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Original Article

Topical estrogen therapy ameliorates bladder estrogen receptor β expression in female patients with overactive bladder

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Abstract: Objective: To explore the effect of topical estrogen therapy on the expression of estrogen receptor β (ERβ) in bladder tissue of female patients with overactive bladder (OAB). Methods: A total of 58 female OAB patients who were treated in the Affiliated Hospital of Qinghai University were included in this retrospective study. The patients were divided into an estrogen group (28 cases) and a tolterodine group (30 cases). In the estrogen group, patients received topical vaginal estrogen treatment (0.5 mg daily) for 3 consecutive weeks and this was followed by a one-week interval. In the tolterodine group, patients received tolterodine (4 mg once daily) for 3 consecutive weeks and this was followed by a one-week interval. All patients underwent cystoscopy and completed Overactive Bladder questionnaire short form (OAB-q SF). The expression of ERβ in the bladder tissue was detected by immunohistochemistry. Results: After 12 weeks, there was no statistical difference in the OAB-q scores between the tolterodine and estrogen groups. However, tolterodine treatment significantly improved urinary incontinence than estrogen treatment ($P = 0.03$). After 12 weeks of estradiol treatment, the expression of ERβ in the bladder tissue was significantly higher than that in the tolterodine group ($P < 0.05$). Conclusion: Topical estrogen therapy ameliorates OAB in female patients, and this may be related to improved ERβ expression in the bladder mucosa.

Keywords: Overactive bladder, estrogen receptor β, topical estrogen therapy, female

Introduction

Abnormal bladder storage and voiding functions are commonly associated with overactive bladder (OAB). According to the definition by the International Continence Society (ICS), OAB is characterized by urgency, with or without urgency incontinence, often accompanied by increased daytime frequency and nocturia [1]. The prevalence of OAB has been reported to be 11.8% in Europe and North America, 20.8% in East Asia, with the highest prevalence found in China at 23.9%, and the prevalence tends to increase with age [2]. OAB patients are primarily affected by lower urinary tract symptoms (LUTS), with urgency and frequent urination being the most common symptoms [3]. Therefore, OAB patients not only experience a significant decrease in quality of life but also psychological problems [4].

The cause of OAB is uninhibited spastic detrusor muscle contractions, but detrusor overactivity is not enough to cause urinary incontinence (UI). Michel et al. suggest that estrogen deficiency caused by menopause is related to the occurrence of OAB, and estrogen can significantly increase bladder capacity and compliance [5]. The female urinary tract arises from the urogenital sinus, which is sensitive to sex steroid hormones [6]. A series of periodic LUTSs and urodynamic changes in premenopausal women suggest that estrogen has a certain physiological effect on the lower urinary tract [7]. Estrogen receptors (ERs) have been identified in the bladder, urethra, and pelvic floor [8]. Estrogen acts through the binding and activation of estrogen receptors α (ERα) and β (ERβ), with ERβ being the major ER subtype in the bladder [9, 10]. Although the current first-line treatment for OAB is antimuscarinic drugs, large literature reports suggest that estrogen replacement therapy (ERT) can also significantly improve OAB symptoms [3, 11]. ERT was first used for OAB-related symptoms as early as 1992 [12]. In addition, a meta-analysis of post-

menopausal women using estrogen to treat OAB symptoms has shown that ERT significantly improved the symptoms of OAB [13].

Although OAB is more common in elderly postmenopausal women, the safety and effectiveness of estrogen in treating OAB have been confirmed in a number of reports [14]. Compared with systemic ERT, the use of topical vaginal estrogen for OAB has more obvious advantages and fewer adverse reactions [11, 15]. The above studies are mainly conducted through questionnaires, so the subjective improvement of OAB symptoms may only represent a local improvement in vaginal atrophy, rather than a description of urothelial changes. Estrogen has been used for the treatment of postmenopausal UI for more than decades. However, there are few basic studies on the role of sex hormones in the lower urinary tract. If the lower urinary tract is indeed the target organ for estrogen action, it should be possible to detect the presence of ERs in tissues and the changes in ERs after the application of estrogen. The purpose of this study was to detect the expression of ERβ in the bladder mucosa tissue of female OAB patients after intravaginal estrogen treatment, so as to provide a theoretical basis for estrogen treatment of OAB.

Subjects and methods

Subjects

This study was approved by the Ethics Committee of the Affiliated Hospital of Qinghai University. A total of 58 female OAB patients who were treated in the Affiliated Hospital of Qinghai University were included in this retrospective study. The patients were divided into an estrogen group (28 cases) and a tolterodine group (30 cases). In the estrogen group, patients received topical vaginal estrogen treatment (0.5 mg daily) for 3 consecutive weeks and were closed for 1 week. In the tolterodine group, patients received tolterodine (4 mg once daily) for 3 consecutive weeks and were closed for 1 week. Inclusion criteria: (1) women aged over 18 years old; (2) women with a history of OAB symptoms for at least 3 months, including an average of 8 or more micturitions per 24 h and at least one urgency incontinence episode per 24 h recorded in 3-day bladder diaries at baseline; (3) women with significant OAB symptoms which reduced quality of life as measured by the Overactive Bladder questionnaire short form (OAB-q SF) [16]. Each item is rated on a six-point scale, with 1 being “not at all” to 6 being “a very great deal”. Exclusion criteria: (1) patients with evidence of chronic urologic inflammation; (2) patients with uncontrolled narrow-angle glaucoma; (3) patients with recurrent urinary tract infection; (4) patients with significant stress incontinence; (5) patients with sexual dysfunction or an anatomic disorder of sexual function of their partner; (6) patients who recently had a major gynecological surgery; (7) patients with abnormal cervical smear results; (8) patients with a history of gynecological malignancy; (9) patients with uncontrolled hypertension; (10) patients with incomplete clinical data.

Cystoscopy

Cystoscopy was performed as a standard procedure in all the patients. A minimum of two bladder biopsies were taken approximately 2 cm superolateral to the ureteric orifices in all patients using cold cup biopsy forceps. Bladder biopsy specimens were sent in 10% formalin solution for histopathologic examination and immunohistochemistry (IHC).

Primary endpoints

The primary endpoints included improvement in OAB symptom severity (measured by the OAB-q SF) after 12 weeks of treatment. Clinic visits took place at 1 and 12 weeks. At baseline and week 12, all participants underwent cystoscopy and completed questionnaires including the OAB-q SF as well as a 3-day bladder diary.

Immunohistochemistry

All formalin-fixed bladder tissue samples were embedded in paraffin. The entire tissue samples were incubated with monoclonal mouse anti-human ERβ antibody (clone 14C8, Abcam®) for final IHC evaluation. IHC analyses were carried out on 4-μm-thick sections placed on tissue bond coated slides using immunoperoxidase method with respective antibodies as per manufacturer instructions. A total red score was used for semi-quantitative analysis of ERβ expression, considering both the proportion score and intensity score. Positively stained

Table 1. Baseline characteristics

Characteristic	Tolterodine (n = 30)	Intravaginal Estrogen (n = 28)	*t/F*	*p*
Years of age, mean ± SD	52.81 ± 9.92	51.88 ± 4.81	3.450	0.631
BMI (kg/m^2), mean ± SD	26.04 ± 4.61	24.34 ± 2.45	7.585	0.837
Serum Estradiol	40.16 ± 12.8	35.58 ± 9.04	12.444	0.124
Marital status, n (%)			15.481	0.130
Single/never married	3 (10.0)	7 (25.0)		
Married	27 (90.0)	21 (75.0)		
Comorbidity, n (%)				
Diabetes mellitus	1 (3.3)	1 (3.6)	8.749	0.961
Hypertension	2 (6.6)	1 (3.6)	1.547	0.595

BMI, body mass index.

target cells were graded on a scale of 0 to 4 based on the proportion score: 0 (none), 1 (1-10%), 2 (11-50%), 3 (51-80%), and 4 (81-100%). Similarly, the intensity (staining intensity) scores were graded on a scale of 0 to 1 based on the score: 0 (no staining), 1 (weak staining), 2 (intermediate), and 3 (strong). The two scores were added to obtain a final score. If it was less than or equal to 2 then this was considered a negative score and a score over 2 was considered positive. Under 40× magnification, areas with the highest intensity were identified. Subsequently, under 400× magnification, the percentage of ERβ was calculated by counting positive stained cells in 100 examined cells. A total of 5 serial sections comprising epithelial tissue were examined and then the mean score (in the form of either positive or negative) was calculated for each specimen (ERβ in epithelium).

Statistical analysis

Statistical analysis was performed with SPSS version 26.0. Fisher's exact test and the chi-squared test were used to evaluate the difference between categorized variables, and a *p*-value of < 0.05 was deemed significant. The categorical data were presented as numbers (percentage) and were compared using the Chi-square test and Odds ratio (95% confidence interval). The quantitative data were presented as mean and standard deviation (SD) and were compared using a Student's *t*-test. Relationships between the quantitative variables were assessed by using Pearson's correlation coefficient. $P < 0.05$ was considered significant.

Results

General clinical information

A total of 65 eligible women (33 premenopausal, 32 postmenopausal) were included between April 2017 and August 2019, with 28 patients in the tolterodine group and 30 patients in the topical vaginal estrogen group completed the 12-week study. Both groups were age-matched, and the serum hormone profile and body mass index were also comparable in both groups ($P > 0.05$). There were no significant differences in marital status and comorbidity between the two groups ($P > 0.05$, **Table 1**).

Analysis of OAB-q SF scores

There were no significant differences in OAB-q SF scores and urgency incontinence episodes between the two groups at baseline (**Table 2**). After 12 weeks of treatment, the differences were in OAB-q SF score (46.7 ± 23.4 vs. 45.4 ± 21.0, $P = 0.84$) and UI episodes (-1.2 ± 8.3 vs. -1.6 ± 7.2, $P = 0.26$) were not significant between the tolterodine and intravaginal estradiol groups (**Table 2**). However, both groups reported reduced OAB-q SF scores after 12 weeks of treatment compared to baseline (tolterodine, $P < 0.0001$; intravaginal estradiol, $P = 0.002$, **Table 2**). In addition, within-group analyses showed a significant decrease in UI episodes in the tolterodine group but not in the intravaginal estradiol group (tolterodine, $P = 0.03$; intravaginal estradiol, $P = 0.26$, **Table 2**).

Immunohistochemistry of ERβ

Immunohistochemistry was performed to examine the expression of ERβ protein on selected

Table 2. Analysis of OAB-q SF scores and changes from baseline to 12 weeks (mean ± SD)

Item	Tolterodine (n = 30)	Intravaginal Estradiol (n = 28)	*t/F*	*p*
Baseline				
OAB-q SF score	65.48 ± 15.37	61.61 ± 20.04	19.375	0.421
UI episodes	10.42 ± 11.64	16.21 ± 10.54	4.594	0.554
12-week Follow-up				
OAB-q SF score	46.71 ± 23.42*	45.44 ± 21.01*	4.058	0.815
p	0.025	0.028		
t/F	5.982	7.531		
UI episodes	-1.27 ± 8.34#	-1.64 ± 7.22	7.251	0.854
p	< 0.014	0.267		
t/F	3.597	6.914		

OAB-q SF, Overactive Bladder questionnaire short form; UI, urinary incontinence. **Compared to the 12-week follow-up OAB-q score within-group; #Compared to the 12-week follow-up UI episodes within-group. All reported P values are paired t test P values.*

tissues. ERβ staining in the squamous epithelium of the bladder trigone (**Figure 1A**) and dome (**Figure 1B**) is presented. However, ERβ was uniformly expressed in all nuclei in bladder transitional epithelium (**Figure 1C**) while underlying stromal cell nuclei showed no immunoreaction (**Figure 1D**). There was no difference in ERβ staining between the tolterodine and intravaginal estradiol groups at baseline ($P > 0.05$, **Table 3**). ERβ was positive in 14 of 30 patients in the tolterodine group (47%; 12 weak and 2 intermediate), and was positive in 14 of 28 patients (50%; 11 weak, 2 intermediate, and 1 strong) in the vaginal estradiol group. Follow-up after 12 weeks of medication, ERβ expression was significantly increased in intravaginal estradiol groups (**Figure 1E**) vs. tolterodine group (**Figure 1F**; **Table 3**). No significant changes were found in ERβ expression in the tolterodine group after 12 weeks of treatment ($P > 0.05$). There, ERβ was positive in 13 of 30 patients in the tolterodine group (43%; 13 weak), while was positive in 23 of 28 patients (73%; 13 weak, 8 intermediate and 2 strong) in the vaginal estradiol group, indicating that the expression score and quantity of ERβ in the vaginal estradiol group were increased. In addition, compared with baseline, ERβ expression also changed significantly after 12 weeks of estrogen treatment ($P < 0.01$, **Table 3**).

Comparison of adverse effects

Adverse reactions to oral tolterodine were observed in 16 cases, resulting in an overall adverse reaction rate of 53%, and were primarily characterized by mild toxic anticholinergic effects, with dry mouth being the most common. Adverse reactions to intravaginal estrogen occurred in 7 cases, with an overall adverse reaction rate of 25%, mainly characterized by irregular vaginal bleeding and local infection (**Table 4**).

Comparison of urodynamic parameters

Urodynamic examination of both groups after 12 weeks of treatment revealed that use of intravaginal estrogen did not differ from oral Tolterodine in terms of maximum urinary flow rate, mean uroflow rate, urination time, and maximum detrusor pressure. However, there was a significant difference in maximum bladder capacity, $P < 0.05$, probably due to the enhanced bladder capacity expansion caused by the anticholinergic effect induced by Tolterodine (**Table 5**).

Discussion

In this study, we explored the expression of ERβ in female OAB patients after topical estrogen therapy. It was found that topical estrogen therapy improved the symptoms of OAB and increased the expression of ERβ.

A number of studies have confirmed that female patients are more susceptible to OAB, which may be related to female physiologic characteristics [17, 18]. However, the etiology of OAB is still unclear and might be multifactorial. The occurrence of OAB is associated with endocrine disorders [19]. Zhu et al. suggested that estro-

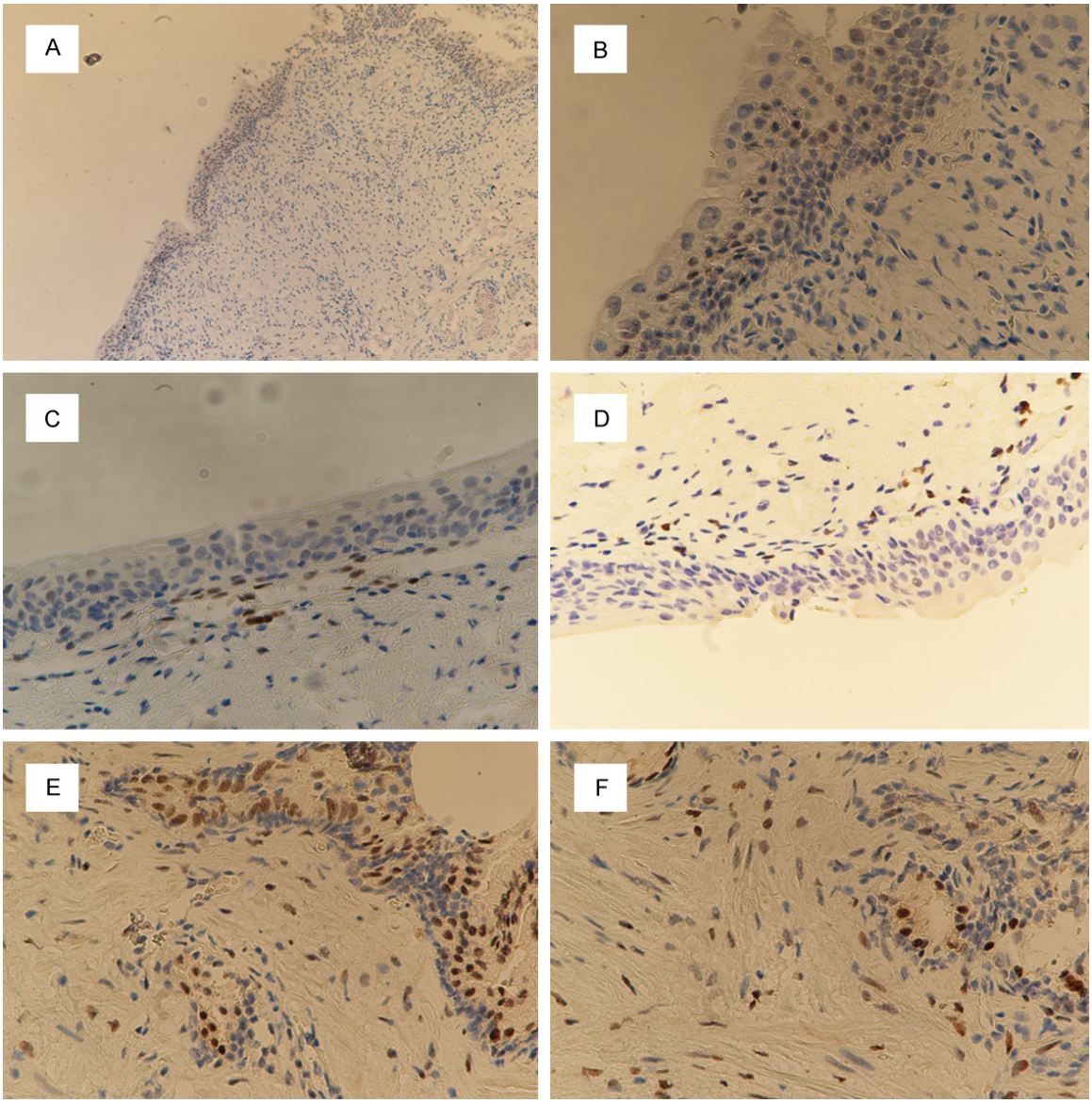

Figure 1. Immunohistochemical staining of ERβ protein in squamous epithelium in the bladder trigone (A) and dome (B). Positive immunoreaction is indicated by brown color. ERβ was mostly confined to nuclei, but cytoplasmic staining was seen in all transitional epithelial cells (C), while underlying stromal cell nuclei showed no immunoreaction (D). Follow-up after 12 weeks of medication, ERβ expression was significantly increased in intravaginal estradiol groups (E) compared with that in tolterodine group (F). Immunonegative structures show the counterstain only (indicated by blue color). (A) was captured at ×100 magnification, (B-F) were captured at ×400 magnification. ERβ, estrogen receptor β.

gen status could directly affect bladder function [20]. The decrease in estrogen after the beginning of the post-menopausal period and pregnancy is believed to lead to the onset of OAB [21]. Estrogen therapy can reverse increase functional bladder capacity and reduce micturition frequency in ovariectomized rats, suggesting that abnormal estrogen may cause frequent urination and dysuria [22]. In addition, estrogen can also improve the nutritional status of the urethra and bladder mucosa, increase blood flow around the urethral mucosa, and elevate the maximum closed pressure of the urethral sphincter [23].

Previous studies have shown that ERβ is much more widely expressed than ERα, and ERβ plays an important role in the development and

Table 3. ERβ expression in the tolterodine and intravaginal estradiol groups and changes from baseline to 12 weeks

Group	ERβ expression				12-week Follow-up ERβ expression				*t/F*	*p*
	0 (%)	1 (%)	2 (%)	3 (%)	0 (%)	1 (%)	2 (%)	3 (%)		
Tolterodine (n = 30)	16 (53)	12 (40)	13.542	2 (7)	0 (0)	17 (57)	13 (43)	0 (0)	0 (0)	0.165
Intravaginal Estrogen (n = 28)	14 (50)	11 (39)	6.821	2 (8)	1 (3)	5 (17)	13 (46)	8 (29)	2 (8)	< 0.014*
p		0.485				0.001				
t/F		12.587				1.685			*p (t/F)*	

ERβ, estrogen receptor β. **Compared to the 12-week follow-up within* Intravaginal Estrogen *group.*

Table 4. Comparison of adverse effects of the two treatment modalities

Adverse effect	Dry mouth	Dysuria	Vertigo	Xerophthalmia	Local inflammation	Enlarged breasts	Vaginal bleeding	Overall incidence
Tolterodine (n = 30)	8	3	2	1	2	0	0	53%
Intravaginal Estrogen (n = 28)	0	1	1	0	3	0	2	25%

Table 5. Comparison of urodynamic parameters between the two groups after treatment

Urodynamic	Qmax (mL/s)	Qave (mL/s)	Vt(s)	Pdet. Qmax (cmH_2O)	MCC (ml)	FS (ml)	BC (ml/cmH_2O)
Tolterodine (n = 30)	27.26 ± 4.43	12.62 ± 1.49	23.6 ± 4.9	32.5 ± 7.8	519.1 ± 81.6	225.3 ± 44.5	4.50 ± 0.73
Intravaginal Estrogen (n = 28)	25.70 ± 4.59	12.36 ± 1.66	23.4 ± 5.0	30.6 ± 7.2	479.6 ± 57.1	209.6 ± 42.1	4.66 ± 0.69
F	1.727	0.392	0.009	0.867	4.503	1.881	0.684
P	0.194	0.534	0.924	0.356	0.038*	0.176	0.412

**Compared to the 12-week follow-up within* Intravaginal Estrogen *group. Qmax: maximum urinary flow rate; Qave: mean uroflow rate; Vt: urination time; Pdet. Qmax: maximal detrusor pressure; MCC: maximum bladder capacity; FS: bladder initial sensing capacity; BC: bladder compliance.*

function of the bladder [24]. Studies have found that female urinary bladder mucosa mainly expresses ERβ [9, 10] in the bladder triangle and bladder dome. This indicates that ERβ is uniformly distributed in the nucleus of the bladder triangle and the squamous metaplasia of the bladder dome.

Antimuscarinic drugs are effective in treating OAB. In recent years, β3 receptor agonist mirabelon has received attention as a novel treatment for OAB [25]. Since OAB occurs predominantly in the elderly, complications should also be recognized, and the use of these drugs in elderly patients is strictly limited because of possible adverse reactions. With the extension of medication time, patients' compliance with anti-muscarinic drugs (mouthfeel, constipation, dysuria, etc.) also decreased [26]. Mirabillon is not suitable for elderly patients with uncontrolled hypertension and severe heart disease [27]. This study employed estrogen to treat OAB patients because the occurrence of OAB in women is related to estrogen deficiency, and the efficacy of estrogen in treating urgency/frequency symptoms of OAB patients has been confirmed in previous studies [13]. Previous animal studies have also shown that estrogen can reduce the frequency and amplitude of detrusor contractions, thereby promoting detrusor relaxation [28]. Postmenopausal women can use estrogen to improve symptoms of urgency, frequent urination, and nocturia [29]. On the one hand, estrogen improves urogenital tract blood supply [30], and on the other hand, we speculate that it may be associated with the ERs in the bladder mucosa. Estrogen has attracted attention as a possible way to treat OAB symptoms. Estrogen may act on the bladder and, in turn, indirectly regulate urothelial bladder sensory-motor function or increase the expression of ERs, as observed in Syrian hamsters [31]. Nelken et al. compared the use of estradiol vaginal rings and oxybutynin in the treatment of OAB and reported that the daily voiding rate was significantly reduced in both groups, but there was no significant difference between the two groups [32]. Tseng et al. con-

ducted a randomized controlled trial in 80 women, comparing the group treated with tolterodine alone with the group treated with tolterodine and intravaginal estrogen cream [33]. The tolterodine plus intravaginal estrogen group showed a significant improvement in the mean day frequency and urine output after treatment, but there were no significant differences in terms of nocturia and urgency. Given that our study showed no difference in the improvement of symptoms between the two groups, topical vaginal estrogen therapy may be an effective and well-tolerated alternative to anticholinergic treatment for female OAB.

Previously observed fluctuations in the menstrual cycle of urinary symptoms may be mediated by ERβ [34]. In this study, while the OAB symptoms were improved in patients treated with estrogen, we observed that the expression of ERβ in the bladder epithelium changed, indicating that estrogen may directly regulate epithelial cells. Thus, exogenous estrogen has an impact on bladder filling and voiding sensation to some extent. Our data indicated that ERβ plays a crucial role in this functional aspect. Interestingly, estrogen stimulation on uterine smooth muscle nerve regeneration through mechanisms dependent on ER indicates that the bladder may be subject to similar control mechanisms [35]. By employing IHC semi-quantitative analysis to assess ERβ expression, we can gain insights into the regulation of ERβ function by estrogen and help understand the biological significance of ERβ in OAB. RhoA/ROCK is a calcium-sensitive pathway involved in mediating changes in bladder contraction, and ERβ is involved in regulating this pathway to improve OAB symptoms [36]. Our data showed that ERβ expression significantly elevated in bladder mucosa after topical vaginal estrogen therapy, and we speculate that increased ERβ expression may inhibit bladder contraction. Although the urinary tract function changes under the long-term effects of estrogen still need further research, our observations suggest that ERβ is an important target in the bladder for improving the subjective symptoms of patients with OAB by estrogen therapy.

There are some limitations in this study. First, the sample size is small. Second, this study is a single-center study. Third, we did not explore the mechanism of the effect of local estrogen therapy on bladder ERβ expression in female OAB patients. Therefore, in future studies, we should execute a multicenter study with a large sample size to validate the results of this study and explore the possible mechanisms.

Conclusion

Topical estrogen therapy can significantly improve the subjective symptoms of OAB, and the effect is no less than that of tolterodine. The possible mechanism is that estrogen supplementation can significantly increase the expression of ERβ in the bladder mucosa.

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Written informed consent was obtained from all patients.

Disclosure of conflict of interest

None.

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References

[1] Lightner DJ, Gomelsky A, Souter L and Vasavada SP. Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: AUA/SUFU guideline amendment 2019. J Urol 2019; 202: 558-563.

[2] Chuang YC, Liu SP, Lee KS, Liao L, Wang J, Yoo TK, Chu R and Sumarsono B. Prevalence of overactive bladder in China, Taiwan and South Korea: results from a cross-sectional, population-based study. Low Urin Tract Symptoms 2019; 11: 48-55.

[3] Coyne KS, Wein AJ, Tubaro A, Sexton CC, Thompson CL, Kopp ZS and Aiyer LP. The burden of lower urinary tract symptoms: evaluating the effect of LUTS on health-related quality of life, anxiety and depression: EpiLUTS. BJU Int 2009; 103 Suppl 3: 4-11.

[4] Bartoli S, Aguzzi G and Tarricone R. Impact on quality of life of urinary incontinence and overactive bladder: a systematic literature review. Urology 2010; 75: 491-500.

[5] Vaughan CP and Markland AD. Urinary incontinence in women. Ann Intern Med 2020; 172: ITC17-ITC32.

[6] Kurita T, Wang YZ, Donjacour AA, Zhao C, Lydon JP, O'Malley BW, Isaacs JT, Dahiya R and Cunha GR. Paracrine regulation of apoptosis by steroid hormones in the male and female reproductive system. Cell Death Differ 2001; 8: 192-200.
[7] Van Geelen JM, Doesburg WH, Thomas CM and Martin CB Jr. Urodynamic studies in the normal menstrual cycle: the relationship between hormonal changes during the menstrual cycle and the urethral pressure profile. Am J Obstet Gynecol 1981; 141: 384-92.
[8] Blakeman PJ, Hilton P and Bulmer JN. Oestrogen and progesterone receptor expression in the female lower urinary tract, with reference to oestrogen status. BJU Int 2000; 86: 32-38.
[9] Kontos S, Kominea A, Melachrinou M, Balampani E and Sotiropoulou-Bonikou G. Inverse expression of estrogen receptor-beta and nuclear factor-kappaB in urinary bladder carcinogenesis. Int J Urol 2010; 17: 801-809.
[10] Tuygun C, Kankaya D, Imamoglu A, Sertcelik A, Zengin K, Oktay M and Sertcelik N. Sex-specific hormone receptors in urothelial carcinomas of the human urinary bladder: a comparative analysis of clinicopathological features and survival outcomes according to receptor expression. Urol Oncol 2011; 29: 43-51.
[11] Raghunandan C, Agrawal S, Dubey P, Choudhury M and Jain A. A comparative study of the effects of local estrogen with or without local testosterone on vulvovaginal and sexual dysfunction in postmenopausal women. J Sex Med 2010; 7: 1284-1290.
[12] Eriksen PS and Rasmussen H. Low-dose 17 beta-estradiol vaginal tablets in the treatment of atrophic vaginitis: a double-blind placebo controlled study. Eur J Obstet Gynecol Reprod Biol 1992; 44: 137-144.
[13] Nappi RE and Davis SR. The use of hormone therapy for the maintenance of urogynecological and sexual health post WHI. Climacteric 2012; 15: 267-274.
[14] Smith AL and Wein AJ. Estrogen replacement therapy for the treatment of postmenopausal genitourinary tract dysfunction. Discov Med 2010; 10: 500-510.
[15] Long CY, Liu CM, Hsu SC, Wu CH, Wang CL and Tsai EM. A randomized comparative study of the effects of oral and topical estrogen therapy on the vaginal vascularization and sexual function in hysterectomized postmenopausal women. Menopause 2006; 13: 737-743.
[16] Coyne K, Revicki D, Hunt T, Corey R, Stewart W, Bentkover J, Kurth H and Abrams P. Psychometric validation of an overactive bladder symptom and health-related quality of life questionnaire: the OAB-q. Qual Life Res 2002; 11: 563-574.
[17] Archer JS. NAMS/Solvay Resident Essay Award. Relationship between estrogen, serotonin, and depression. Menopause 1999; 6: 71-78.
[18] Milsom I, Coyne KS, Nicholson S, Kvasz M, Chen CI and Wein AJ. Global prevalence and economic burden of urgency urinary incontinence: a systematic review. Eur Urol 2014; 65: 79-95.
[19] Ho CH, Chang TC, Guo YJ, Chen SC, Yu HJ and Huang KH. Lower urinary tract symptoms and urinary flow rates in female patients with hyperthyroidism. Urology 2011; 77: 50-54.
[20] Zhu Q, Ritchie J, Marouf N, Dion SB, Resnick NM, Elbadawi A and Kuchel GA. Role of ovarian hormones in the pathogenesis of impaired detrusor contractility: evidence in ovariectomized rodents. J Urol 2001; 166: 1136-41.
[21] Weber MA, Kleijn MH, Langendam M, Limpens J, Heineman MJ and Roovers JP. Local oestrogen for pelvic floor disorders: a systematic review. PLoS One 2015; 10: e0136265.
[22] Cheng CL and de Groat WC. Effects of agonists for estrogen receptor α and β on ovariectomy-induced lower urinary tract dysfunction in the rat. Am J Physiol Renal Physiol 2014; 306: F181-F187.
[23] Legendre G, Ringa V, Fauconnier A and Fritel X. Menopause, hormone treatment and urinary incontinence at midlife. Maturitas 2013; 74: 26-30.
[24] Taylor AH and Al-Azzawi F. Immunolocalisation of oestrogen receptor beta in human tissues. J Mol Endocrinol 2000; 24: 145-155.
[25] Nitti VW, Auerbach S, Martin N, Calhoun A, Lee M and Herschorn S. Results of a randomized phase III trial of mirabegron in patients with overactive bladder. J Urol 2013; 189: 1388-95.
[26] Wagg A, Compion G, Fahey A and Siddiqui E. Persistence with prescribed antimuscarinic therapy for overactive bladder: a UK experience. BJU Int 2012; 110: 1767-1774.
[27] Kuo HC, Lee KS, Na Y, Sood R, Nakaji S, Kubota Y and Kuroishi K. Results of a randomized, double-blind, parallel-group, placebo- and active-controlled, multicenter study of mirabegron, a β3-adrenoceptor agonist, in patients with overactive bladder in Asia. Neurourol Urodyn 2015; 34: 685-92.
[28] Matsubara S, Okada H, Shirakawa T, Gotoh A, Kuno T and Kamidono S. Estrogen levels influence beta-3-adrenoceptor-mediated relaxation of the female rat detrusor muscle. Urology 2002; 59: 621-625.
[29] Zullo MA, Oliva C, Falconi G, Paparella P and Mancuso S. Efficacy of estrogen therapy in urinary incontinence. A meta-analytic study. Minerva Ginecol 1998; 50: 199-205.

[30] Matarazzo MG, Cianci S, Rampello L, Presti LL and Caruso S. Urethral sphincter innervation and clitoral blood flow after the transobturator (TOT) approach. Int Urogynecol J 2013; 24: 621-625.
[31] Lin YC, Talley DJ and Villee CA. Increased progesterone receptor concentrations in bladder lesions of estrogen-treated Syrian hamsters. Cancer Res 1979; 39: 2614-2617.
[32] Nelken RS, Ozel BZ, Leegant AR, Felix JC and Mishell DR Jr. Randomized trial of estradiol vaginal ring versus oral oxybutynin for the treatment of overactive bladder. Menopause 2011; 18: 962-6.
[33] Tseng LH, Wang AC, Chang YL, Soong YK, Lloyd LK and Ko YJ. Randomized comparison of tolterodine with vaginal estrogen cream versus tolterodine alone for the treatment of postmenopausal women with overactive bladder syndrome. Neurourol Urodyn 2009; 28: 47-51.
[34] Hextall A, Bidmead J, Cardozo L and Hooper R. The impact of the menstrual cycle on urinary symptoms and the results of urodynamic investigation. BJOG 2001; 108: 1193-1196.
[35] Bjorling DE, Beckman M, Clayton MK and Wang ZY. Modulation of nerve growth factor in peripheral organs by estrogen and progesterone. Neuroscience 2002; 110: 155-167.
[36] Chavalmane AK, Comeglio P, Morelli A, Filippi S, Fibbi B, Vignozzi L, Sarchielli E, Marchetta M, Failli P, Sandner P, Saad F, Gacci M, Vannelli GB and Maggi M. Sex steroid receptors in male human bladder: expression and biological function. J Sex Med 2010; 7: 2698-2713.