

Immunohistochemical expression of hormonal receptors, collagen, elastin, and proteoglycans in genuine urinary incontinence

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Summary

Purpose: To study the expression of hormonal receptors, collagen, elastin, proteoglycans, and VIP in the vaginal wall of women with stress urinary incontinence (SUI). **Materials and Methods:** Fifty-eight specimens of the anterior vaginal wall (28 women with SUI) were processed by Ventana immunostaining method. **Results:** Both groups were compatible for age, BMI, and obstetric history. Positive ER- α and ER- β immunoreaction was observed in 46.4% and 3.6% of SUI (43.3% and 33.3 % of controls) ($p < 0.05$), respectively, and PR immunoreaction in 39.3% of SUI (46.7% of controls). Collagen I and III immunoreaction was observed in 28.6% and 21.4 % of SUI (30.% and 36.7% of controls), respectively, and elastin, decorin, and fibromodulin immunoreaction in 10.7 %, 10.7%, and 10.7% of SUI (50%, 33.3%, 33.3% of controls) ($p < 0.05$), respectively. VIP immunoreaction was observed in 7.1% of SUI (36.7% of controls). **Conclusion:** Immunoexpression of ER- β , elastin, decorin, fibromodulin, and VIP was significantly lower in SUI than controls, showing that the ER- β dependent re-modeling of the extracellular matrix of vaginal tissues is the main mechanism of SUI.

Key words: Incontinence; Hormones; Collagen; Elastin; Proteoglycans.

Introduction

Genuine stress urinary incontinence (SUI) is defined as the involuntary loss of urine in conditions that increase intra-abdominal pressure such as coughing, laughing, lifting weight, *et al.* [1]. The pathophysiology of genuine SUI includes deficits in the supporting tissues of pelvic floor, mainly of collagens and elastin, factors important in maintaining the elasticity of pelvic floor (hammock hypothesis) [2]. Proteoglycans, such as decorin and fibromodulin, interact with the collagen and elastic fibers to form a network supporting the fibers in place [3-5].

Vasoactive intestinal peptide (VIP) is a neurotransmitter, component of a group of molecules responsible in changing the voltage of the supporting tissues and its deficiency contributes to the pathogenesis of SUI [6].

Estrogens and progesterone are important in various functions of the lower urinary tract via their receptors. Estrogen deficiency is considered to be a main factor of the atrophic changes of epithelial and stromal tissues of vaginal wall, producing SUI. Estrogen administration is considered as a therapeutic option in these cases [7].

There is evidence that the two subtypes of ER, ER- α , and ER- β , have different actions, and because of the fact that there is little information about the ER- β function, the present authors considered it important to study separately their

expression in the various vaginal wall components [8, 9].

The purpose of this study was to highlight the distribution and changes of the estrogen and progesterone receptors expression, by immunohistochemical method, and their relation with the distribution and changes of collagen, elastin, proteoglycans, and the VIP, at the vaginal wall of women with or without SUI.

Materials and Methods

Biopsy specimens from the anterior vaginal wall of 58 women were obtained: 30 specimen from women without SUI who underwent surgery for benign disease, like hysterectomy for leiomyomas, and 28 specimens from women with SUI who underwent surgery therapy with tension-free vaginal tape. Definition of SUI was according to criteria of International Continence Society [10]. Both groups were compatible for age, BMI, and obstetric history. All women were postmenopausal, aged 55-70 years and had one to three children. Their BMI was between 18.5 to 30. Cases reporting hormone therapy, history of pelvic inflammatory disease, malignancy or previous surgical procedures were excluded from the study.

This research was approved by Aretaieion Hospital Research and Ethics Committee and informed consent was obtained from patients participating to this study. All cases were protected by special codes to protect the anonymity of patients.

After routine procedure sections from paraffin embedded tissues were stained by Hematoxylin-Eosin for basic study. Additional sections were obtained for immunohistochemical study by

Table 1. — Demographic characteristics of the two study groups.

	Group		<i>p</i>
	Non-SUI n=30 (n)	SUI n=28 (n)	
Age (years), mean (SD)	61.5 (4.1)	62.6 (4.2)	0.317 ⁺
Parity, median (IQR)	2 (2 - 2)	2 (1.5 - 2)	0.827 ⁺⁺
Parity (n)			0.974 [*]
1	7 (23.3)	7 (25)	
2	17 (56.7)	16 (57.1)	
3	6 (20.0)	5 (17.9)	
BMI (kg/m ²), mean (SD)	26.7 (2.1)	27.3 (1.6)	0.212 ⁺
BMI (kg/m ²) Normal	6 (20.0)	3 (10.7)	0.473 ^{**}
Overweight	24 (80.0)	25 (89.3)	

*Pearson's Chi-square test; **Fisher's Exact test; ⁺Student's *t*-test;

⁺⁺Mann-Whitney test.

Ventata semi-automatice immunostaining method. The authors used ER- α and ER- β antibodies by clone N19, PR antibodies (clone AB-52), for collagen, for elastin (monoclonal clone BA-4), and for decorin, fibromodulin, and VIP. Evaluation of results was made by semi-quantitative method and the authors considered as negative all cases where positive immunoreaction was observed in < 10% of cells and as positive when > 10 % of cells were stained.

Statistical analysis

Normal distributed variables are expressed with mean and standard deviation (SD), while variables with skewed distribution are expressed with median and interquartile range (IQR). Qualitative variables are expressed as absolute and relative frequencies. For the comparison of proportions, chi-square and Fisher's exact tests were used. If the normality assumption was satisfied for the comparison of means between two groups, Student's *t*-tests were used. Mann-Whitney test was used for the comparison of continuous variables between two groups when the distribution was not normal. All reported *p*-values are two-tailed. Statistical significance was set at *p* < 0.05 and analyses were conducted using SPSS statistical software (version 19.0).

Results

The present sample consisted of 58 women (28 with SUI and 30 without SUI). Demographic characteristics for the two study groups are presented in Table 1.

The mean age for the SUI group was 62.6 (SD = 4.2) years and for the non-SUI group was 61.5 (SD = 4.1) years (*p* = 0.317). The two study groups were similar in terms of parity and BMI. The proportion of overweight women was 89.3% in the SUI group and 80% in the non-SUI group.

The percentage of women with positive ER- α was 43.3% for women without incontinence and 46.4 % for women with incontinence. The proportion of women with positive ER- α and progesterone was not significantly different between the two groups. The percentage of women with positive ER- β was significantly higher in women without incontinence (33.3%) compared with participants with incontinence (3.6%) (*p* < 0.05). The percentage of women

Table 2. — Comparison of study parameters between the SUI and non-SUI group.

		Group		<i>p</i>
		Non-SUI n=30 n (%)	SUI n=28 n (%)	
ER- α	Negative	17 (56.7)	15 (53.6)	0.813
	Positive	13 (43.3)	13 (46.4)	
ER- β	Negative	20 (66.7)	27 (96.4)	0.004
	Positive	10 (33.3)	1 (3.6)	
Progesterone	Negative	16 (53.3)	17 (60.7)	0.571
	Positive	14 (46.7)	11 (39.3)	
Collagen I	Negative	21 (70)	20 (71.4)	0.905
	Positive	9 (30)	8 (28.6)	
Collagen III	Negative	19 (63.3)	22 (78.6)	0.203
	Positive	11 (36.7)	6 (21.4)	
Elastin	Negative	15 (50)	25 (89.3)	0.001
	Positive	15 (50)	3 (10.7)	
VIP	Negative	19 (63.3)	26 (92.9)	0.007
	Positive	11 (36.7)	2 (7.1)	
Decorin	Negative	20 (66.7)	25 (89.3)	0.039
	Positive	10 (33.3)	3 (10.7)	
Fibromodulin	Negative	20 (66.7)	25 (89.3)	0.039
	Positive	10 (33.3)	3 (10.7)	

with positive PR was 46.7% for women without incontinence and 39.3% for women with incontinence. Percentage of women positive to collagen I was 30.0% for women without incontinence and 28.6% for incontinent women. The percentages of women positive to collagen III was 36.7% for women without incontinence and 21.4% for women with incontinence. Elastin was found in 10.7% of incontinent women and in 50.0% of those without incontinence (*p* < 0.05).

The percentage of women without incontinence positive with decorin and fibromodulin was 33.3% and 33.3 %, and 10.7% and 10.7%, respectively, for incontinent women (*p* < 0.05). Women without incontinence who had positive VIP were 36.7% compared with 7.1% of women with incontinence. The comparison of study indexes between the SUI and non-SUI group is shown in Table 2. Graphic presentation of the results is shown in Figure 1.

Discussion

Urinary incontinence is a symptom that affects many women and especially after menopause. The pathophysiology of genuine SUI include deficits in the supporting tissues. Differences have been found in expression of estrogen receptors in vaginal wall of women with SUI according to the study of Xie *et al.* [11], less expression of ER- β in the present study, which explains the benefit in use of estrogen in therapy of SUI [12]. The concentration of elastin is described to be less in women with SUI, which shows the changes of the tissues that explain dysfunction of the elas-

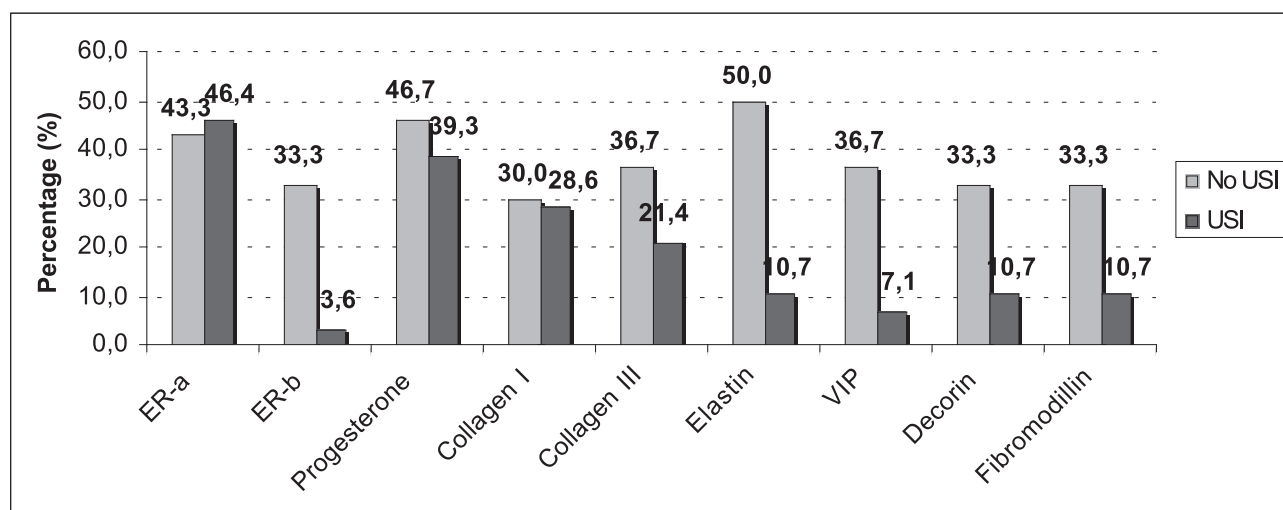


Figure 1. — Comparative expression of hormone receptors, collagens, elastin, and proteoglycans in normal subjects and women with SUI.

ticity of pelvic floor, which have been described by Goepel *et al.* [13]. The present findings regarding the alterations of proteoglycans, decorin, and fibromodulin, show the difficulty in demonstration and remodeling which in leads to abnormal forming of the tissues and loss of their mechanical role in women with SUI, which Chen *et al.* also reported [14]. The role of VIP as neurotransmitter, component of a group of molecules responsible in changing the voltage of the supporting tissues, was established by the finding of less expression in the tissue of women with SUI, as Wang *et al.* also showed by their study [15, 16].

Conclusions

The number of women with SUI who had positive ER- β was statistically significantly lower than the group of women without urinary incontinence. Participants with incontinence who had positive reaction to elastin were statistically significantly fewer compared with those without incontinence. The samples of women without urinary incontinence with positive reaction to proteoglycans (decorin and fibromodulin) were statistically significantly more compared to samples from women with SUI. VIP was detected statistically significantly in more samples of women without stress SUI.

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