Urothelial Barrier Deficits, Suburothelial Inflammation and Altered Sensory Protein Expression in Detrusor Underactivity

Yuan-Hong Jiang and Hann-Chorng Kuo*

From the Department of Urology, Buddhist Tzu Chi General Hospital and Tzu Chi University, Hualien, Taiwan, Republic of China

Purpose: The pathophysiology of detrusor underactivity remains unclear and impaired bladder afferent function is considered one of the important etiologies. We investigated urothelial barrier deficits, suburothelial inflammation and sensory proteins expressed in the bladder mucosa of patients with detrusor underactivity.

Materials and Methods: Bladder mucosa biopsies were performed in 34 patients with videourodynamic proven detrusor underactivity as the study group and in 10 women with stress urinary incontinence as controls. The expression of zona occuldens-1, E-cadherin in the urothelium, tryptase and apoptosis levels in the suburothelium, β3-adrenoceptor, M2 and M3 muscarinic receptors, P2X3 receptor, and inducible and endothelial nitric oxide synthase were compared between study patients and controls.

Results: Study patients included 22 women and 12 men with a mean ± SD age of 56.3 ± 19.7 years, of whom 15 had a history of diabetes. Study patients had significantly lower E-cadherin expression, and a higher number of mast cells and apoptotic cells than controls. Additionally, lower expression of M2 and M3 muscarinic receptors, P2X3 receptors and endothelial nitric oxide synthase was detected in study patients but higher expression of β3-adrenoceptor. In study patients a positive correlation was noted between tryptase and apoptosis levels ($r = 0.527$) and between the expression of M2 muscarinic receptor and P2X3 receptor ($r = 0.403$). However, β3-adrenoceptor expression negatively correlated with E-cadherin expression ($r = -0.490$, each $p < 0.05$).

Conclusions: Urothelial dysfunction, increased suburothelial inflammation and altered sensory protein expressions in bladder mucosa were prominent in patients with detrusor underactivity. Impaired urothelial signaling and sensory transduction pathways appear to reflect the pathophysiology of detrusor underactivity.

Key Words: urinary bladder neck obstruction, muscle contraction, inflammation, afferent pathways, protein

Abbreviations and Acronyms

BOO = bladder outlet obstruction
CBC = cystometric bladder capacity
DO = detrusor overactivity
DU = detrusor underactivity
eNOS = endothelial nitric oxide synthase
iNOS = inducible nitric oxide synthase
OAB = overactive bladder
Pdet = detrusor voiding pressure
PVR = post-void residual volume
Qmax = maximal urinary flow rate
UAB = underactive bladder
VUDS = videourodynamic study
ZO-1 = zonula occludens-1

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* Correspondence: Department of Urology, Buddhist Tzu Chi General Hospital, 707, Section 3, Chung Yang Rd., Hualien, Taiwan, Republic of China (telephone: 886-3-8561825, extension 2117; FAX: 886-3-8560794; e-mail: hck@tzuchi.com.tw).

DETRUSOR underactivity or UAB is an important but under researched issue. DU was defined as "a contraction of reduced strength or duration resulting in prolonged [and/or] incomplete emptying of the bladder" by ICS (International Continence Society).[1] Nonetheless, it has received only minimal attention. DU can be observed in many conditions, including aging, BOO, neurological insults and myogenic failure, but its pathophysiology remains unclear.[2] Altered or impaired bladder afferent function with impaired subsequent activation of detrusor

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contraction has been considered an important etiology of DU.[3,4]

The bladder urothelium serves not only as a physical barrier but also as a sensor and transducer of sensory signaling.[5] Urothelial cells express various receptors and ion channels, including purinergic receptors, muscarinic receptors, adrenoceptors and transient receptor potential ion channels, which sense and respond to various intravesical stimuli. In addition, the activation of β3-adrenoceptors in the urothelium and the suburothelium can inhibit bladder afferent signaling and facilitate bladder storage.[6] The urothelium and the underlying suburothelium are in close communication and are thought to be an integrated sensory unit. Urothelial dysfunction, increased suburothelial inflammation and apoptosis develop in many lower urinary tract diseases, including OAB and neurogenic DO,[7–9] and probably also in DU.

BOO is also considered a contributor to the development of DU.[2,10] In BOO chronic bladder ischemia and repeat ischemia/reperfusion cycles cause excessive oxidative stress, which could be responsible for the development of bladder dysfunctions such as DO and DU.[11] Augmented purinergic and muscarinic receptors in the urothelium are considered important in the pathophysiology of DO by mediating augmented/altered bladder afferent neurotransduction.[12,13] We also found significant urothelial dysfunction and alterations of sensory proteins, including higher β3-adrenoceptor and lower iNOS expression, in the bladder mucosa of patients with BOO and urodynamic DU.[14] Impaired urothelial signaling and sensory transduction pathways through β3-adrenoceptors and the nitric oxide synthase pathway could advance DU.

Urothelial function and sensory proteins in the bladder mucosa could participate in the pathophysiology of DU. Evidence of this hypothesis is rare in humans. We investigated urothelial barrier deficits, suburothelial inflammation and the expression of sensory proteins in the bladder mucosa of patients with DU. The relationships between altered sensory protein expression and bladder dysfunction were also examined.

MATERIALS AND METHODS

Patients

From June 2012 to March 2014 we prospectively and consecutively enrolled patients in study who were in chronic urinary retention and required clean intermittent catheterization and in whom DU was proved by VUDS. Additionally, the selected patients were candidates for transurethral resection of the prostate or external urethral sphincter onabotulinumtoxinA injection to improve self-voiding efficiency. Clinical investigations included transrectal sonography of the prostate in male patients, and cystourethroscopy and VUDS in all patients before surgery. Study exclusion criteria were active urinary tract infection, significant evidence indicative of BOO on VUDS or cystourethroscopy, interstitial cystitis, occult or overt neuropathy such as cerebrovascular accident, spinal cord injury, multiple sclerosis and Parkinson's disease, a history of bladder surgery or injury and a history of pelvic floor or spine surgery. As controls we also invited into the study 10 women with genuine stress urinary incontinence who had no other storage or voiding dysfunction on VUDS and who were candidates for a pubovaginal sling procedure.

Videourodynamics and Biopsy Procedures

VUDS was performed and the results were interpreted according to ICS recommendations.[1,15] VUDS parameters included Qmax, Pdet, CBC, voided volume and PVR. DU was defined as low voiding pressure, low flow rate, PVR volume greater than 300 ml and less than 33% voiding efficiency (voided volume/CBC) as well as a relaxed external urethral sphincter on electromyography during voiding. During the filling phase DO was defined as an involuntary detrusor contraction associated with urgency symptom. Cystourethroscopy was performed to exclude anatomical BOO, including bladder neck obstruction or contracture, benign prostatic obstruction in men and urethral stricture.

Cold cup bladder biopsies were performed concurrently in all patients with DU after surgery to enhance self-voiding efficiency and in controls after anti-incontinence surgery. Only bladder mucosa was taken to prevent bladder perforation. Bladder specimens were obtained from the posterior wall around 2 cm above the ureteral orifice. Any erythematous or inflammatory bladder mucosa was avoided as the biopsy site. One bladder specimen was sent to the pathology department for histological examination to exclude the possibility of carcinoma in situ.

This study was approved by the Buddhist Tzu Chi General Hospital institutional review board and ethics committee. Each patient was informed about the study rationale and procedures, and written informed consent was obtained before surgery.

Immunofluorescence Staining and Protein Expression

Bladder tissue samples from patients with DU and controls were investigated for urothelial adhesive function by E-cadherin expression, urothelial integrity by ZO-1 expression, mast cell activation by tryptase level and cellular apoptosis by TUNEL assay. Laboratory procedures were performed similarly to those in our previous study.[8] Tissue sections were 6 μm. Immunofluorescence quantification was determined in 4 consecutive high power fields (magnification 400×) in the area with the greatest density. Immunofluorescence (tryptase and TUNEL) assays were quantified by counting the number of positively stained cells per 100 cells per unit area (4 μm^2) and are shown as the percent. The intensity of E-cadherin using fluorescence microscopy and ZO-1 using confocal microscopy was quantified with Image J (https://imagej.nih.gov/ij/) processing.[16]