Urothelial dysfunction and sensory protein expressions in patients with urological or systemic diseases and hypersensitive bladder

Hueih-Ling Ong, Hann-Chorng Kuo

Department of Urology, Tzu Chi University, Hualien, Taiwan
Department of Urology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

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ABSTRACT

Objective: To investigate the underlying pathophysiology in the urothelium of different lower urinary tract diseases (LUTDs) and in patients with overactive bladder (OAB) or hypersensitive bladder (HSB), including chronic inflammation, barrier proteins, and sensory functional receptors.

Materials and Methods: A total of 156 patients, including 14 with idiopathic OAB, 11 with detrusor overactivity and inadequate contractility (DHIC), 19 with end-stage renal disease (ESRD) and HSB, 26 with spinal cord injury (SCI) and detrusor overactivity (DO), 26 with bladder outlet obstruction (BOO) and DO, 23 with diabetes mellitus (DM) and OAB, 19 with interstitial cystitis (IC), and 24 with ketamine cystitis (KC) were investigated for urothelial dysfunction and sensory protein expressions. Twenty control patients without LUTD were invited and separated into two groups for comparative studies. All participants had urodynamically proven DO or increased bladder sensation on video urodynamic studies. Urothelial dysfunction and functional receptor expressions were investigated and compared between patients with LUTD and controls.

Results: All patient subgroups had significant increases in mast cell activation and apoptotic cell counts and a decrease in E-cadherin expression. P2X3 expression was significantly decreased in DHIC but was increased in BOO/DO. Urothelial M3 expression was significantly increased in patients with OAB, BOO/DO, DM/OAB, and KC. M2 expression was significantly decreased in DHIC but increased in patients with BOO/DO. β3-AR expression was significantly decreased in patients with OAB and increased in patients with DHIC, ESRD/HSB, DM/OAB, and KC. Patients with OAB and BOO/DO had significantly increased M2/β3-AR. Lower M2/β3-AR was associated with lower voiding efficiency and large postvoid residual (PVR) in DHIC, ESRD/HSB, and SCI/neurogenic detrusor overactivity (NDO).

Conclusion: Patients with OAB or HSB showed increased urothelial inflammation and lower barrier protein expression. Increased M3/β3-AR or M2/β3-AR in the urothelium was associated with OAB, whereas decreased M3/β3-AR or M2/β3-AR was associated with poor voiding efficiency and large PVR in LUTD.

1. Introduction

The urothelium of the urinary bladder plays a significant role in bladder sensation during urine storage and mediates detrusor contraction during voiding. The urothelium detects physiological and chemical stimuli and releases various signaling molecules. Under several conditions, the urothelial barrier is disrupted and results in a cascade of events in the bladder, leading to symptoms of lower urinary tract diseases (LUTD), such as in interstitial cystitis (IC) and ketamine-related cystitis (KC). Several LUTDs, such as bladder outlet obstruction (BOO), IC, KC, spinal cord injury (SCI) with neurogenic bladder, and systemic diseases, such as diabetes mellitus (DM), end-stage renal disease (ESRD), and congestive heart failure may cause overactive bladder (OAB) or hypersensitive bladder (HSB). There are several similarities in serum biomarkers and bladder urothelial histopathologies in these LUTDs. Biomarkers, such as urinary nerve growth factor or urine cytokines, may be useful for the diagnosis and monitoring of LUTD patients.
serum C-reactive protein, are elevated in OAB, IC, and men with benign prostatic hyperplasia and lower urinary tract symptoms (LUTS), suggesting chronic inflammation is involved in these LUTDs. Previous studies have shown mast cell infiltration in both OAB and IC bladder epithelia, suggesting that suburothelial inflammation was involved in IC as well as in OAB. Nonetheless, the adhesion protein E-cadherin and tight junction protein zonula occludens-1 (ZO-1) expressions were decreased only in IC. The urothelial dysfunction is likely to be a part of the pathogenesis for the LUTDs that possess bladder hypersensitivity or overactivity. Altered regulation of urothelial homeostasis in IC is likely to cause epithelial dysfunction, activation of mast cells, neurogenic inflammation, and autoimmunity. IC bladder biopsies showed abnormal expression of uroplakin, chondroitin sulfate, and ZO-1, strongly suggestive of abnormal cell differentiation in bladders with IC. Investigation of the relationships between chronic inflammation and urothelial dysfunction, such as urothelial apoptosis, expression of junction proteins, and inflammatory re- actions in the suburothelium, could advance our understanding of the pathophysiology of bladder hypersensitivity in LUTDs.

Bladder urothelium expresses all subtypes of muscarinic receptors. M3 receptors are primarily responsible for detrusor contraction. M2 receptors have a role in mediating indirect contractions or inhibition of detrusor relaxation. In the human detrusor muscle, M2 and M3 receptors are predominant. Although the density of M2 receptors is three times that of M3 receptors M3 receptors mediate detrusor contraction in vitro. In M3 knockout mice study, 95% of bladder strips contractility induced by carbachol was found to be mediated by M3 receptors. Evidence also suggests that antimuscarinic drugs affect muscarinic receptors within the urothelium and on bladder sensory nerves, and indirect actions in the suburothelium, could advance our understanding of the pathophysiology of bladder hypersensitivity in LUTDs.

Bladder urothelium plays a crucial role in the transmission of the sensation of fullness and pain and induces reflex changes in the bladder. Bladder pathology often results in augmented ATP release from the urothelium, causing excitation of purinergic receptors (P2X) on the sensory fibers. Activation of cholinergic receptors in the feline epithelium cells facilitates ATP release and activates adjacent afferent nerves near myofibroblasts, which results in sensory urgency. The immunoreactivity of M2, M3, and P2X3 receptors was greatest in the urothelium of rats with BOO compared with the control rats.

Beta-3 adrenoceptors (β3-AR) are abundant in the detrusor of the urinary bladder and play a major role in detrusor relaxation during the storage phase of bladder function. The β3-ARs promote urine storage by inducing detrusor relaxation in animal and human bladders. In humans, the β3-ARs are recognized as the predominant β-receptor subtype in the urinary bladder, representing 97% of total β-adrenoceptor mRNA expression in the bladder. β3-AR agonists relax the detrusor smooth muscle during the bladder storage phase and increase bladder capacity. β3-ARs in the human urothelium appear to be functional such that receptor density changes in disease states alter bladder storage function.

This study aims to investigate the underlying pathophysiology in the urothelium of several LUTDs and systemic diseases with OAB or HSB. We speculate that chronic inflammation and altered sensory protein expression in the bladder urothelium is involved in the pathogenesis of OAB and HSB with systemic diseases. The urothelial dysfunction markers (apoptosis, mast cell activity, E-cadherin, and ZO-1) and sensory receptors in the urothelium (M2, M3, β3-AR, and P2X3) were explored to define the underlying pathophysiology of the OAB/HSB symptoms in these LUTDs.

2. Materials and methods

2.1. Patients

The patients investigated in this study were selected from the Tzu Chi General Hospital Department of Urology, Hualien, Taiwan. The enrolled patients included: (1) 20 control patients with superficial bladder cancer without LUTS, stress urinary incontinence undergoing anti-incontinence surgery, or microscopic hematuria undergoing ureteroscopic examination; the 20 control patients were separated into two groups for comparative study with different LUTD subgroups; (2) patients with idiopathic OAB; (3) patients with detrusor overactivity and inadequate contractility (DHC); (4) patients with ESRD with HSB; (5) patients with SCI and DO; (6) patients with BOO and urodynamic detrusor overactivity (DO) due to benign prostatic hyperplasia; (7) patients with DM and OAB; (8) patients with IC and HSB; and (9) patients with KC and HSB. All bladder specimens were obtained at cystoscopic examinations or transurethral surgeries to determine a diagnosis or treatment procedure.

The diagnosis of LUTDs was based on the recommendations of the International Continence Society. DHC was defined when patients had urodynamic DO with low voiding pressure and post-void residual (PVR) greater than 33% of the cystometric bladder capacity (CBC). The research ethics committee of Buddhist Tzu Chi General Hospital approved this study (IRB: TCGH 101-061). Each patient was informed about the study rationale and procedures and written informed consent was obtained before any bladder procedure. Bladder biopsies at the posterior wall were performed before any cystoscopic procedure and from controls after anti-incontinence procedures.

2.2. Bladder tissue study

Four bladder biopsies were obtained from the posterior wall about 2 mm above the ureteral orifice, and only bladder mucosa was taken. One of the bladder biopsy specimens was sent to the pathology department for hematoxylin and eosin staining to exclude the possibility of carcinoma in situ. The other specimens were embedded in optimum cutting temperature medium and were stored frozen at −80°C for further investigations.

2.3. Immunofluorescence staining

The urinary bladder specimens were immersed and fixed for 1 hour in an ice-cold solution of 4% formaldehyde in phosphate-buffered saline (PBS; pH 7.4). Next, they were rinsed with ice-cold PBS containing 15% sucrose for 12 hours. Biopsy specimens were embedded in optimum cutting temperature medium and stored at −80°C. Four sections per specimen were cut using a cryostat at a thickness of 8 μm and were collected onto new silane III-coated slides (Muto Pure Chemicals Co., LTD, Tokyo, Japan). Sections were postfixed in acetone at −20°C and blocked with rabbit serum. The sections were incubated overnight at 4°C with primary antibodies to antihuman ki-67 (DakoCytomation Denmark A/S, Glostrup, Denmark), antihuman E-cadherin (BD Biosciences, San Diego, CA, USA), antihuman ZO-1 (Invitrogen, Burlington, Canada), or antihuman tryptase (Chemicon, Temecula, CA, USA) after rinsing the sections with 0.1% Tween-20 in PBS, rabbit antihouse-conjugated fluorescein isothiocyanate secondary antibodies (DakoCytomation Denmark A/S) were applied to the sections and incubated for 1 hour. Finally, the sections were counterstained with DAPI (Sigma Chemical Company, St. Louis, MO, USA). The negative controls included the isotypes of the
primary antibodies. We recorded the mean, maximum, range, and standard deviation of staining intensity and percent positive area measurements using three randomly chosen hot spots within each specimen. Immunofluorescence staining images of E-cadherin and mast cell tryptase were captured using fluorescence microscopy with a digital imaging system (Zeiss, Oberkochen, Germany). The distribution and fluorescence intensity of ZO-1 was measured using a confocal microscope (Zeiss). Expression of E-cadherin and ZO-1 in the urothelium were quantified using Image J Software, which was developed by the National Institutes of Health. The percentages of tryptase-positive mast cells were calculated from five consecutive high-power fields (>400) from areas with the highest density cell infiltration.

For Immunohistochemistry (IHC) of β3-AR, the urinary bladder specimens were fixed and processed for immunohistochemical analyses as previously described. Sections were incubated overnight at 4°C with an antibody against human β3-AR (Abcam, Cambridge, UK). Streptavidin-biotin peroxidase complex technology (super sensitive Immunohistochemistry detection systems; BioGenex Laboratories, Fremont, CA, USA) was used. Images were obtained using a digital imaging system (Zeiss). The results of immunofluorescence (tryptase) staining were quantified by counting the numbers of positive cells/total cells per unit area (4 μm²) shown as the percentage of positive cells per 100 total cells. Three tissue sections per sample from diseased bladders and controls were evaluated, and the mean values were used in the subsequent statistical analyses.

2.4. Terminal deoxynucleotidyl transferase dUTP nick end labeling assay

The urinary bladder specimens were fixed by immersion for 1 hour in an ice-cold solution of 4% formaldehyde in PBS (pH 7.4). They were then rinsed with ice-cold PBS containing 15% sucrose for 12 hours. The specimens were embedded in optimum cutting temperature medium (Miles, Elkhart, IN, USA) and then stored at −80°C. The tissues were cut into 8 μm-thick slices and were collected onto new silane-III-coated slides. The sections were incubated with 100 μL of 20 μg/mL proteinase K (Calbiochem, Darmstadt, Germany) at room temperature for 20 minutes and washed with PBS. The sections were covered with 100 μL of terminal deoxynucleotidyl transferase (TdT) equilibration buffer (Calbiochem) and then incubated at room temperature for 30 minutes. After carefully blotting the 1X TdT equilibration buffer from the specimens, we applied TdT labeling reaction mixture (Calbiochem) onto the specimens and incubated them for 90 minutes at 37°C. The positive control, apoptotic HL-60 cells (Calbiochem), was treated with 1 μg/mL DNase I in Tris-buffered saline/1mM MgSO4 (Promega Corporation, Madison, WI, USA) at room temperature for 20 minutes. The negative control was generated by substituting dH2O for the TdT enzyme in the reaction mixture. After washing with PBS, the cells were mounted using Fluorescein-FragEL mounting media (Calbiochem). The total cell population was visualized using a 330–380 nm filter for DAPI while the labeled nuclei were visualized using a standard fluorescein filter (465–495 nm).

2.5. Western blotting

Western blotting was done to identify sensory proteins in the urothelium including the muscarinic receptors M2 and M3, β3-AR, and purinergic receptor P2X3. First, the bladder tissues were homogenized with liquid nitrogen and the proteins were separated using lysis buffer with a protease inhibitor cocktail and phosphatase inhibitor cocktail (Roche Diagnostics, Mannheim, Germany) for 60 minutes. The soluble proteins were determined using the Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Inc., Hercules, CA, USA) by adding 10 μg to 8–16% Precise Tris-Glycine Gel (Thermo Scientific, Rockford, IL, USA) for electrophoresis. After gel electrophoresis, the blots were transferred to 0.2 μm polyvinylidene fluoride membranes, and the extraneous proteins were blocked using 3% skim milk as the blocking buffer for 1 hour. Then, primary antibodies to P2X3 (GeneTex, Irvine, CA, USA), M2 (GeneTex), M3 (GeneTex), β3-AR (Abcam, Cambridge, UK), and GAPDH (positive control; Cell Signaling Technology, Danvers, MA, USA) were applied. The samples were incubated overnight at 4°C then washed with Tris-buffered saline with Tween 20 and incubated with secondary antibody (goat antirabbit immunoglobulin G-HRP, 1:3000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membranes were probed with enhanced chemiluminescence reagent (ECL; Millipore Corporation, Billerica, MA, USA) and exposed to X-ray film.

2.6. Statistical analysis

The differences in the urodynamic parameters, urothelial dysfunction, and sensory protein expressions between patients with LUTDs and controls were analyzed using the Kruskal–Wallis test. All calculations were performed using SPSS for Windows, version 10.0 (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

3. Results

A total of 20 controls and 156 patients, including 14 with idiopathic OAB, 11 with DHIC, 19 with ESRD/HSB, 26 with SCI/neurogenic detrusor overactivity (NDO), 23 with BOO/DO, 19 with DM/HSB, 20 with IC, and 24 with KC were investigated for urothelial dysfunction and sensory protein expression. Table 1 lists the urodynamic parameters. All patient subgroups had increases in bladder sensation either at the first sensation of filling or during filling or during bladder filling, indicating bladder hypersensitivity in these patients. All patients with BOO had DO. Patients with KC also presented with very small CBC and DO. Additionally, the maximum flow rate was decreased in all subgroups, but the detrusor pressure was not significantly different from that of the controls, except in patients with BOO. PVR volume was significantly greater in patients with DHIC, SCI, and IC but was significantly lower in patients with KC. Table 2 provides the protein expressions in the urothelium of the urinary bladder in patients with different LUTDs and controls. All subgroups of patients had significantly increased mast cell activation and apoptotic cell counts in the urothelium, except for DHIC. All subgroups had significantly decreased E-cadherin expression, except for ESRD/HSB. ZO-1 expression was significantly decreased in patients with ESRD/HSB, SCI/NDO, IC, and KC. P2X3 expression was significantly decreased in DHIC but was significantly increased in patients with BOO/DO.

Confocal immunofluorescence microscopy of the β3-AR in the controls and patients with LUTDs is shown in Figure 1. The immunohistochemistry results indicated that β3-AR was mainly expressed in the urothelium. The apical surface of the urothelium in particular had the highest expression. Suburothelial tissues express lower levels of β3-AR.

Table 3 shows the expressions of muscarinic receptors M3, M2, and β3-AR in the urothelium of the urinary bladder in patients with LUTDs and controls. The urothelial M2 expression was significantly increased in patients with OAB, BOO/DO, DM/OAB, and KC. M2 expression was significantly increased only in patients with BOO/DO. β3-AR expression was significantly increased in patients with DHIC, ESRD/HSB, DM/OAB, and KC but was significantly decreased in patients with OAB. When we calculated the ratio of M3/β3-AR...
and M2/3-AR, patients with OAB had significantly increased ratios. Patients with DHIC, ESRD/HSB, and SCI/NDO had significantly decreased M3/3-AR and M2/3-AR. Patients with BOO/D0 had significantly decreased M3/3-AR, but M2/3-AR showed a significant increase. Patients with IC had significant reductions, and KC patients had significant increases of M3/3-AR.

4. Discussion

In several LUTDs and systemic diseases with bladder overactivity or hypersensitivity, urothelial inflammation and functional proteins showed significant changes. The muscarinic receptors and β3-AR and the ratio of M3/3-AR and M2/3-AR also showed alterations in certain diseases affecting bladder function. These changes of urothelial expression contributed to sensory disorders in the bladder storage phase and affected detrusor contractility.

In the patients with LUTDs and systemic diseases, bladder inflammation and urothelial apoptosis increased. In previous studies of IC, we demonstrated that inflammation led to increased urothelial apoptosis, which resulted in lower expression of the adhesion protein E-cadherin and tight junction protein ZO-1. Additionally, chronic inflammation inhibited normal basal cell proliferation and affected the apical urothelial function. Bladder inflammation caused by intravesical irritants in patients with IC led to intense afferent nerve activity and to long-term plasticity that lowered the thresholds for nociceptive and mechanoreceptive afferent fibers. Chronic inflammation is considered the primary pathophysiology responsible for LUTS. Moreover, urinary nerve growth factor is increased in patients with OAB, BOO, urinary tract infection, IC, Parkinson’s disease, and cerebrovascular accident. It is possible that OAB symptoms are associated with chronic inflammation of the bladder wall and impaired urothelial function. In patients with OAB, DHIC, ESRD/HSB, SCI/NDO, BOO/D0, DM, OAB, IC, and KC, we observed decreased E-cadherin expression in the urothelium. In patients with OAB, SCI/NDO, IC, and KC, we observed decreased ZO-1 expression. Decreased E-cadherin in the urothelium results in increased bladder pain scores in patients with IC. These urothelial dysfunctions could be associated with...
Figure 1. Confocal immunofluorescence microscopy of the β3-adrenoceptors in the patients with (A) a normal bladder; (B) overactive bladder; (C) detrusor overactivity and inadequate contractility; (D) end-stage renal disease and hypersensitive bladder; (E) spinal cord injury and detrusor overactivity; (F) bladder outlet obstruction and detrusor overactivity; (G) diabetes mellitus and overactive bladder; (H) interstitial cystitis; and (I) ketamine cystitis. White lines represent basement membrane of the uroepithelium. Green color indicates immunofluorescent staining of the β3-adrenoceptors.

Table 3

Muscarinic receptor M3 and M2 and β3-AR adrenoceptor expression in the urothelium of the urinary bladder in patients with different LUTDs and controls.

<table>
<thead>
<tr>
<th>LUTD (n)</th>
<th>M3</th>
<th>M2</th>
<th>β3-AR</th>
<th>M3/β3</th>
<th>M2/β3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10)</td>
<td>0.24 ± 0.23</td>
<td>0.31 ± 0.43</td>
<td>0.37 ± 0.19</td>
<td>0.90 ± 1.36</td>
<td>0.87 ± 1.33</td>
</tr>
<tr>
<td>OAB (14)</td>
<td>0.90 ± 0.61*</td>
<td>0.58 ± 0.68</td>
<td>0.24 ± 0.25*</td>
<td>9.83 ± 10.9*</td>
<td>5.02 ± 4.93*</td>
</tr>
<tr>
<td>Control (10)</td>
<td>1.55 ± 1.03</td>
<td>1.22 ± 0.59</td>
<td>0.57 ± 0.48</td>
<td>3.44 ± 2.28</td>
<td>4.03 ± 4.45</td>
</tr>
<tr>
<td>DHIC (11)</td>
<td>0.82 ± 0.35</td>
<td>0.56 ± 0.62*</td>
<td>0.86 ± 0.30*</td>
<td>1.08 ± 0.51*</td>
<td>0.61 ± 0.57*</td>
</tr>
<tr>
<td>Control (10)</td>
<td>1.55 ± 1.03</td>
<td>1.22 ± 0.59</td>
<td>0.57 ± 0.48</td>
<td>3.44 ± 2.28</td>
<td>4.03 ± 4.45</td>
</tr>
<tr>
<td>ESRD/HSB (19)</td>
<td>1.57 ± 1.89</td>
<td>1.61 ± 1.96</td>
<td>1.55 ± 1.0*</td>
<td>0.88 ± 0.70*</td>
<td>0.85 ± 1.13*</td>
</tr>
<tr>
<td>Control (10)</td>
<td>1.55 ± 1.03</td>
<td>1.22 ± 0.59</td>
<td>0.57 ± 0.48</td>
<td>3.44 ± 2.28</td>
<td>4.03 ± 4.45</td>
</tr>
<tr>
<td>SCI/NDO (26)</td>
<td>0.37 ± 0.27</td>
<td>0.79 ± 0.65</td>
<td>0.78 ± 0.54</td>
<td>0.69 ± 0.71*</td>
<td>1.38 ± 1.35*</td>
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<tr>
<td>Control (10)</td>
<td>1.59 ± 0.70</td>
<td>0.41 ± 0.30</td>
<td>0.88 ± 0.58</td>
<td>2.15 ± 1.42</td>
<td>0.61 ± 0.55</td>
</tr>
<tr>
<td>BOO/DO (23)</td>
<td>0.70 ± 0.31*</td>
<td>1.07 ± 1.18*</td>
<td>0.86 ± 0.27</td>
<td>0.86 ± 0.50*</td>
<td>1.42 ± 1.65*</td>
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<tr>
<td>Control (10)</td>
<td>0.24 ± 0.23</td>
<td>0.31 ± 0.43</td>
<td>0.37 ± 0.19</td>
<td>0.90 ± 1.36</td>
<td>0.87 ± 1.33</td>
</tr>
<tr>
<td>DM/OAB (19)</td>
<td>0.84 ± 0.56*</td>
<td>0.41 ± 0.59</td>
<td>0.86 ± 0.60*</td>
<td>3.06 ± 5.96*</td>
<td>0.51 ± 0.46</td>
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<td>Control (10)</td>
<td>1.92 ± 2.23</td>
<td>1.53 ± 1.83</td>
<td>0.84 ± 1.10</td>
<td>3.08 ± 1.82</td>
<td>3.72 ± 3.83</td>
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<tr>
<td>IC (20)</td>
<td>0.60 ± 0.32*</td>
<td>1.13 ± 0.42</td>
<td>0.70 ± 0.40</td>
<td>1.64 ± 1.89*</td>
<td>2.64 ± 2.67</td>
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<tr>
<td>Control (10)</td>
<td>0.14 ± 0.93</td>
<td>0.12 ± 0.21</td>
<td>1.06 ± 0.13</td>
<td>0.12 ± 0.07</td>
<td>0.11 ± 0.18</td>
</tr>
<tr>
<td>KC (16)</td>
<td>0.81 ± 0.46*</td>
<td>0.18 ± 0.21</td>
<td>2.41 ± 1.79*</td>
<td>0.47 ± 0.38*</td>
<td>0.12 ± 0.17</td>
</tr>
</tbody>
</table>

* Indicates significant difference from the control.

BOO = bladder outlet obstruction; DHIC: detrusor overactivity and inadequate contractility; DM = diabetes mellitus; DO = detrusor overactivity; ESRD: end-stage renal disease, HSB = hypersensitive bladder; IC = interstitial cystitis; KC = ketamine related cystitis; LUTD = lower urinary tract disease; NDO = neurogenic detrusor overactivity; OAB = overactive bladder; SCI = spinal cord injury.
with increased bladder sensation, or they could induce DO. Interestingly, the P2X3 expressions showed significant increases only in BOO/DO and decreases in DHIC, but no change in the other diseases, suggesting the P2X3 expression in the urothelium is associated with bladder sensation as well as detrusor contractility. During bladder storage, ATP is released from the urothelial cells triggering bladder fullness sensations or inducing bladder activity.21 In patients with DHIC, the decrease in P2X3 receptors could diminish the trigger effect on sustained detrusor contraction, resulting in large PVR volumes and poor bladder sensation when the bladder is not completely emptied.

β3-AR is expressed strongly in the umbrella cells compared to cells in the intermediate urothelial layers.22 Stimulation of β3-AR relaxes detrusor smooth muscle, decreases afferent signaling from the bladder, and improves bladder compliance, thus increasing bladder capacity.23 Unlike the mechanism of antimuscarinic agents, β3-AR agonists increase bladder capacity without accompanying changes in micturition pressure, PVR volume, or voiding contraction.24 Normal bladder sensation is important in the initiation of the detrusor reflex during voiding and sustained detrusor contractility during voiding. β3-AR agonists could stimulate the urethral release of unidentified factors that inhibit detrusor contractility, either directly or indirectly.25 Stimulation of M2 receptors could inhibit sympathetic βAR-mediated relaxation, leading to more efficient bladder emptying.26 Both muscarinic receptors and β3-AR are involved in the urotheiogenic signaling on enhancing or inhibiting detrusor contractility.34,36 If the mucosal expressions of these receptors are inadequate, patients might have altered bladder sensation or inadequate bladder emptying.

Both M3 and M2 receptors are highly expressed in the urothelium of the bladder.15 The functional role of M2 receptors may emerge and increase in density in certain bladder diseases, such as BOO in rats,26 neurogenic bladder disease in humans,39 and denervated bladder disease in rats.40 In addition, muscarinic receptors in the urothelium or suburothelium may affect sensory fibers and contribute to the pathophysiology of OAB.16,41 Acetylcholine released from parasympathetic postganglionic neurons or nonneuronal sources, such as the urothelium and suburothelium during bladder filling, stimulates subsequent micromotion in the detrusor resulting in afferent stimulation, which contributes to the pathophysiology of OAB.19,42

In this study, urothelial M2 and M3 receptors were significantly increased in patients with OAB and BOO/DO but were decreased in patients with DHIC and SCI/NDO. β3-AR expression was significantly decreased in patients with OAB, but significantly increased in patients with DHIC, ESRD, and DM/OAB; the change in β3-AR density could be related to low voiding efficiency and large PVR volume in these diseases. When we calculated the M3/β3-AR and M2/β3-AR ratios, only patients with OAB had increases in both ratios. Patients with BOO/DO had lower M3/β3-AR than patients with OAB, but M3/β3-AR was significantly increased. Patients with DHIC, SCI/NDO, and ESRD/HSB all showed significant decreases in both ratios. An imbalance of urothelial M3, M2 receptors, and β3-AR is associated with OAB. In patients with BOO/DO, the increase in expression of urothelial M2 was greater than that of M3 receptors, without a change in β3-AR and resultant in DO. In patients with low M3/β3-AR and M2/β3-AR in the urothelium, poor voiding efficiency and large PVR volumes were noted, which could be attributed to decreased mucasirnic stimulation in association with an increase in β3-AR density. We also found M3 receptors and M3/β3-AR increased in IC and decreased in IC. The increase of M3/β3-AR in IC bladders might be associated with more detrusor overactivity and smaller CBC in IC patients than those in IC bladders. Patients with IC or KC could have denuded urothelium resulting in inconsistent protein expressions based on observations of the bladder biopsy samples. Nevertheless, severe inflammation and urothelial dysfunction were evident in both IC and KC patients, which could have led to increased bladder sensation and small bladder capacity.

The strength of this study was the use of multiple LUTD with hypersensitive bladder to investigate the urotheilial dysfunction and sensory protein expressions in human bladders. However, the limitation of the study is the small case number in each LUTD subgroup that may have wide variations in clinical and urotheilial characteristics.

5. Conclusion

Patients with LUTDs and nonurological diseases and bladder overactivity or hypersensitivity showed increased urothelial inflammation and lower barrier protein expressions. The urotheilial muscarinic receptors and β3-AR expression were also altered. Increased M3/β3-AR or M2/β3-AR is associated with OAB, whereas decreased M3/β3-AR or M2/β3-AR is associated with poor voiding efficiency and large PVR volume. Treatment of bladder hypersensitivity or low voiding efficiency in these LUTDs might target the altered receptor ratio.

Conflicts of interest

None.

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