ratio ( $433.2 \pm 519.0$  to  $290.0 \pm 296.6$ ) and BDNF/Cr ratio ( $1023.8 \pm 1757.2$  to  $489.6 \pm 627.8$ ). After 3 months of oxybutynin treatment, NGF had an additional, marginal decrease to  $240.7 \pm 422.5$ , while BDNF/Cr had a marked reduction to  $231.1 \pm 311.2$ . At 6 months, BDNF/Cr ratio was significantly lower than at baseline ( $p<0.05$ ), whereas NGF variation had no statistical significance.

Moreover, a significant correlation was only found between BDNF/Cr ratio and IUSS score variations, from baseline to 3 months (after life-style counseling:  $r=0.681$ ,  $p<0.01$ ) and from baseline to 6 months (after antimuscarinic treatment:  $r=0.729$ ,  $p<0.01$ ).

Considering baseline evaluation, using receiver-operator characteristic (ROC) analysis, the area under the curve was higher for BDNF/Cr (0.81) compared to NGF/Cr (0.72). While a threshold urinary BDNF/Cr value of 300pg/mg provided a sensitivity of 71.4% and a specificity of 89.5%, a threshold urinary NGF/Cr value of 200pg/mg provided a sensitivity of 61.9% and a specificity of 68.4%.

### Interpretation of results

According to these results, urinary NFs constitute potential biomarkers of OAB, with eventual diagnostic and monitoring interest. This statement is even more evident for BDNF, with higher sensitivity and specificity than NGF and a correlation with the severity of symptoms. In only 2 out of 21 patients, both neurotrophins were low, which confers to the association of urinary NGF and BDNF extremely high sensitivity in the diagnosis of OAB. Furthermore, these data suggest NFs as key elements in the pathogenesis of OAB, which might be relevant both from the pathophysiological and therapeutic point of view.

### Concluding message

To our knowledge, this is the first comprehensive study of urinary NGF, BDNF and GDNF in OAB patients and healthy volunteers.

In OAB patients, urinary NGF and BDNF levels were significantly higher, compared to controls, while no significant differences were found for GDNF.

Urinary NGF and BDNF decreased after lifestyle counseling and antimuscarinic treatment. This variation was more pronounced for BDNF, with statistical significance after pharmacological intervention. BDNF also showed a significant correlation with the reduction of OAB symptoms.

The differences observed between OAB patients and controls clearly point that urinary NFs, in particular BDNF, may serve as potential biomarkers of OAB syndrome.

### References

1. European Urology 2010; 58:360-5

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<b><i>Is this a clinical trial?</i></b>	<b>Yes</b>
<b><i>Is this study registered in a public clinical trials registry?</i></b>	<b>No</b>
<b><i>Is this a Randomised Controlled Trial (RCT)?</i></b>	<b>No</b>
<b><i>What were the subjects in the study?</i></b>	<b>HUMAN</b>
<b><i>Was this study approved by an ethics committee?</i></b>	<b>Yes</b>
<b><i>Specify Name of Ethics Committee</i></b>	<b>Comissão de Ética para a Saúde do Hospital de São João, E.P.E.</b>
<b><i>Was the Declaration of Helsinki followed?</i></b>	<b>Yes</b>
<b><i>Was informed consent obtained from the patients?</i></b>	<b>Yes</b>