

ARTICLE



Overactive bladder syndrome – focus onto detrusor overactivity

Taras Ptashnyk^a , Martin Hatzinger^a, Federico L. Zeller^b and Ruth Kirschner-Hermanns^b

^aDepartment of Urology, Diakonissen Hospital, Mannheim, Germany; ^bClinic of Urology/Neuro-Urology, University Clinic Rheinischen Friedrich-Wilhelms Universität Bonn and Neurologisches Rehabilitationszentrum Godeshoehe e.V. Bonn, Bonn, Germany

ABSTRACT

Introduction and hypothesis: To determine in patients with overactive bladder (OAB), urodynamic differences as well to compare the characteristics of patient's, with presence of detrusor overactivity on urodynamics with those with absence of detrusor overactivity.

Methods: Taking into account the urodynamic findings, the patients with OAB symptoms were categorized into one of two groups: group 1 (with detrusor overactivity) or group 2 (without detrusor overactivity), and comparative analyses for both groups were performed (epidemiological data, patient history, urodynamic criteria, bladder diaries, IC-OAB, IC-OABqol – questionnaires).

Results: There was a significant difference in age as well in the disease duration between group 1 and group 2. Although the mean number of micturitions and nocturia episodes was comparable, the numbers of urgency episodes differed significantly. The number of 'wet' patients was significantly higher in the group 1 with the significantly higher number of incontinence episodes. Group 1 demonstrated higher OAB symptom scores and higher impact on the patients' quality of life.

Conclusions: More than half of the patients complaining of urgency-frequency do not have detrusor overactivity upon urodynamic testing, and only half have detrusor overactivity that correlates with urge perception. The patients with no detrusor overactivity appear to be significantly younger and have fewer symptoms, with a less pronounced impact on quality of life. They also have significantly higher maximal bladder capacity. This data supports the hypothesis that both conditions are different phases (early and late) of the same pathological state, or may be two different subtypes of OAB.

ARTICLE HISTORY

Received 23 March 2020
Revised 5 October 2020
Accepted 14 October 2020

KEYWORDS

OAB; detrusor overactivity; detrusor instability; urodynamic

Introduction

Overactive bladder (OAB) syndrome is a collection of urinary tract symptoms that affects millions of people worldwide. It is characterized by urgency, increased micturition frequency, and nocturia, and in one-third of the cases is accompanied by urgency incontinence. The underlying cause can vary [1].

Due to an ICS definition these symptoms are usually 'suggestive of urodynamically demonstrable detrusor overactivity but can be due to other forms of urethralesical dysfunction' [2].

Epidemiological surveys show that OAB is present in approximately 16% of the general population aged 40 years and older [3]. Due to our ageing society, this syndrome will become more significant in the future, both from a medical and from a socioeconomical perspective [4]. Although OAB is an important issue in healthcare, it has become clear that OAB relates to many different clinical entities, with or without well-defined causes for the signs and symptoms that patients experience [5]. Patients presenting with symptoms of OAB have different findings upon urodynamic testing. Whereas some patients present with the typical signs of detrusor overactivity, others have stable bladder filling. Previously, these conditions were referred to as sensory urgency (stable bladder filling) or motor urgency (bladder filling with detrusor overactivity [6,7]. According to the

International Continence Society (ICS) definition [2], the diagnosis of OAB does not require urodynamic confirmation of detrusor overactivity, and clinically, empirical therapy for OAB with anticholinergics is most commonly initiated without urodynamic testing. However, some neurologists and gynaecologists are concerned that a correct diagnosis will be missed in many patients and they will not receive the appropriate treatment because the bladder has been described as an unreliable witness [8].

Therefore, in the present study, we sought to determine all of the urodynamic differences between OAB patients with and without detrusor overactivity, with the aim of better understanding whether these are two different conditions, or just different phases (early and late) of the same pathological state. In addition, we compared patient characteristics, such as age, disease duration, and correlation to incontinence. Better understanding of the natural history of both conditions may help us to improve the rationale for their treatment.

Patients and methods

Between January 2012 and December 2015, 55 patients seeking medical help and complaining of OAB symptoms for at least 6 Month underwent physical investigation

and urodynamic testing, including urodynamic cystometry and pressure/flow studies. A detailed history and evaluation of bladder diaries, as well as questionnaires were carried out. The following inclusion criteria were used to recruit patients to the study: female, at least 18 years of age, who were suffering with symptoms of OAB (urgency – at least 1 episode per day, frequency >8 micturitions per day, nocturia – at least 1 episode per night), with and without urgency incontinence. None of the participants had been treated with antimuscarinics, or drugs that could affect bladder activity at least one month before entering the study. None of patients participated in another study, treating the OAB-Symptoms or using antimuscarinics. Patients having painful bladder syndrome or known neurological illness (paraplegic women, women with multiple sclerosis, morbus Parkinson, or history of stroke) were excluded from the study. Urin microscopy was used to rule out the urinary tract infection.

Patients having stress urinary incontinence (SUI) were excluded from the study. The stress urinary incontinence was ruled out by anamnesis and vaginal investigation.

In order to rule out secondary OAB due to infravesical obstruction, the patients having prolapse categorized as grade II or above, or showing obstructive voiding during pressure-flow studies (i.e. having an obstruction coefficient suggested by Schäfer ($OCO = P_{det,Qmax}/(40 + 2Q_{max})$) greater than 0.35 [9]) were also excluded from the study. Patient having residual urine over 100 ml were excluded from the study as well.

In order to achieve the comparable results, the patients were advised to have a constant fluid intake around two liters per day during the study.

All patients had a sufficient understanding of the language and were able to complete the questionnaires without any assistance, and gave fully informed and written consent for their participation and use of their data. The approval of local ethical committee was obtained (approval number 23/11).

The urodynamic evaluation was performed according to the guidelines of good urodynamic practice [10] by a single investigator, who was a senior-urologist. 30 mL/min bladder filling speed was applied. The filling was stopped when the patient developed a strong desire to void. All the procedures were performed in a sitting position. In all patients who did not develop detrusor overactivity (wave-shaped rise in detrusor pressure >5 cmH₂O) [7], additional provocative measures were undertaken to reveal any hidden detrusor overactivity (listening to running water and washing hands in cold water) at the bladder capacity, exceeding the capacity at which there was a normal desire to void.

Taking into account the urodynamic findings, the patients were categorized into one of two groups: group 1 (with detrusor overactivity) or group 2 (without detrusor overactivity), and detailed comparative analyses of both groups were performed afterwards. Besides epidemiological factors such as age, the following three urodynamic criteria were compared: (1) bladder volume at normal desire to void, (2) maximal functional bladder capacity, and (3) detrusor pressure at maximal flow. In the group with detrusor overactivity, the

correlation between detrusor contraction and urge or desire to void was also evaluated. The following two characteristics of the patients' history were compared: presence of urinary incontinence and duration of the complaints.

The mean number of voidings, mean number of nocturia episodes, mean number of incontinence episodes, mean number of urgency episodes, and mean voided volume were evaluated using bladder diaries. The OAB-symptom score, OAB-symptom bother, and impact on the quality of life was evaluated from questionnaires (IC-OAB, IC-OABqol).

Depending on the distribution, an unpaired *t*-test or Mann–Whitney *U* test were used for statistical analyses.

Results

Upon urodynamic testing, detrusor overactivity was found in 27 (49%) of the 55 investigated patients ('unstable group'); the other 28 patients (51%) had stable filling ('stable group').

There was a significant difference in age between the 'stable group' and 'unstable group' (47.3 years vs. 65 years; $p < 0.05$).

Evaluating the distribution of patients dividing in different age groups, we revealed significantly higher number of patients having no the detrusor overactivity in age under 50 years (13 vs. 2) and conversely significantly higher number of patients having DO in age over 50 years (25 vs. 15; Figure 1). Interestingly, we did not see any patient having DO in age under 30 years as well any having no DO in age over 70 years.

The disease duration of the two groups differed markedly too, with the stable group being shorter (9 months vs. 15 months; $p < 0.05$; Table 1).

The mean number of micturitions was comparable in both groups; 13.8 in the 'stable group' vs 13.2 in the 'unstable group'. The number of nocturnal micturitions was also comparable with 3.14 in the 'unstable group' vs. 2.96 in the 'stable group'. The number of urgency episodes was significantly higher in the 'unstable group' (4.9 vs. 3.7; $p < 0.05$). The number of patients with urge incontinence was significantly higher in the 'unstable group' compared with the

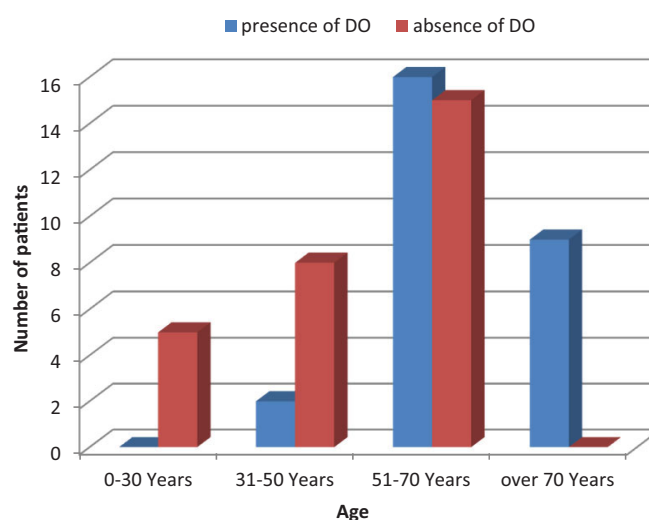


Figure 1. Distribution of the patients in different age groups.

Table 1. Demographic parameters and questionnaire results.

Parameter	Presence of detrusor overactivity	Absence of detrusor overactivity	<i>p</i> Value
Number of patients (%)	27 (49%)	28 (51%)	
Mean age, years	65.0 ± 11.5	47.3 ± 12.7	<0.001
Disease duration, months	15.0 (15.0; 28.5)	9.0 (9.0; 15.0)	<0.001
OAB Symptoms score	11.3 ± 2.6	8.7 ± 2.0	<0.001
OAB Symptoms bother	8.4 (6.8; 10.0)	7.0 (4.6; 9.8)	<0.05
QoL score	90.4 ± 26.1	74.7 ± 22.2	<0.05

Table 2. Bladder diary parameters.

Parameter	Presence of detrusor overactivity	Absence of detrusor overactivity	<i>p</i> Value
Mean voided volume, mL	127 ± 56	119 ± 48	>0.05
Mean number of micturitions	13.2 ± 6.5	13.8 ± 3.5	>0.05
Nocturia	3.14 ± 2.3	2.96 ± 1.3	>0.05
Number of urgency episodes	4.9 ± 4.8	3.7 ± 3.8	<0.05
Urinary incontinence, number of patients [% (95% CI)]	13 [59.1 (38.1 – 80.1)]	6 [27.3 (8.3–46.3)]	<0.05
Number of episodes of urinary incontinence/ day	5.3 (4.7; 9.3)	2.9 (1.4; 3.0)	<0.001

Table 3. Urodynamic parameters.

Parameter	Presence of detrusor overactivity	Absence of detrusor overactivity	<i>p</i> Value
First desire to void, mL	116 ± 59	113 ± 47	>0.05
Maximal bladder capacity, mL	216.5 (152.3; 304.0)	373.0 (337.0; 442.0)	<0.001
Pdet at Flow max, cm/H2O	21.4 ± 7.6	21.2 ± 9.8	>0.05

'stable group' (13 vs. 6; $p < 0.05$). There was a significant difference between the number of urgency incontinence episodes in the 'stable group' and the 'unstable group' (2.9 vs. 5.3; $p < 0.05$; Table 2).

The 'stable group' demonstrated significantly lower OAB symptom scores compared with the 'unstable group' (8.7 vs. 11.3; $p < 0.05$). Furthermore, it was found that OAB symptoms have a lower impact on the quality of life in the 'stable group' patients compared with the 'unstable group' (74.7 vs. 90.4; $p < 0.05$; Table 1).

During the urodynamic studies, the first desire to void occurred at comparable volumes of 113 mL in the 'stable group' vs. 116 mL in the 'unstable group', whereas the maximal bladder capacity differed significantly between the in the 'stable group' and the 'unstable group' (373 mL vs. 216 mL; $p < 0.05$; Table 3). Detrusor pressure at the maximal flow was comparable in both groups 21.4 cm/H2O in the 'unstable group' vs. 21.2 cm/H2O in the 'stable group' (Table 3). Only 12 Patients (54%) in the 'unstable group' correlated their 'feeling of urge or desire to void' to detrusor overactivity episodes registered during the filling.

Discussion

Based on the urodynamic findings of detrusor overactivity, the former classification by the ICS divided OAB into: *sensory urgency*, defined as increased perceived bladder sensation during filling, a low first desire to void, and low bladder capacity in the absence of recorded urinary tract infection (urodynamic profile without detrusor instability); or *motor urgency*, defined as detrusor overactivity characterized by involuntary detrusor contraction during the filling phase, which may be spontaneous or provoked and which the patient cannot completely suppress (unstable filling) [11].

It has been suggested that the diagnosis of sensory urgency is an early form of detrusor overactivity and may

just be earlier in the spectrum of disease [12]; however, this theory has not been adequately tested [13]. This classification had only a formal meaning since it had no consequences for either the further workup or the treatment algorithm. Sensory urgency has been criticized due to an unclear definition, not sufficient researched aetiology, and lack of treatment regimens [11,13,14]. In addition, due to its subjective nature, sensation is difficult to quantify and study [15]. As a result, in 2002 the diagnosis of sensory urgency was eliminated by the Standardization Sub-Committee of the ICS, citing it as having 'little intuitive meaning' [2]. Both conditions (sensory urgency and detrusor overactivity) were placed under the umbrella term of overactive bladder syndrome. These two subtypes are not distinguishable in terms of treatment recommendation because antimuscarinics are designated as pharmacological treatment for both types of OAB. These drugs were awarded the 1st (best possible) 'level of evidence' and the A (best possible) 'grade of recommendation' [5]. Currently these medications are considered the therapy of choice, despite their high costs, number of side effects, and the fact that the efficacy is confounded by the placebo response [16].

Historically, the myogenic theory was used to describe the pathophysiology of OAB syndrome [17,18]. Thus, antimuscarinic therapy, assuming that antimuscarinics work by blocking M3 receptors in the bladder and suppressing involuntary bladder contraction, has remained the main focus of pharmacological actions for many years.

The uncertainty regarding the mechanism of action in cases where detrusor overactivity is not present was often explained by the consideration that the 'real' diagnosis of detrusor overactivity might have been missed at the time of urodynamic testing. New focus into afferent action abnormalities associated with OAB, as well as acknowledgment regarding the mechanism of action of antimuscarinics on afferent M2 receptors in the bladder (urotheliogenic theory)

has strengthened the needlessness of separating into sensory and motor urgency. Therefore, there has been no further development in the understanding of this pathology [19–21]. Moreover, the most recent reports show the equal efficacy of antimuscarinics on OAB, with or without detrusor overactivity, strengthening the role of antimuscarinics as a therapy of choice for both these conditions [22].

A dysfunctional urethral (i.e. urethral pressure variation during bladder filling) has been discussed as a possible cause of OAB. The urethra-driven urgency may and may not be accompanied by detrusor contractions [23]. This is supported by the fact that many patients experience urgency when moving from a sitting or lying position to a standing position, whereas these patients may not display DO on urodynamics, as the investigation is usually performed only in the sitting position [24]. However up to now most known pharmaceutical therapies target the bladder. However a dysfunctional urethra could be an explanation for failure of pharmacological therapy.

Very few efforts have been undertaken to revive sensory urgency as an independent pathology. Haylen [13] believes that the abovementioned reasons to eliminate this definition were not adequate enough without any explanations for cancelling the well-researched pathological state, and depriving an entire generation of women the diagnosis that was given to them. In defence of the diagnosis of sensory urgency, the author claims that sensory urgency is an early form of detrusor overactivity, or an earlier stage in the spectrum of disease.

In support of this statement, other reports cite better efficacy of bladder training (drill) in OAB patients without detrusor overactivity [25,26]. Aitchison et al. [27] believes that the natural history of the diagnosis trends towards spontaneous resolution. Our attempt was to undertake a deeper investigation into the different subtypes of OAB in order to reveal all the differences between them, in terms of urodynamic findings, as well natural history of the disease. This would allow us to gain some new insights into the approach for OAB because the costs and adverse effects of antimuscarinics are substantial.

The results of our study revealed several significant differences: the patients with OAB symptoms who did not have detrusor overactivity were much younger, with significantly shorter disease duration. It may be due to, that the presence detrusor overactivity may depend solely on the ability of the body to suppress it, with this ability disappearing with disease progression or with the age. We would also suggest that white matter disease could play role here.

Although our investigation is a cross sectional analysis only, our findings support the aforementioned theory of sensory urgency as an early form of detrusor overactivity, or being an earlier stage in the spectrum of disease. Furthermore, despite the comparable mean number of voidings, as well as the nocturia episodes, the patients without detrusor overactivity demonstrated significantly lower numbers of urgency episodes and episodes of urgency urinary incontinence. The OAB symptom score and bother, and the perceived impact on patients' quality of life was also

significantly lower in those patients having no detrusor overactivity. Therefore, we suggest that this group of patients make up a subgroup of the overall OAB cohort that has a milder disease profile. To support this is also the fact that in young patients we often see a discrepancy of mean voided volume, from micturition diary and maximum bladder capacity during an urodynamic study. In our opinion, this is a sign that those patients might especially benefit from early behavioural treatment. This statement could also justify starting therapy in this group rather with non-medical treatments, such as dietary modifications, biofeedback, or peripheral nerve stimulation before giving the antimuscarinics. In other words, in younger patients with shorter disease duration and milder symptoms we could expect to have a better outcome with alternative treatments that may not be as effective as antimuscarinics, but have no side effects. Alternatively, the reduced dose of antimuscarinics could be tried in this cohort of patients. The psychological counselling might be also of benefit to this group of patients. The further questions should be answered: what role does oestrogen substitution have in this subtype of OAB patients? Will behavioural therapy have an effect, or even what is the role placebo effect? [27,28]. We believe that the approach to OAB in the future will be more individualized, regarding diagnosis and treatment algorithms. Based on our current understanding of OAB pathophysiology, the proposed approach may be characterized by a diagnosis algorithm that subcategorizes patients based on OAB symptomatology, as defined by the presence or absence of sensory or motor pathology.

The urodynamic picture of OAB is not homogenous. More than half of the patients complaining of symptoms of urgency-frequency do not have a finding of detrusor overactivity, which was believed to be the main reason for development of OAB syndrome, and was considered the main target of action of antimuscarinic agents. Moreover, only half of the patients who have detrusor overactivity correlate it to the urge perception. The patients who do not have any detrusor contractions appear to be significantly younger, have fewer symptoms, and a less pronounced impact on their quality of life. They also have significantly higher maximal bladder capacity. All these factors support the hypothesis that both conditions are different phases (early and late) of the same pathologic conditions, and may suggest the gradual onset of the disease or could be regarded as different subtypes of OAB. Longitudinal studies with repeated measurements including continuous urethral pressure measurements of the same patients, continuing suffering OAB over longer periods of time might prove this hypothesis.

Undoubtedly the simultaneous evaluation of urethral pressure could bring a valuable information and help to refine the classification (for example 'bladder-driven OAB with detrusor overactivity'; 'bladder-driven OAB without detrusor overactivity'; 'urethra-driven OAB with detrusor overactivity'; 'urethra-driven OAB without detrusor overactivity'). Taking into account the positive effect of stress urinary incontinence surgery and prolapse surgery in reducing the signs of OAB

this classification will allow much more precise tailoring of OAB therapy-strategies [29,30].

This study has generated more questions than answers. However, despite of that it does shed more light into the understanding of the pathophysiology of urgency, especially the role of detrusor overactivity. Answering these questions may lead to the improvement in therapy algorithms of treatment for this widespread pathology.

Although to date there is some knowledge about the impact of antimuscarinics in the patients in both OAB-groups described, there are gaps in the knowledge regarding how other non-medical therapies (e.g. biofeedback, tibialis stimulation, psychological training), as well as Botox-injection, work in these cases?

Disclosure statement

The authors have no relevant financial or nonfinancial relationships to disclose.

ORCID

Taras Ptashnyk  <http://orcid.org/0000-0003-2576-9855>

References

- [1] Stewart WF, Van Rooyen JB, Cundiff GW, et al. Prevalence and burden of overactive bladder in the United States. *World J Urol.* 2003;20:327–336.
- [2] Abrams P, Cardozo L, Fall M, et al.; Standardisation Subcommittee of the International Continence Society. The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. *Urology.* 2003;61:37–49.
- [3] Milsom I, Abrams P, Cardozo L, et al. How widespread are the symptoms of an overactive bladder and how are they managed? A population-based prevalence study. *BJU Int.* 2001;87:760–766.
- [4] Kelleher CJ. Economic and social impact of OAB. *Eur Urol Suppl.* 2002;1:11–16.
- [5] Wyndaele JJ, Van Meel TD, De Wachter S. Detrusor overactivity. Does it represent a difference if patients feel the involuntary contractions? *J Urol.* 2004;172:1915–1918.
- [6] Sekido N, Hinotsu S, Kawai K, et al. How many uncomplicated male and female overactive bladder patients reveal detrusor overactivity during urodynamic study? *Int J Urol.* 2006;13:1276–1279.
- [7] Hashim H, Abrams P. Is the bladder a reliable witness for predicting detrusor overactivity? *J Urol.* 2006;175:191–194.
- [8] Flisser AJ, Blaivas JG. Role of cystometry in evaluating patients with overactive bladder. *Urology.* 2002;60:33–42.
- [9] Schaefer W, Clarkson B, Griffiths D, et al. The urodynamics of voiding function in females: grading of bladder outflow conditions on a continuous scale. *J Urol.* 2011;185:682–683.
- [10] Schäfer W, Abrams P, Liao L, et al.; International Continence Society. Good urodynamic practices: uroflowmetry, filling cystometry, and pressure-flow studies. *Neurourol Urodyn.* 2002; 21:261–274.
- [11] Abrams P, Blaivas JG, Stanton SL, et al. The standardisation of terminology of lower urinary tract function. The International Continence Society Committee on Standardisation of Terminology. *Scand J Urol Nephrol Suppl.* 1988;114:5–19.
- [12] Creighton SM, Dixon J. Bladder hypersensitivity. In: Stanton SL, Monga AK, editors. *Clinical urogynecology.* London (UK): Churchill Livingstone; 2000. p. 321–327.
- [13] Haylen BT, Chetty N, Logan V, et al. Is sensory urgency part of the same spectrum of bladder dysfunction as detrusor overactivity? *Int Urogynecol J Pelvic Floor Dysfunct.* 2007;18:123–128.
- [14] Shutherst JR, Frazer MI, Richmond DH, et al. Sensory syndromes of bladder and urethra. In: *Introduction to clinical gynaecological urology.* London (UK): Butterworth; 1990. p. 128–139.
- [15] Wise B. Frequency/urgency syndromes (sensory urgency section). In: Cardozo LD, Staskin D, editors. *Textbook of female urology and urogynaecology.* London (UK): Isis Medical Media; 2001. p. 912.
- [16] Herbison P, Hay-Smith J, Ellis G, et al. Effectiveness of anticholinergic drugs compared with placebo in the treatment of overactive bladder: systematic review. *BMJ.* 2003;326:841–844.
- [17] Brading AF. A myogenic basis for the overactive bladder. *Urology.* 1997;50:57–67.
- [18] Maake C, Landman M, Wang X, et al. Expression of smoothelin in the normal and the overactive human bladder. *J Urol.* 2006;175: 1152–1157.
- [19] Yoshimura N. Lower urinary tract symptoms (LUTS) and bladder afferent activity. *Neurourol Urodyn.* 2007;26:908–913. Review.
- [20] Yoshimura N, Chancellor MB. Current and future pharmacological treatment for overactive bladder. *J Urol.* 2002;168:1897–1913.
- [21] Rapp DE, Lyon MB, Bales GT, et al. A role for the P2X receptor in urinary tract physiology and in the pathophysiology of urinary dysfunction. *Eur Urol.* 2005;48:303–308.
- [22] Nitti VW, Rovner ES, Bavendam T. Response to fesoterodine in patients with an overactive bladder and urgency urinary incontinence is independent of the urodynamic finding of detrusor overactivity. *BJU Int.* 2010;105:1268–1275.
- [23] Shafik A, Shafik AA, El-Sibai O, et al. Role of positive urethrovaginal feedback in vesical evacuation. The concept of a second mic-turition reflex: the urethrovaginal reflex. *World J Urol.* 2003;21: 167–170.
- [24] Hubeaux K, Deffieux X, Deseaux K, et al. Stand up urgency: is this symptom related to a urethral mechanism? *Prog Urol.* 2012; 22:475–481.
- [25] Holmes DM, Stone AR, Bary PR, et al. Bladder training-3 years on. *Br J Urol.* 1983;55:660–664.
- [26] Ferrie BG, Smith JS, Logan D, et al. Experience with bladder training in 65 patients. *Br J Urol.* 1984;56:482–484.
- [27] Aitchison M, Carter R, Paterson P, et al. Is the treatment of urgency incontinence a placebo response? Results of a five-year follow-up. *Br J Urol.* 1989;64:478–480.
- [28] Cardozo LD1, Wise BG, Benness CJ. Vaginal oestradiol for the treatment of lower urinary tract symptoms in postmenopausal women—a double-blind placebo-controlled study. *J Obstet Gynaecol.* 2001;21:383–385.
- [29] Jain P, Jirschele K, Botros SM, et al. Effectiveness of midurethral slings in mixed urinary incontinence: a systematic review and meta-analysis. *Int Urogynecol J.* 2011;22:923–932.
- [30] Petros PE, Woodman PJ. The integral theory of continence. *Int Urogynecol J.* 2008;19:35–40.