

Association between Blood Vessel Changes of Bladder Mucosa and Storage Symptoms in Female Stress Urinary Incontinence Patients

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Aims of Study: Although midurethral sling (MUS) surgery is a very effective procedure to relieve stress urinary incontinence (SUI) as well as mixed incontinence, urgency/frequency and urge incontinence remain in some patients post-operatively and bother the patients. In this study, we investigated that the cystoscopic findings of blood vessel changes such as neovascularization and telangiectasia in bladder mucosa associated with the development of storage symptoms in stress urinary incontinence before and after operation (Fig. 1).

Materials and Methods: 60 patients who were admitted for MUS surgery included in our study. 20 patients were genuine stress incontinence (Group 1), 20 were SUI with urgency/frequency (Group 2) and 20 were mixed incontinence (Group 3). Average age was 53 year-old (range 38-70). Cystoscopic examination were done under anesthesia before midurethral sling procedure and blood vessel changes was classified as neovascularization, telangiectasia. Association between blood vessel changes and presence of storage symptoms was evaluated and storage symptoms was defined as documentation of urgency/frequency, nocturia.

Results: There was no operation-related complications such as bladder perforation, mesh erosion and urethral injury. 4 cases in group 1, 16 cases in group 2, 18 cases in group 3 showed blood vessel changes. Persistent storage symptoms observed in 1 case (Group 1), 3 cases (Group 2) and 4 cases (Group 3) post-operatively (Table 1). There was no case of new development of storage symptoms in which did not showed mucosal blood vessel changes pre-operatively. As storage symptoms severe in stress urinary incontinence, the incidence of mucosal blood vessel changes increased. Persistence of storage symptoms was limitedly observed in blood vessel changed patients.

Conclusions: The incidence and severity of storage symptoms were closely related with bladder mucosal vessel changes and this finding suggest that storage symptoms in stress urinary incontinence may be associated with factors which provoke the over-expression of mucosa blood vessel changes.

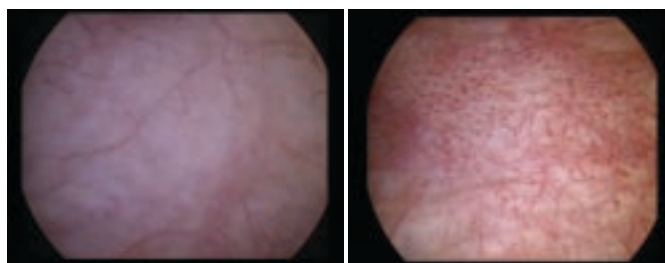


Fig. 1. Telangiectasia in cystoscopic findings.

Table 1. Correlation between storage symptom and telangiectasia

		Genuine stress incontinence		Stress urinary incontinence with urgency/frequency		Mixed incontinence	
		Pre-op.	Post-op.	Pre-op.	Post-op.	Pre-op.	Post-op.
Bladder mucosa vessel changes	Positive	4	1*	16	3*	18	4*
	Negative	16	0*	4	0*	2	0*
		Total		Total		Total	
		20		20		20	

Interleukin-4 Gene Therapy Using Herpes Simplex Virus Vectors for Bladder Pain and Overactivity

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Aims of Study: Although the etiology bladder pain syndrome/interstitial cystitis (BPS/IC) is not fully understood, bladder inflammation associated with production of inflammatory cytokines or chemokines have been proposed as potential pathogenesis of the disease. The expression levels of interleukin (IL)-2, IL-6, TNF α or other chemokines in the bladder of IC patients are significantly greater than controls, which might be used as a specific marker for IC. On the other hands, IL-4 is a prototypical anti-inflammatory cytokine, which inhibits secretion of cytokines like IL-1 β , TNF α and IL-6. However, because of its pleiotropic effects on the immune system, it could not be used as systemic treatment of pain in humans. We therefore examined the effects of localized and targeted gene therapy using replication-deficient herpes simplex virus (HSV) vectors (S4IL4) expressing murine IL-4, on bladder overactivity and pain behavior induced by intravesical application of resiniferatoxin (RTx) in rats.

Materials of Methods: Replication-deficient HSV vector S4IL4 express-

ing IL-4 (HSV-IL4) was produced using Cre-lox recombination of a plasmid that contains ICP4 promoter driving the murine IL-4 gene in addition to the HCMV IE promoter- β -galactosidase expression cassette and a lox combination site into the thymidine kinase (tk) locus of d120, which is a replication-defective KOS strain HSV mutant, containing a deletion of both copies of the immediate-early ICP4 gene. As an isogenic control vector, we employed the SHZ (HSV-*lacZ*) virus that has the HCMV IE promoter- β -galactosidase expression cassette recombined into thymidine kinase (tk) locus of d120 by similar techniques. One or 2 weeks prior to experiments, 20 μ L of viral suspension (4×10^9 pfu/mL S4IL4 or 5×10^9 pfu/mL SHZ) were injected to the bladder wall of female SD rats with midline incision under pentobarbital anesthesia. (1) Virus infection was examined by immunohistochemistry using β -galactosidase staining technique. (2) Murine IL-4 in L6 DRGs and the bladder of S4IL4 injected rats were quantified by ELISA. (3) Cystometry was performed under urethane anesthesia (1.2 g/kg, s.c.). After 2 hours of saline infusion, 10 nM RTx was continuously infused into the bladder to induce bladder overactivity (n=9 each). Intercontraction intervals (ICI) were compared before and after RTx administration. (4) A behavioral study was performed to evaluate the effects of IL4 gene delivery through the bladder on bladder pain induced by RTx. In a conscious condition, 3 μ M RTx (0.3 mL, 1 min) was injected to the bladder through a temporally indwelled urethral catheter to evaluate nociceptive behaviors such as licking (lower abdominal licking) and freezing (motionless head-turning to the lower abdomen) were counted and recorded every 5 sec for 15 min (n=7-8). Based on our previous observation, licking behavior represents urethral pain, which is mainly mediated by activation of the pudendal nerve. On the other hands, freezing behavior is predominantly related to bladder pain, which is mainly mediated by activation of the pelvic nerve. (5) HSV vector-treated rats were sacrificed 6 hours after 3 μ M RTx application into the bladder (0.3 mL, 1 min) to quantify IL-1 β in the bladder using ELISA (n=4 each).

Results: (1) Lac Z-positive staining was identified in the bladder and L6 DRG neurons of SHZ injected rats. (2) Murine IL4 was detected in the bladder (5.6 ± 1.6 pg/mg total protein [TP]) and L6 DRG (13.3 ± 3.8 pg/mgTP) in S4IL4 injected rats. (3) There were no significant difference in ICI between two groups during saline infusion ($p=0.15$). After RTx administration, SHZ injected rats showed a significant reduction in ICI ($p<0.05$) while ICI did not change significantly ($p=0.47$) in S4IL4 injected rats. (4) RTx-induced freezing behavior was significantly less in S4IL4 injected rats by 47% than in SHZ rats (30 ± 5 vs 57 ± 6 times, $p<0.05$). (5) Bladder IL-1 β after 3 μ M RTx administration was significantly reduced in S4IL4 injected rats compared to SHZ injected rats (42.1 ± 8.0 vs 84.4 ± 9.6 pg/mgTP, $p<0.05$).

Conclusions: The results of this study indicate that: (1) HSV vectors injected into the bladder wall are transported into L6 DRG neurons via afferent nerves; (2) significant increases of murine IL-4 were detected in the bladder and L6 DRG from S4IL4 vector-injected rats, (3) in S4IL4-treated rats, the ICI was not reduced during RTx intravesical administration, which induced bladder overactivity in SHZ-treated control rats; (4) S4IL4-injected rats showed a reduction in freezing behavior; and (5) increased expression of IL-1 β elicited by RTx-induced bladder irritation was reduced in S4IL4-treated rats.

Therefore, IL-4 gene therapy using replication-deficient HSV vectors could be a new strategy for treating bladder pain and/or urinary frequency in patients with BPS/IC.

Chronic Inflammation but not Urothelium Dysfunction in Patients with Overactive Bladder Syndrome

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Aims of Study: Recent investigations have linked overactive bladder syndrome (OAB) with chronic inflammation. Urinary nerve growth factor, cytokines and serum C-reactive protein have been demonstrated to increase in patients with OAB and interstitial cystitis/painful bladder syndrome (IC/PBS). Previous reports have suggested that IC/PBS is associated with increased activated mast cell numbers in the bladder and disruption of the barrier function of the urothelium. However, there is no study investigating the mast cell activation and urothelium barrier dysfunction in OAB. Since there are similarities in the inflammatory protein expression between OAB and IC/PBS, therefore, in this study the Infiltration of mast cells and the distribution of protein involved in barrier function were explored by immunohistochemical assessment of E-cadherin and ZO-1 in the bladder tissue of patients with OAB and IC/PBS.

Materials and Methods: Bladder wall biopsies were performed in 27 patients with OAB, 18 patients with IC/PBS, and 19 patients with stress urinary incontinence but without urgency frequency symptoms and served as controls. The expression of junction protein E-cadherin, tight junction protein ZO-1 and activated mast cell in bladder wall from these patients were evaluated quantitatively using immunofluorescence staining. The percentages of tryptase-positive mast cells were calculated from 5 consecutive high-power fields (X400) in the area with the highest dense infiltrate. Confocal microscopy was used to capture ZO-1 image. Density of E-cadherin and ZO-1 expression were quantified with Image J software. Two to three sections per sample from OAB or IC/PBS bladders and controls were examined. Statistical analysis was performed using one-way ANOVA and p value small than 0.05 was considered as significance. This study was approved by the Institution Review Board of the hospital.

Results: The OAB group consisted of 6 women and 21 men aged from 42 to 85 years old (mean 72). Patients with IC/PBS were 16 women and 2 men aged 21 to 72 years old (mean 42). Control patients were 19 women aged 29 to 71 years old (mean 54). All OAB patients presented with urgency and urgency incontinence (OAB wet) and the bladder tissue were obtained during procedure of intravesical botulinum toxin injection. All patients with IC/PBS had been proven by cystoscopic hydrodistention and had characteristic glomerulation. The bladder tissues of the controls were obtained during anti-incontinence surgery.

The number of mast cells in suburothelium and detrusor area was low in the control group (mean±standard error 1.77±0.47, range 0.00-9.00). But a highly significant increase of the mast cells infiltration was observed in the specimens from IC/PBS (4.64±0.72, range 1.00-10.00) and OAB patients (4.00±0.55, range 0.00-11.00) (p=0.008 and p=0.024, respectively). The expression level of E-cadherin and ZO-1 in the IC/PBS bladder (mean±standard error 59.05±9.48 and 7.45±0.99) were significantly down regulated compared with that in the control group (96.30±9.15 and 14.55±2.08; p=0.009 and p=0.013). However, compare the density of E-cadherin and ZO-1 between control bladder and OAB patients (79.41±6.90 and 13.46±1.32), there were no significant difference between the two group (p=0.334 and p=0.876) (Table 1, Fig. 1). These results suggest that the pathophysiology of IC/PBS and OAB both might linked with chronic inflammation. However, IC/PBS is associated with impairment of the barrier function of the urothelium but the bladder urothelium barrier in OAB is not disrupted.

Conclusions: In our study, patients with OAB and IC/PBS all had significantly greater number of mast cells in the bladder wall compared with controls. The bladder biopsies from IC/PBS patients reported previously have confirmed the involvement and presence of mast cells in the detrusor. Mast cells have been considered as crucial effector cells for the immune response implicated in the pathogenesis of IC/PBS. Bladder mast cell activation has been reported as a characteristic pathological finding in a subset of IC/PBS patients. Measurement of surrogate mast cell-related products in urine has been previously studied to assess the disease extent in patients. Since patients with OAB and IC/PBS all had elevated mast cell activities compared with that of the controls in this study, It is possible that a common pathway of chronic inflammation exists in the pathogenesis between these two diseases.

The results of this study suggest that both IC/PBS and OAB are associated with chronic inflammation and the role of bladder tissue mast cells in the pathogenesis and pathophysiology of OAB and IC/PBS is worthy of further investigation.

Table 1. Expression level of mast cell, E-cadherin and ZO-1 protein in bladder tissue of OAB and IC/PBS patients

	Control (n=19)	IC (n=18)	OAB (n=27)	Control vs IC Control vs OAB OAB vs IC
Age	53.63±11.88 29~71	41.78±13.48 21~72	71.63±10.77 42~85	
Gender	F: 19	F: 16 M: 2	F: 6 M: 21	
Mast cells	1.77±0.47 0.00~9.00	4.64±0.72 1.00~10.00	4.00±0.55 0.00~11.00	p=0.008** p=0.026* p=0.740
E-cadherin	96.30±9.15 44.83~166.24	59.05±9.48 7.5~155.56	79.41±6.90 17.50~150.25	p=0.009** p=0.334 p=0.143
ZO-1	14.55±2.08 2.19~37.69	7.45±0.99 1.44~14.05	13.46±1.32 5.17~27.79	p=0.013* p=0.876 p=0.024*

*: p<0.05; **: p<0.01

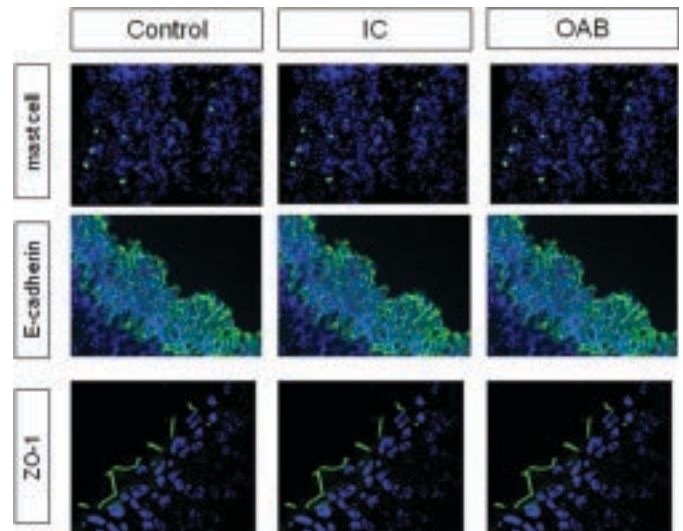


Fig. 1. Immunofluorescence staining of mast cell activity, E-cadherin and ZO-1 in OAB, IC/PBS and control bladder.

The Relaxant Effect of Ginseng Saponin on the Bladder and Prostatic Urethra: In Vitro and in Vivo Study

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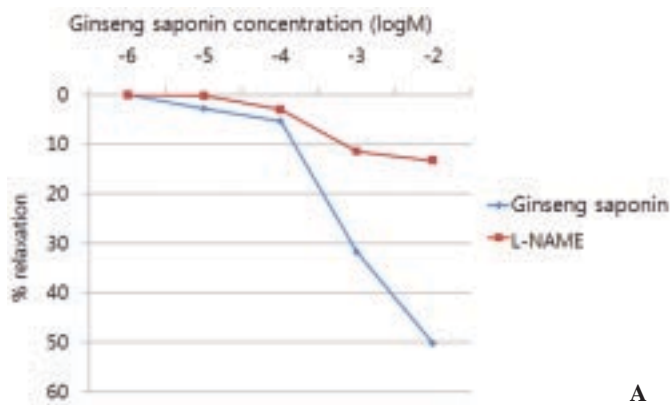
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Aims of Study: We performed in vivo and in vitro analysis of ginseng saponin on the bladder and prostatic urethra to investigate its relaxation effect.

Materials of Methods: In vitro study, prostatic urethra muscle strips were harvested from 18 male New Zealand rabbits. The strips were mounted in organ baths and connected to force displacement transducers. After stabilization, maximal tissue contractions were obtained by the addition of phenylephrine for urethra strips. When the contraction was stabilized, a dose-response curve of ginseng saponin was constructed (10⁻⁶ to 10⁻² mol/L) (Fig. 1, Table 1). After pretreatment urethra strips with N-nitro-L-arginine methyl ester (L-NAME), a dose-response curve of ginseng saponin was repeated. For the in vivo study using adult male Sprague-Dawley rats composed of three groups (control, partial bladder outlet obstruction and saponin fed groups) intravesical pressure and urethral perfusion pressure were monitored.

Results: The ginseng saponin induced significant relaxant effect on the prostatic urethra strips in a dose-dependent manner. At 10⁻² M, ginseng saponin induced a significant relaxation of the urethra strips by 50.2%. The relaxant effect was partially inhibited with L-NAME pretreatment. In the in vivo study, the UPPΔ was significantly higher in



A



B

Fig. 1. (A) Dose-response curves of ginseng saponin in rabbit prostatic urethral strips relaxation. The relaxing effect was partially inhibited with pretreatment of L-NAME. (B) Dose-response relaxation tracing curves of ginseng saponin.

Table 1. In vitro, organ bath study showed significant relaxation effect of KRG saponin on prostatic urethra strips. And this effect was partially blocked after L-NAME pretreatment

% Relaxation	Saponin concentration (logM)				
	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³	10 ⁻²
Prostatic urethra	0.18±0.1	2.78±3.89	5.28±6.18	31.75±13.11	50.21±20.26
L-NAME pretreatment	0.20±0.13	0.20±0.37	3.00±4.39	11.46±10.60	13.32±11.18
p-value	0.189	0.102	0.581	0.011	0.012

Wilcoxon signed ranks test

Table 2. Urodynamic parameters of three rat groups. (Group I: the control group, Group II: the group of partial bladder outlet obstruction (PBOO) with standard diet, Group III: the group of PBOO with ginseng saponin administration)

	Group I	Group II	Group III	
UPP _{basal} , cmH ₂ O	0.91±0.01	0.89±0.01	0.91±0.019	
UPP _{nadir} , cmH ₂ O	0.89±0.02	0.88±0.01	0.88±0.01	
UPP _Δ , cmH ₂ O	-0.017±0.01	-0.008±0.01	-0.022±0.01	p=0.001*
P _{ves} , cmH ₂ O	0.080±0.01	0.114±0.04	0.086±0.02	p=0.026+
P _{pves} , cmH ₂ O	0.10±0.01	0.13±0.04	0.11±0.01	
P _{vesgap} , cmH ₂ O	0.02±0.01	0.02±0.01	0.02±0.02	

UPP_{basal}: baseline urethral perfusion pressure; UPP_{nadir}: urethral perfusion pressure during relaxation; UPP_Δ: difference between UPP_{basal} and UPP_{nadir}; P_{ves}: baseline vesical pressure; P_{pves}: vesical pressure during bladder contraction; P_{ves Δ}: vesical pressure change between peak and baseline pressure; *Statistically significant among group 1, 2 and 3; +Statistically significant between group 2 and 3

the group III than group I or II ($p < 0.001$). And group III showed significant lower baseline vesical pressure than group II (Table 2).

Conclusions: Significant relaxation effect of ginseng saponin on the bladder and prostatic urethra was shown in the in vitro and in vivo studies. And this mechanism was thought to pass nitric oxide/nitric oxide synthase (NO/NOS) pathway.

Long-term Efficacy of Silodosin on the Lower Urinary Tract Function in Patients with Benign Prostatic Hyperplasia According to Prostate Size: Prospective Investigation Using Pressure-flow Study

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Aims of Study: Prostate size is an important factor in selecting treatment option and predicting therapeutic outcome in benign prostatic hyperplasia (BPH). α_1 -adrenoceptor (AR) antagonists are widely used as the drug of first choice for lower urinary tract symptoms with BPH. On the other hand, the efficacy of single use for patients with large prostate is debatable. We prospectively investigated the long-term effects of silodosin, a new α_1 -AR antagonist purely selective for the α_{1A} -AR subtype, on the lower urinary tract function in patients with BPH according to prostate size, using pressure-flow study (PFS).

Materials and Methods: This study is an open-labeled and prospective study in 140 men with lower urinary tract symptoms associated with BPH. Silodosin was administered at 8 mg/day to these patients. The patients were classified into 2 groups according to prostate size; small prostate (SP) group (prostate volume < 35 mL) and large prostate (LP) group (≥ 35 mL). Primary endpoints were changes in parameters of voiding and storage function as measured by PFS from baseline to 4 weeks and 1 year after administration. Secondary endpoints were changes in international prostate symptom score (IPSS), IPSS-quality of life (QoL), overactive bladder symptom score (OABSS).

Results: Of the 140 patients, 8 discontinued treatment because of adverse reactions, 6 patients were performed transurethral resection of the prostate, and 6 patients did not undergo PFS after treatment. Accordingly, 120 patients were included in analysis. Mean age of the analyzed patients was 69.2 years, ranging from 51 to 84. The prostate volume ranged from 20 to 84 mL, with mean of 43.9 mL. In the 63 case LP group, mean prostate volume was 52.5 mL and in the 57 case SP group, it was 30.2 mL.

At baseline, no significant difference was detected in IPSS or IPSS-QoL between LP group and SP group, but LP group had a significantly

higher OABSS (6.4 vs 5.0). On PFS, detrusor pressure at maximum flow rate (PdetQmax), bladder outlet obstruction index, and incidence of detrusor overactivity were significantly higher in LP group. After treatment, both groups showed significant improvement in IPSS, IPSS-QoL and OABSS at both 4 weeks and 1 year. For the long-term examination, SP group showed further improvement in both IPSS-total and QoL, while LP group reduced the effectiveness of improvement in both parameters. PFS during the voiding phase at 4 weeks demonstrated a significant improvement in maximum flow rate, postvoid residual, PdetQmax and bladder outlet obstruction index, regardless of prostate size. However, although the improvement in voiding parameters at 4 weeks was maintained to 1 year in SP group, these parameters changed for the worse compared with that of 4 weeks in LP group. In storage phase on PFS, both groups showed a significant increase in first desire to void and maximum cystometric capacity at 4 weeks and further improvement at 1 year.

Conclusions: Silodosin quickly improves bladder storage function and relieves bladder outlet obstruction, regardless of prostate size. However, in long-term administration, an improvement of bladder outlet obstruction may be reversed in patients with large prostate, despite preservation of an improvement in storage function.

Serum C-reactive Protein Levels are Associated with Residual Urgency Symptoms in Patients with Benign Prostatic Hyperplasia after Medical Treatment

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Aims of study: Urinary urgency is a common and bothersome symptom in patients with benign prostatic hyperplasia (BPH); this symptom may persist even after medical treatment. Chronic inflammation has been reported to be associated with the pathogenesis of BPH and lower urinary tract symptoms. We investigate the association between serum C-reactive protein (CRP) level and residual urgency symptoms in BPH patients after medical treatment.

Materials and Methods: A total of 205 men undergoing stable medical treatment for BPH, defined as a total prostate volume of ≥ 40 mL, were enrolled. Patients with acute infection or those taking non-steroid anti-inflammatory drugs or aspirin were excluded. Uroflowmetry, postvoid residual volume, transrectal ultrasound parameters, serum prostate specific antigen (PSA), and CRP level were measured. A three-day void diary was recorded to identify the presence of urinary urgency.

Results: The mean serum CRP level was 0.24 mg/dL (range, 0.01-2.84), and residual urgency was identified in 90 patients (43.9%). Patients with residual urgency were older and had significantly higher serum CRP levels (0.39 ± 0.54 mg/dL) than those without urgency (0.13 ± 0.20 , $p < 0.001$). On multivariable logistic regression analysis, men with CRP levels of ≥ 0.3 mg/dL were more likely to have urgency (odds ratio, 8.22; 95% confidence interval, 3.34-20.3) after adjusting for age, serum PSA level, and antimuscarinic use. Patients with serum CRP levels ≥ 0.30 mg/dL had more urgency (82.1%) than those with serum CRP levels < 0.30 mg/dL (34.9%).

Conclusions: Serum CRP level is significantly associated with residual urgency in BPH patients after medical treatment. Chronic inflammation may play a role in the occurrence of residual urgency in BPH patients.

A Comparative Study of the Efficacy of Solifenacin Succinate for Frequency and Frequency with Urgency Patients

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Aims of Study: The overactive bladder (OAB) was defined as urgency with or without urge incontinence, usually with frequency and nocturia. But several studies presented that patients reported that the most troublesome OAB symptom was frequency. It is difficult to perception urgency and to distinguish between urgency and frequent desire to void. So we assumed that antimuscarinic drug was effective for frequency without urgency as well as urgency. We compared of the efficacy of solifenacin for frequency and frequency with urgency.

Materials and Methods: This study is a multi-center, 12-week, open label, comparative study and non-inferior study based on the hypothesis, "The efficacy of solifenacin for frequency is non-inferior to the efficacy of solifenacin for frequency with urgency". We classified the patients with frequency of micturition > 8 voids/day without urgency as group 1 and the patients with frequency of micturition > 8 voids/day with urgency (urgency grade ≥ 3 (5 scales) & ≥ 2 /day) as group 2. The primary efficacy variable was change of daily micturition frequencies from baseline and secondary efficacy variables included change in Patients' Perception of Bladder Condition (PPBC), OAB Symptom Score (OABSS) and Benefit, Satisfaction, Willingness to continue (BSW) questionnaire at the end of treatment. Safety was evaluated by adverse events, maximal urinary flow rate (MFR) and post-void residual (PVR). **Results:** Of 286 patients enrolled, 240 (83.9%) were completed (Group 1: 115, group 2: 125). The change of daily micturition frequencies of group 1 was -1.9 ± 3.0 and group 2 was -2.4 ± 3.2 , respectively ($p =$

0.176). There was no significant difference between two groups. However, the lower limit of confidence interval set were -1.33 and -1.80, respectively and didn't meet the predetermined non-inferiority limit (-0.8). There was no significant difference between the portions of patients who improved in PPBC. The portion of patients who improved to mild grade from moderate and severe grade in OABSS was higher in group 1 than group 2 (88% and 62.8%, respectively, $p=0.016$). There was no significant difference in BSW questionnaire at the end of treatment. There were no significant difference in changes of MFR and PVR from baseline between two groups (-1.4 ± 11.3 vs -0.11 ± 10.3 and 5.5 ± 32.1 vs 9.3 ± 44.1 , respectively). Treatment with solifenacin was well tolerated in group 1 and group 2.

Conclusions: We couldn't verify that the efficacy of solifenacin for frequency without urgency was non-inferior to the efficacy of solifenacin for OAB. However solifenacin was also effective in patients with frequency without urgency after 12 week treatment.

Implantation of Bone Marrow-derived Cells Recovers Radiation Injured Urinary Bladders in Rats

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Aims of Study: We indicated that bone marrow-derived cells implanted into frozen-injured urinary bladders and urethral sphincters differentiated into smooth or striated muscle cells, and then restored functional tissues (Imamura et al, Cell Trans, 2008; Tissue Eng, 2011). In addition, the frozen-injured tissues had an ideal microenvironment to bring out the potentials of bone marrow-derived cells (Imamura et al, Tissue Eng, 2009). Thus, we targeted irreversibly damaged, radiation-injured urinary bladders. In this study, we investigated to determine if the implantation of bone marrow-derived cells could recover the radiation-injured urinary bladders in rats.

Materials and Methods: This study used female 10-weeks old Sprague-Dawley (SD) rats, and male 17-weeks old Tg-SD (green) rats as recipient and donor animals, respectively. Thirty SD rats were anesthetized, and retained and protected with an iron board. The region including urinary bladder within 1 cm-diameter circle bordering on pubis was exposed through the iron board. The region was radiated with 2 Gy (wherein Gy is the absorbed energy of 1 J/kg). The radiation was performed every week, and repeated 5 times. After the radiation, the rats were maintained with freely available food and water under a 12-hour alternating light-dark cycle for 2 weeks. After 14 days of radiation, the radiated rats ($n=20$) were separated into two groups: cell implantation group ($n=10$), and cell-free injection control group ($n=10$). Other rats

were used as pre-implantation group to estimate the radiation-injured urinary bladders ($n=10$). Six female SD rats received non-radiation treatments were used as sham group.

Six male green rats were anesthetized, and then their both femurs of each rat were harvested. We harvested bone marrow cells from the femurs with culture medium. The cells were cultured in type I collagen-coated 10 cm culture dishes for 7 days. During culture, the medium was completely replaced every day, and non-attached cells were discarded. Seven days after seeding, adherent proliferating cells were used as bone marrow-derived cell.

Seven weeks after the radiation, the urinary bladders of cell-implantation and cell-free injection control rats were exposed. For the cell implantation group, the 2.0×10^6 bone marrow-derived cells were implanted into the bladder wall. For the cell-free injection control group, 200 μ L of cell-free culture medium was similarly injected.

At 4 weeks after implantation, cystometric investigations of the rats were performed, and then their urinary bladders were analyzed by immunohistochemistry using GFP-antibody to detect implanted cells, and smooth muscle actin (SMA)-, or S100-antibody, as a smooth muscle cell or nerve cell marker. In addition, the tissue sections were performed with hematoxylin and eosin and masson trichrome stain. To estimate proportion of smooth muscle area, the masson trichrome stain images were analyzed with image pro software. Results were expressed as means \pm standard error of the means. Statistical differences were determined by non-repeated measures ANOVA followed by the Scheffe's test. Differences with $p<0.05$ were considered significant.

Results: After 7 days after of culture, the adhered proliferating cells were positive for STRO1 (CD34)-antibody, which was a mesenchymal cell marker, but not positive for smooth muscle and nerve cell differentiation marker antibodies.

Just prior to the cell-implantation, smooth muscle layers of the radiation-injured urinary bladders were disorganized (Fig. 1A). The proportion of smooth muscle area (0.08 ± 0.01) was significantly decreased compared to the non-radiated sham urinary bladders (0.21 ± 0.01 , $p<0.01$). At 4 weeks after, although the control urinary bladders injected with the cell-free solution had smooth muscle cells, the smooth muscle layers were similar to the pre-implantation radiation-injured urinary bladders (Fig. 1B). However, the cell-implanted urinary bladders had numerous smooth muscle cells that were organized into distinct smooth muscle layers (Fig. 1C). The proportion of the smooth muscle area of cell-implanted urinary bladders (0.19 ± 0.01) was significantly higher than that of the cell-free control ones (0.11 ± 0.01 , $p<0.01$).

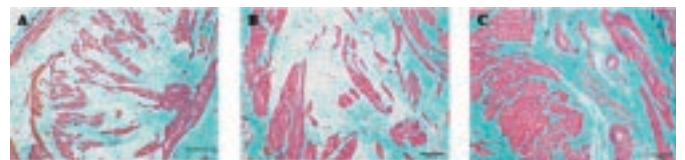


Fig. 1. Smooth muscle layers were stained with masson trichrome. (A) Just prior to implantation, smooth muscle layers of radiation-injured urinary bladders were disorganized. (B) After 4 weeks, the smooth muscle layers of cell-free control injection did not alter. (C) After 4 weeks, the smooth muscle layers of cell-implantation were reconstructed. Bar=100 μ m.

The reconstructed smooth muscle layers had some GFP-positive cells that were positive for SMA-antibody appeared to form contacts among themselves, creating smooth muscle layer structures. Similarly, other implanted GFP-labeled cells were positive for S100-antibody.

The cystometric investigations showed that basal, threshold and micturition pressure, and micturition volume did not have any statistical differences among four groups. The voiding interval (7.92 min) and residual volume (0.13 mL) of radiation-injured urinary bladders tended to lengthen and increase compared to the sham non-radiated sham ones (6.18 min and 0.06 mL, respectively). These values of cell-free control urinary bladders (7.39 min and 0.15 mL, respectively) did not alter compared to the radiation-injured ones. However, the voiding interval (5.46 min, $p < 0.05$) and residual volume (0.04 mL, $p < 0.01$) of cell-implanted urinary bladders were significantly decreased compared to the cell-free control and radiation-injured ones, and these values were as same as the non-radiation sham ones.

Conclusions: The radiation-injured urinary bladders showed disorganization of smooth muscle layers and dysuria including lengthened voiding interval and increased residual volume. However, bone marrow-derived cells implanted into the radiation-injured urinary bladders reconstructed smooth muscle layers, and improved the voiding interval and residual volume. Therefore, implantation of bone marrow-derived cells may recovery the irreversibly damaged urinary bladders.

Position of Suburethral Sling at the Bladder Neck May Predict a Higher Recurrent Rate of Stress Urinary Incontinence

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Aims of Study: Many surgical procedures for stress urinary incontinence (SUI) have been reported, and the best one remains controversial. Most of the research focuses on the comparison of the effectiveness and safety among different surgical techniques of suburethral sling. However, little data are available on the influence of the position of the suburethral sling on the treatment outcomes of SUI. The aim of this study was to investigate the relationship between the position of the suburethral sling and the outcomes of anti-incontinence surgery.

Materials and Methods: From 1998 to 2010, 154 women with SUI who received pubovaginal sling procedure using a polypropylene suburethral sling were retrospectively reviewed. Patients with preoperatively urodynamic proven detrusor overactivity, detrusor underactivity, neurogenic bladder dysfunction, high grade cystocele requiring concomitant colporrhaphy or pelvic floor reconstruction were not included. All patients had been investigated preoperatively and post-

operatively by transrectal sonography of the bladder and urethra (TRUS-B). The bladder neck (BN) incompetence at resting status and urethral incompetence during straining were also examined in TRUS-B to identify the BN and urethral condition after sling procedure. The suburethral sling positions in TRUS were classified as at the BN (BN, 0-20% of the urethral length), proximal urethral (PU, 21-40% of the urethral length), middle urethral (MU, 41-60% of the urethral length), and distal urethral (DU, 61-80% of the urethral length) (Fig. 1). Their post-operative continent status and the lower urinary tract symptoms such as urgency/urgency urinary incontinence or difficult voiding were compared among different groups of patients with different suburethral sling position.

Results: All patients received the pubovaginal sling procedures by the same urologist and the procedure was standardized as previously published technique. The mean age was 60.7 years (ranged from 30 to 85 years), and the median follow-up was 48 months (from 6 to 124). Postoperatively, the sling was found to locate at BN in 18, PU in 82, MU in 45, and DU in 9 patients. The overall rate of recurrent SUI was 15.5% (24 patients). Among the 154 patients, there was a significant higher recurrent rate of SUI in the group of BN (BN 38.9%, PU 8.5%, MU 17.8%, and DU 22.2%) ($p = 0.012$) (Table 1). The occurrence of BN incompetence at the resting status in TRUS-B was significantly higher in the group of DU (BN 27.8%, PU 40.2%, MU 71.1% and DU 88.9%) ($p < 0.001$). The occurrence of incompetent urethra in TRUS-B was significantly higher in the groups of BN and DU (BN 22.2%, PU 2.4%, MU 6.7%, and DU 22.2%) ($p = 0.007$). De novo urge symptoms occurred in 14.9% of overall patients, and de novo difficult voiding symptoms occurred in 18.2% of patients. There was no significant difference among these four groups in either de novo urge symptoms ($p = 0.571$) or voiding symptoms ($p = 0.934$).

Conclusions: The patient group with sling at the BN had a higher recurrence rate of SUI, and the groups with sling at the PU and MU had

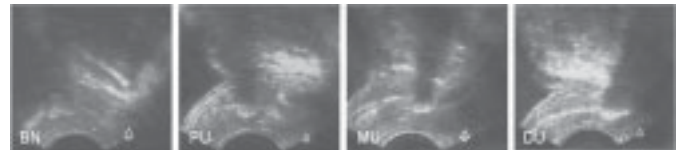


Fig. 1. The sling position after pubvaginial sling procedure.

Table 1. Outcomes of pubovaginal sling procedure among groups with different sling positions

	Bladder neck	Proximal urethra	Middle urethra	Distal urethra	Total	p-value
No. of patients	18	82	45	9	154	
Recurrence of SUI	7 (38.9)	8 (8.5)	8 (17.8)	2 (22.2)	24 (15.6)	0.012
De novo urge symptoms	1 (5.6)	14 (17.1)	6 (13.3)	2 (22.2)	23 (14.9)	0.571
De novo voiding symptoms	3 (16.7)	15 (18.3)	9 (20.0)	1 (11.1)	28 (18.2)	0.934
BN incompetence in resting TRUS-B	5 (27.8)	33 (40.2)	32 (71.1)	8 (88.9)	78 (50.6)	<0.001
Urethral incompetence in straining TRUS-B	4 (22.2)	2 (2.4)	3 (6.7)	2 (22.2)	11 (7.1)	0.007

(): percentage

a better continence rate. When the suburethral sling was located at the more distal position, the BN incompetence rate in TRUS-B was higher. In the groups of BN and DU, there was higher incidence rate of urethral incompetence in TRUS-B. The data indicated that suburethral slings at the BN contributed more in the BN competence during resting, but cannot assure a competent urethra during straining. On the other hand, the sling position at the PU and MU gave more contribution in maintaining urethra competence and therefore, a higher continence rate in long-term follow-up. Nevertheless, no significant difference was noted in the aspects of de novo urge or voiding symptoms among different groups, suggesting the de novo urge might be from the bladder origin and difficult urination was result from sling tension on the bladder outlet rather than the sling position.

Positioning of the suburethral sling at the BN may predict a higher recurrent rate of SUI. The position of the suburethral sling played an important role in the effect of anti-incontinence surgery. Sling locating at the PU and MU had the best continence rate, which implies a good hammock effect can be achieved when placing the suburethra sling at these positions. The sling position has no effect on de novo urge or difficult voiding symptoms.

Change of Urodynamics and Incontinence Following Augmentation Cystoplasty in Patients with Neurogenic Bladder Secondary to Spinal Cord Lesion

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Aims of Study: It is well known augmentation cystoplasty has a significant role in preventing upper urinary tract deterioration and acquiring urinary continence in patients with neurogenic bladder refractory to conservative treatment. In this study, we evaluate the alteration of urodynamic parameters and continent status in patients underwent augmentation cystoplasty due to spinal cord lesion in Korean.

Materials and Methods: A retrospective study included patients who underwent augmentation enterocystoplasty from Jan 2006 to Sep 2010 in the Seoul National University Hospital. Preoperative and postoperative fluoroscopic urodynamic parameters and incontinence questionnaires (ICI-Q Korean version) were analyzed.

Results: Thirty-six patients (21 males and 15 females) were analyzed. Fourteen patients had spinal cord injury, 17 congenital myelodysplasia, and 5 acquired spinal disease (mean age 32.4±17 yr, SD). For enterocystoplasty, ileum was utilized in 29 cases, sigmoid in 7 cases. Postoperative urodynamics were performed at mean 9.6 months after surgery. Preoperatively involuntary detrusor contractions (IDC) were found in 62.9% (22/35 cases), decreased bladder capacity in 65.7%

(23/35), poor compliance 77.1% (27/35), sphincteric incompetency demonstrated by fluoroscopy in 57.1% (20/35), and vesicoureteral reflux (VUR) in 60.0% (21/35), including bilateral in 6, unilateral in 15. Postoperatively 89.5% (17/19), 87.5% (14/16) and 81.3% (13/16) of patients showed normalization in compliance, capacity and IDC respectively. In 13 patients (15 postoperatively investigated), VUR improved in 84.2% (16/19 ureters; disappeared in 14, down-graded in 2). Out of 24 patients who complained preoperative subjective symptom, 29.2% (7/24) patients had small amount of incontinence, 70.8% (17/24) did more than moderate preoperatively, whereas postoperatively 72.0% (18/25) patients replied as dry. The incontinence-related bother score (visual analogue scale) declined from 8.3 (±2.3) preoperatively to 1.3 (±2.3) postoperatively (p<0 .001).

Conclusions: Both objective urodynamic parameters and subjective incontinence symptom improved significantly after augmentation cystoplasty in patients with neurogenic bladder caused by spinal cord lesion.

Keywords: neurogenic bladder, urinary incontinence, urondynamics, augmentation cystoplasty

The Muscarinic Receptor Subtype of M₂ Mediates Ca²⁺ Sensitization Via Indirect Activation of Rho Kinase Pathway in Human Detrusor Smooth Muscle

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Aims of Study: In urinary bladder, carbachol (CCh) induces contraction through M₂ and mainly M₃ receptor subtypes. However, the contribution of the muscarinic receptor subtypes to the Ca²⁺ sensitization, so called, rho-kinase (ROK) pathway and protein kinase C (PKC) pathway are still unknown. In particular, there is absolutely no report regarding the contribution of M₂ receptor subtype on the Ca²⁺ sensitization. It is very likely and of our interest that the down-regulation of cyclic adenosine monophosphate (cAMP) via M₂ receptor subtype would an indirect influence on contraction since it has been suggested that cAMP might antagonize ROK pathway. The aim of our present study is to clarify the role and signaling cascades of these M₂ and M₃ receptor subtypes on Ca²⁺ sensitization using alpha-toxin permeabilized human detrusor smooth muscle (DSM).

Materials and Methods: The DSM specimens were obtained from urinary bladder of human who underwent radical cystectomy due to urinary bladder cancer.

Alpha-toxin permeabilized human DSM strips were prepared and mounted horizontally for isometric force recording. The role and signalling cascade of M₂ and M₃ receptor subtypes in Ca²⁺ sensitization was studied using selective antagonists of M₂ (AF-DX116) and M₃ (4-DAMP) receptor subtypes. The effects of a selective inhibitor of ROK,

Y-27632, and a selective inhibitor of PKC, GF-109203X were also studied in contraction induced by 10 μM CCh with 100 μM GTP at fixed 1 μM $[\text{Ca}^{2+}]_i$ after pre-application with 1 μM AF-DX 116 or with 1 μM 4-DAMP. The effect of a cAMP specific phosphodiesterase (PDE IV) inhibitor Rolipram on Ca^{2+} sensitization was also investigated. All protocols were carried out after pre-treatment with thapsigargin (1 μM) and with cyclopiazonic acid (CPA; 1 μM).

All data and graphs are expressed as the mean \pm SEM of n (the number of samples) and N (the number of patients). Student's t-test was used for statistical analyses.

Results: As preliminary information, the small inhibitory effects by 5 μM Y-27632 (10.6 \pm 2.2%, n=4; N=4) and greater inhibitory effect by 5 μM GF-109203X (39.7 \pm 1.5%, n=4; N=4) were observed the contraction induced by only 1 μM Ca^{2+} . After the increase of Ca^{2+} sensitization was observed by the application of 10 μM CCh with 100 μM GTP at the fixed concentration of 1 μM Ca^{2+} , 1 μM AF-DX116 and 1 μM 4-DAMP inhibited the contraction by 13.0 \pm 1.5% and 41.4 \pm 2.9%, respectively (n=6; N=4, p<0.001). 5 μM Y-27632 attenuated the increase of Ca^{2+} sensitization by 10 μM CCh with 100 μM GTP at 1 μM Ca^{2+} for 54.7 \pm 6.0% (n=6). Not only 1 μM 4-DAMP but also 1 μM AF-DX 116 remarkably inhibited this attenuation effect of Y-27632 from 54.7 \pm 6.0% to 4.4 \pm 1.0% (n=6; N=4) and to 14.7 \pm 1.1% (n=6; N=4), respectively although the inhibition by 1 μM 4-DAMP was significantly stronger than that by 1 μM AF-DX116 (p<0.001). Likewise, 5 μM GF-109203X attenuated the increase of Ca^{2+} sensitization by 10 μM CCh with 100 μM GTP at 1 μM Ca^{2+} for 70.7 \pm 3.2% (n=6; N=4). Both 1 μM AF-DX116 and 1 μM 4-DAMP inhibited this attenuation effect of GF-109203X from 70.7 \pm 3.2% to 16.4 \pm 5.8% (n=6; N=4) and 35.9 \pm 2.7% (n=6; N=4), respectively. The inhibition by 1 μM 4-DAMP was significantly stronger than that by 1 μM AF-DX116 (p<0.001). The above result that M₂ selective inhibitor AF-DX116 (1 μM) certainly inhibited the attenuation effect of Y-27632 was on the contrary to the probable effect of AF-DX 116 on the ROK pathway. Therefore, the indirect effect of M₂ receptor on Ca^{2+} sensitization via down regulation of cAMP was speculated. Accordingly, the effect of PDE IV specific inhibitor Rolipram on the contraction was investigated. 5 μM Rolipram attenuated the Ca^{2+} (1 μM) induced contraction by 35.1 \pm 1.0% (n=4, N=3). This attenuation by Rolipram was significantly relatively decreased from 35.1 \pm 1.0% to 15.5 \pm 1.0% (n=4) in the condition where Ca^{2+} sensitization was activated by 10 μM CCh with 100 μM GTP (p<0.001).

Conclusion: The inhibition by Y-27632 and GF-109203X on only Ca^{2+} -induced contraction indicated that ROK and PKC pathways have already been activated in Ca^{2+} -induced contraction of permeabilized DSM. The inhibition by 1 μM 4-DAMP was significantly stronger than that by 1 μM AF-DX116 on the Ca^{2+} sensitization by CCh with GTP indicating that the predominant role of M₃ receptor in Ca^{2+} sensitization of human DSM. Slightly AF-DX 116 but mainly 1 μM 4-DAMP inhibited the attenuation effect of GF-109203X on the Ca^{2+} sensitization by CCh with GTP indicating the coherent understanding that M₃ receptor plays a predominant role in PKC pathway. Regarding ROK pathway, from the fact that M₂ selective inhibitor AF-DX 116 certainly inhibited the attenuation effect of Y-27632 although AF-DX116 induced little inhibition in Ca^{2+} sensitization activated by CCh with GTP, the indirect effect of M₂ receptor was suggested. To support this hypothesis,

the attenuation by PDE IV inhibitor Rolipram on the contraction just by Ca^{2+} was relatively decreased by the activation of Ca^{2+} sensitization by CCh with GTP. These results suggested that cAMP formation had already been inhibited in advance by muscarinic stimulation via M₂ receptor.

Our present study demonstrated the first evidence in human DSM that both M₂ and M₃ receptor subtypes mediate Ca^{2+} sensitization. Our study also revealed the predominant role of M₃ receptor subtypes to ROK and PKC pathways with comparable contribution which induced Ca^{2+} sensitization. Further, interestingly, the contribution of M₂ receptor subtype is indirectly and preferably to ROK pathway via the down-regulation of cAMP but little to PKC pathway. These findings might be important in the clinical application of highly selective antimuscarinic treatment for lower urinary tract dysfunction.

First Voided Volume in the Morning as the Prediction of Overactive Bladder

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Aims of the Study: Frequency-volume chart (FVC) provides objective information on voiding parameters, serving as an important tool in the evaluation and management of overactive bladder (OAB). However, many patients cannot record FVC well due to a variety of reasons. We suspect that a simple and convenient measurement, such as the first morning void volume (FMV) might be a useful alternative. The aim of the study is to determine the usefulness of measuring the FMV in evaluating patients with OAB.

Materials and Methods: We prospectively recruited patients with OAB symptoms between January 2009 and April 2010. All patients completed a 3-day FVC. Mean FMV was obtained by averaging 3-days' FMV. Symptom severity was evaluated with Overactive Bladder Symptom Score (OABSS) questionnaire proposed by Homma et al. We also defined small volume bladder as the maximal bladder volume \geq 300 mL recorded in the FVC. All patients underwent either conventional pressure-flow urodynamic study or videourodynamic study. The correlation between FMV, urodynamic parameters and symptoms severity was analyzed with Spearman correlation. We also compared the sensitivity and specificity in predicting small volume bladder with FMV and cystometry (CMG) capacity.

Results: A total of 102 patients, including 43 men and 59 women, were recruited into the study. The mean age of the patients was 66.8 \pm 14.0 years (range: 23-85). The mean FMV was 196.6 \pm 106.5 mL (range: 20-650). The FMV was the maximum voided volume of the day in 24 patients (23.5%) and was significantly correlated with the all-day mean void volume (p<0.001). FMV was negatively correlated with daytime

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frequency ($p=0.013$) and nighttime frequency ($p<0.001$). FMV was significantly correlated with cystometric capacity ($p=0.026$), which was significantly correlated with the total score of OABSS ($p=0.031$). Although there was no significant correlation between FVM and total score of OABSS, FMV was negatively correlated with the sub-score for nighttime frequency of the OABSS ($p=0.004$). As in predicting small volume bladder, FMV showed its superiority either in sensitivity or specificity compared to CMG capacity (100% vs 95.9%, 43.3% vs 15.1%,

respectively).

Conclusions: In patients with OAB FMV might represent the severity of daytime and nighttime frequency and the cystometric capacity. The FMV also is a better tool to predict if a OAB patient has small volume bladder comparing to CMG capacity. A simple measurement of FMV seems to be helpful in evaluating OAB patients, especially for those with poor compliance in recording FVC.