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Increased Nerve Growth Factor Production in Suburothelium in Patients with Chronic Interstitial Cystitis

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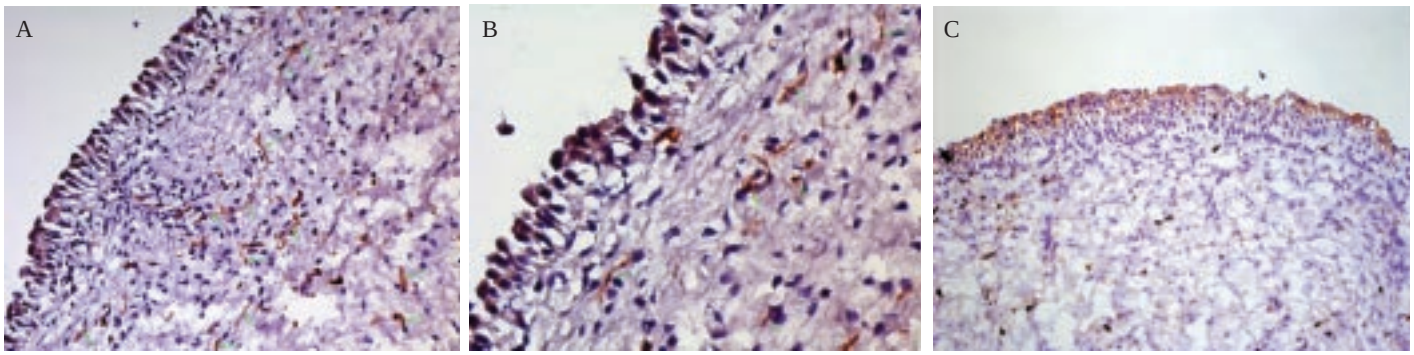

Fig. 1. Immunohistochemistry of NGF in bladder mucosa of CIC patient (A: 200× ; B: 400×) and control (C: 200×).

Recent studies have shown increased levels of nerve growth factor (NGF) in the bladder tissue and urine of patients with painful inflammatory conditions of the lower urinary tract, such as sensory urgency and chronic interstitial cystitis (CIC) [1,2]. Intravesical botulinum toxin A (BTX-A) reduces levels of NGF in the bladder tissue of patients with idiopathic and neurogenic detrusor overactivity [3]. Although the mechanism responsible for this reduction of bladder NGF has not been elucidated, prevention of neural plasticity by blockade of NGF production has been suggested as a potential treatment to reduce urge incontinence and symptoms of detrusor overactivity.

Urinary bladder specimens were immersion fixated for 1 hour in an ice-cold solution of 4% formaldehyde in phosphate buffered saline (PBS) (pH 7.4) and rinsed with ice-cold PBS containing 15% sucrose for 12 hours. The specimens were embedded in OCT medium (Ames Inc., Iowa, USA) and then store at -80 ˚C. The tissues were sectioned into 6 μm thick slices and were collected on poly-L-Lysine-coated slides (SuperFrost® Plus). They were then fixed for 10 minutes in cold acetone before use. Endogenous peroxidase activity was first blocked by pretreatment of tissue sections with 0.3% hydrogen peroxide for 30 minutes, followed by rinsing in PBS. Background staining was blocked by incubating the sections with casein solution (Power Block, BioGenix) for 10 minutes, followed by rinsing with PBS. Sections were incubated overnight at 4 ˚C with primary antibodies of anti-human beta NGF (goat polyclonal antibody, R&D). After rinsing the sections with TPBS (PBS contain 0.1% Tween-20), rabbit anti-goat secondary antibodies (Stressgen, Canada) were applied to the sections followed by incubation for 1 hour. Subsequently, horseradish peroxidase-conjugated secondary antibodies were added for 30 minutes (Polymer-HRP IHC Detection System, BioGenix) and substrate 3,3'- diaminobenzidine (DAB, BioGenix) was added for 3 minutes. Sections were finally counterstained with hematoxylin (Sigma) for 10 seconds. Negative controls included omission of primary antibody.

NGF-immunoreactivity study of the bladder of CIC patients showed significant increased density in submucosal and urothelial cells at baseline (Fig. 1A,1B). By contrast, controls had NGF staining only in apical urothelial cells (Fig. 1C).

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