

INCREASED URINARY NERVE GROWTH FACTOR LEVELS IN PATIENTS WITH IDIOPATHIC DETRUSOR INSTABILITY

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REZUMAT

Objective: Determinarea nivelului de factor de creștere neuronală (FCN) la pacienții diagnosticați cu instabilitate detrusoriană idiopatică și compararea acestora cu valorile FCN în urina lotului martor. **Material și metode:** Am recrutat 6 pacienți (5 femei și un bărbat) cu diagnosticul de instabilitate detrusoriană idiopatică stabilit anterior. Pentru lotul martor am folosit urina a șapte voluntari (toate femei). Nivelele de FCN au fost măsurate folosind metoda ELISA. **Rezultate:** Valorile FCN au fost semnificativ mai mari în grupul cu instabilitate detrusoriană comparat cu lotul martor. **Concluzii:** Nivele crescute ale FCN în urina pacienților cu instabilitate detrusoriană idiopatică pot explica simptomatologia și alterările morfopatologice severe întâlnite în această boală. Totodată ele pot fi motivul pentru care întâlnim un număr crescut de mastocite, precum și creșterea sensibilității fibrelor nociceptive. Acest lucru poate pleda pentru o etiologie neuropată a acestei patologii.

Cuvinte cheie: factor de creștere neuronală, instabilitate detrusoriană

ABSTRACT

Objectives: To determine whether the nerve growth factor (NGF) is elevated in the urine of the patients diagnosed with idiopathic detrusor instability (IDI). **Material and methods:** We have recruited 6 patients (five women and one man) previously diagnosed with idiopathic detrusor instability. For control we used urine from seven healthy volunteers (all women). The NGF levels were measured using an ELISA kit. **Results:** NGF levels were significantly higher in the IDI group compared to the control group. **Conclusions:** Urinary raised levels of NGF in the patients with idiopathic detrusor instability may explain the severe clinical and pathological features in this conditions, including sensitization of the nociceptor fibers and the increased number of mast cells. It also pleads for a neuropathic etiology of the condition.

Key Words: nerve growth factor, detrusor instability

INTRODUCTION

Painful conditions of the urinary bladder include idiopathic detrusor instability (IDI), chronic cystitis and interstitial cystitis. IDI is a urodynamic diagnosis and is characterized by the presence of uninhibited detrusor contractions during the filling phase of the cystometric reading. Clinically this translates into urgency which could lead to urge incontinence.

Known causes of IDI include urinary tract infections (UTI), bladder calculi and bladder tumors, radiation cystitis as well as chronic inflammatory conditions of the bladder. Urologic workup of these patients includes urine microscopy, culture and cytology as well as cystoscopy and bladder biopsy where needed.

However, there is a group of patients, mostly women, suffering of IDI in which all these findings are within normal limits. The etiology in these cases remains unknown and there is little we can do in terms of treatment, besides placebo therapy.

Recent studies have suggested an increased role of the nerve growth factor (NGF) in the inflammatory and painful conditions of the bladder.¹ NGF is a protein normally produced by cells in the target organs, such as skin, blood vessels and urinary bladder. NGF is then absorbed by the sensory fibers via a high affinity receptor (trkA) and retrogradely transported

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Received for publication: Apr. 23, 2004. Revised: Dec. 8, 2005.

to the cell body.

In adults, NGF is necessary for the survival of the sympathetic fibers as well as for phenotypic properties of small sensory fibers, such as the expression of neuropeptides and capsaicin sensitivity.² There is evidence that increased NGF levels may lead to hyperalgesia by: (1) directly sensitizing the nociceptors; (2) increasing levels of substance P and calcitonin-gene related peptide (CGRP), which may play a role in central sensitizing and neurogenic inflammation; (3) local effects, such as the release of histamine from increased numbers of mast cells.³

NGF is elevated in animal models and there are studies that document the raised levels of NGF in obstructive uropathy.

MATERIAL AND METHODS

For the IDI Group we have recruited a number of six patients (five women and one man). All of the patients were suffering of irritative lower urinary tract symptoms. The most frequent complaint was urinary urgency which in two patients led to urge incontinence. Four of the patients also complained of pain relating to bladder filling. Average age was 34 years (range: 25-51 years) for the women, while the man was 79 years old. Informed consent was obtained from both the patients, as well as from the controls.

All patients underwent basic laboratory workup (including urine culture, and ultrasound examinations to assess the urinary residue). All of them were also investigated with basic urodynamic studies.

Three of the women displayed involuntary contractions on the ascendant filling phase of the cystometric time – detrusor instability; the other two women as well as the man had a first voiding sensation at less than 120 ml (average of 70 ml) and a maximal cystometric capacity of less than 270 ml, without signs of involuntary contractions – urgency. The urodynamic investigations were performed in the Department of Urology of the Clinical Emergency Hospital Timisoara, using a ANDROMEDA Ellypse 9000 Urodynamic Unit.

All of the urinary cultures were negative and none of the patients had a significant post voiding residue. None of the patients were taking chronic medication. One woman from the study group was taking a combined contraceptive pill.

For the control group we used fresh urine from voluntary medical personnel. We have taken urine from seven nurses with the average age of 39.2 years (range: 19-55 years). The urinary cultures were all negative.

None of them was taking chronic medication.

The urine samples were then refrigerated at -70°C until analysis.

The NGF assay we used was an ELISA kit with 96 wells (Nunc, Denmark), which we covered with 150 µl of monoclonal anti-NGF antibody 0.5 µg/ml diluted in 50 mM buffer sodium carbonate, pH 9.6; we then incubated the plates for 2 hours at 36°C.

The plate was then washed 1x1 than 3x5 minutes with 250 µl washing solution (0.4 M NaCl and 0.1% Triton X-100 diluted in 0.1 M phosphate buffer pH 7.4). We then added 150 µl from the urine samples. For each sample we used 4 wells.

The plate was then incubated overnight at room temperature. The next day we washed the plate for 1x1 and 3x20 minutes on a rocking platform. Next, we placed the antibodies (anti-NGF), 100 µl for each well, and then we incubated it for 2 hours at 36°C. This was followed by the washing of the plate 1x1 minute, 3x1 hours on the rocking platform to remove the excess antibody.

After the last washing we dissolved 44 mg of chlorophenol red-Beta-D-galactopyranosid (CPRG) – Boehringer Mannheim – in 22 ml of buffer 7.0 pH. We added 250 µl of this in each well and incubated the plate overnight at 36 degrees.

The following morning we read the color reaction with a Dynatech MR5000 plate reader (ICN/Flow Laboratories) using a 575 nm filter. The standard curve was determined using the average of the wells with known NGF concentration. We then calculated the values for each well using this curve.

RESULTS AND DISCUSSION

The NGF level was significantly higher (more than twice) in the patient group compared to the control group: 5.19 ± 0.6 pg/ml vs. 2.12 ± 0.5pg/ml (p < 0.05).

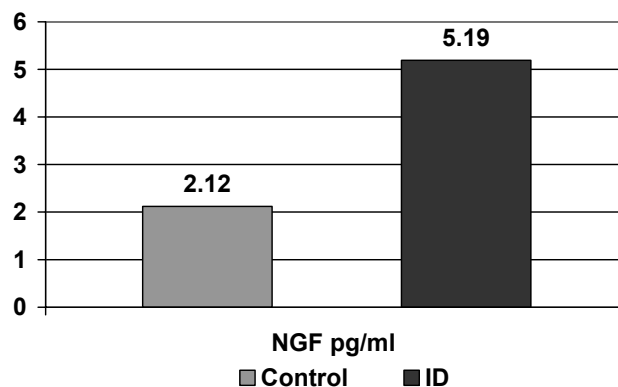


Figure 1. Levels of urinary NGF in controls and IDI patients.

Detrusor instability which is clinically expressed as urgency or even urge incontinence is diagnosed in 9% of the cases referred to the urologist. Approximately 30% of these have an identifiable cause – usually inflammatory. For the rest of 70% the cause remains unknown. These patients represent a challenge for the physician. Numerous treatments have been tried – anticholinergics, antidepressants, dimethyl sulfoxide (DMSO) and even psychotherapy. More recent capsaicin was used with promising results.⁴

Anatomical studies have recently revealed a nervous network located just under the urothelium and presumed to be sensitive.⁵ This nervous plexus can react to heat, pain and can also serve as a receptor for the detrusor stretch. These fibers could also have an efferent function through the release of neuropeptides. The thickening of this layer was described in the interstitial cystitis.⁶

The present study reveals an increase of the NGF level in the urine of the patients suffering of a chronic painful bladder condition. The mechanism underlying this increase remains obscure.

Most patients suffering of idiopathic detrusor instability are women. It is also known that estrogens increase the production of NGF as do the neural fibers themselves.

What we propose is a possible neuropathogenic cause of the detrusor instability. For unknown reasons the density of nervous fibers decreases and thus the rise in the NGF production could be as well the result of a negative feed-back of the muscle in its attempt to re-innervate itself.⁷ We believe that this aspect of urinary incontinence is worth further investigation.

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