Do Oestrogen and Progesterone Receptors ın Anterior Vaginal Wall Affect Success of Sling Procedures?

Turgay Akgül[1], Ali Ayyıldız[1], Bariş Nuhoğlu[1], Firdevs Şahin[2], Hüseyin Üstün[2] and Cankon Germiyanoğlu[1]
Ministry of Health Ankara, Training and Research Hospital, Second Urology Clinic [1] and Department of Pathology [2], Ankara, Turkey

Abstract

Objective: To investigate the expression of oestrogen receptors (ER) and progesterone receptors (PR) in anterior vaginal wall of women with stress urinary incontinence (SUI) and find out the effects of these expressions on histopathological and clinical parameters and on the success of surgical treatment.
Methods: Forty-two women with SUI were enrolled in the study. Twenty-one patients were menopausal with a mean age of 61.38 ± 7.54 (52-74 years) and twenty-one patients were in fertile period with a mean age of 43 ± 5.93 (32-53 years). All women underwent pubovaginal sling for SUI and the biopsies were taken from anterior vaginal wall.
Results: There was a significant difference in age between two groups (p=0.0001). Histopathological features were epithelial hyperplasia, subepithelial fibrosis and lymphoplasmocyt infiltration. The expressions of ER and PR were significantly higher in vaginal walls of fertile group (p=0.016 and p=0.038 respectively). The overall success rate was 86% after sling procedure. Six patients without ER and PR expressions in anterior vaginal wall did not show symptomatic recovery.
Conclusion: ER and PR expressions in anterior vaginal are related with age. Also there is a significant difference in expression rate between fertile and menopausal women. The expressions of ER and PR in anterior vaginal wall may play a role in prognosis of surgical procedures like sling.

Key Words: Oestrogen receptor, progesterone receptor, stress urinary incontinence, sling [Trop J Obstet Gynaecol, 2006, 23:105-109]

Introduction

Stress urinary incontinence (SUI) is a disease which results urinary leakage when the abdominal pressure is increased. The prevalence of SUI has been reported to range from 10 and 58.4% [1].

Urethral hypermobility and intrensec sphincter deficiency are pathophysiological factors that lead to SUI and it has been reported that 80-90% of patients with SUI have the disease as a result of hypermobility [2]. As the incidence of SUI increases significantly in postmenopausal women, oestrogen deficiency might be, at least in part, responsible for this condition [3]. Supporting the urethra and bladder base is one of the functions of anterior vaginal wall [4] and relaxation of anterior vaginal wall due to lack of oestrogen may result in incontinence and prolapse [5-8]. Therefore, it is important to investigate the expression of estrogen receptors (ER) in anterior vaginal wall.

The aim of the present study was to investigate the expression of ER and progesterone receptors (PR) in the vaginal wall of menopausal women and of fertile women with SUI and find out the effects of these expressions on histopathological and clinical parameters and on the success of surgical treatment.

Materials and Methods

After obtaining the approval of the local ethics committee, forty-two women with SUI who applied to our outpatient clinics between December 2004 and December 2005 were enrolled in the study. Main criteria for selection were; visible SUI and urethral hypermobility at the physical examination, absence of acontractile bladder or obstruction**.** Twenty one patients were menopausal (mean age 61.38 ± 7.54, 54-74 years) and twenty one patients were in fertile period with regular menstruation (mean age 43 ± 5.93, 32-51 years). In fertile group only 2 patients (9%) had a prior anti-incontinence surgery and in menopause group 4 patients (19%) had undergone anti-incontinence procedures. There were 3 patients (14%) with pelvic organ prolapse (POP) grade 1 in fertile group and 3 patients (14%) in menopause group. The stage of POP was determined on the basis of the International Pelvic Organ Prolapse Quantitative (POP-Q) system which is provided by International Continence Society [9]. None of the patients in both groups were taking hormone replacement treatment (HRT) or contraceptives. Patients with POP grade II or greater and neurological disorders were excluded. SUI was defined by gynecologic examination, stress test, Bonney test, pad test, urine culture and urodynamic examination. The preoperative urodynamic investigation consisted in a cystometry and urine flow rates. Valsalva leak point pressure, post-void residual urine and other data were recorded. Urge incontinence was present in 33 patients (33/42, 78%) in both groups. None of women in the study showed any signs of urinary infection or oestrogen-related diseases (endometriosis, myoma, or

Correspondence: *Turgay Akgül, MD*
11.Sokak 18/4 06500 Bahçelievler, Ankara / Turkey
Fax: *+ 903122402966* ***GSM:*** *+ 905052293859*
E-mail: *turgayakgul@gmail.com Turgayakgul@yahoo.com*

ovary tumor). The fertile women were operated on at the middle of their menstrual cycle.

All women underwent pubovaginal sling for SUI and the biopsies were taken from the anterior vaginal wall covering the mid-urethra by one senior urologist. Biopsy samples were 0.5 cm x 0.5 cm x 0.5 cm sized. The patients were informed and obtained permission for our study.

Pathology

Specimens

Tissue samples which were collected from anterior vaginal wall were immediately fixed in 10% formol saline and embedded in paraffin wax. The sections were prepared and stained with hematoxylin and eosin for the assessment of epithelial hyperplasia, subepithelial fibrosis and lymphoplasmocyt infiltration under Nikon® Optiphot light microscope (Figure 1).

Immunohistochemistry

After embedding samples in paraffin wax, 5µm sections were mounted onto poly-L lysine (PLL) coated slides. These sections were allowed to wait at 56° C for 12 hours. They were then deparaffinized and taken into distill water after passing ethanol chain from high to low density in 20 minutes.

Before application of oestrogen and progesterone receptor antibodies antigen retrieval procedure is performed. Trisodium citrate buffer solution (antigen retrieval solution) which is 0.01 molar and has pH of 6 is prepared for this purpose. The sections were taken into this solution and boiled in a pressurized container for 20 minutes. After cooling for 20 minutes at room temperature, the sections are taken in phosphate-buffered saline solution (PBS, pH=7.6). The endogenous peroxidase activity was quenched with 0.3 % H_2O_2 in PBS for 20 minutes. The non-specific bindings were quenched with Ultra V Block Nonspecific blocking for 10 minutes. The sections were washed with PBS and Estrogen and Progesterone antibodies (Lab vision Corporation, USA) were added to the sections. Sections were rinsed with PBS and then biotinylated goat anti-mouse antibodies (1.5 mg/ml, Vector Laboratories) were added at dilution of 1:1000 for 20 minutes. Streptavidine peroxidase added to the sections for 20 minutes and after rinsing with PBS, buffer solution was used as the chromogen. The sections were then counterstained with hematoxylin and then mounted.

Table1. Patients' characteristics and comparison of two groups according to histopathological findings (p<0.05)

	Menopausal (n=21)	Fertile (n=21)	p
Age	61.38 + 7.54	43.00 + 5.93	0.0001
Gravidity	5.71	4.87	0.583
Parity	3.9	3.4	0.806
No. of vaginal delivery	3.8	3.2	0.461
No. pads used daily	3.8	3.2	0.666
Epithelial Hyperplasia	16(76%)	21 (100%)	0.060
Subepithelial Fibrosis	11 (52%)	12 (57%)	0.780
Lymphoplasmocyt Infiltration	14 (67%)	16(76%)	0.787
Estrogen Receptor	3 (14%)	12 (57%)	0.019
Progesteron Receptor	0 (0%)	6 (28%)	0.038

Table2. Comparison of ER and PR with histopathological findings and clinical parameters

	Age p	EH † p	SEF † p	LPI † p	ER ‡ p	PR ‡ p	UI ‡ p	POP‡ p
ER	0.040*	0.400	0.736	0.437	1	0.014*	0.769	0.653
PR	0.022*	0.463	0.817	0.221	0.007*	1	0.159	0.552

*According to One way ANOVA test p<0.05

†EH= Epithelial hyperplasia, SEF=Subepithelial fibrosis, LPI= Lymphoplasmocyt infiltration ‡ ER= Estrogen receptor, PR=Progesterone receptor, UI= Urge incontinence, POP= Pelvic organ prolapse

Figure 1.
Hematoxylin and eosin staining in epithelium cell (x 100)

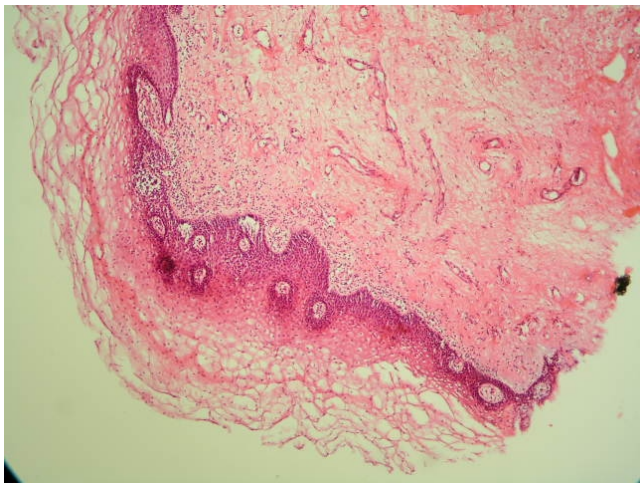


Figure 2.
ER positive immunohistochemical staining in epithelium cell (x 200)

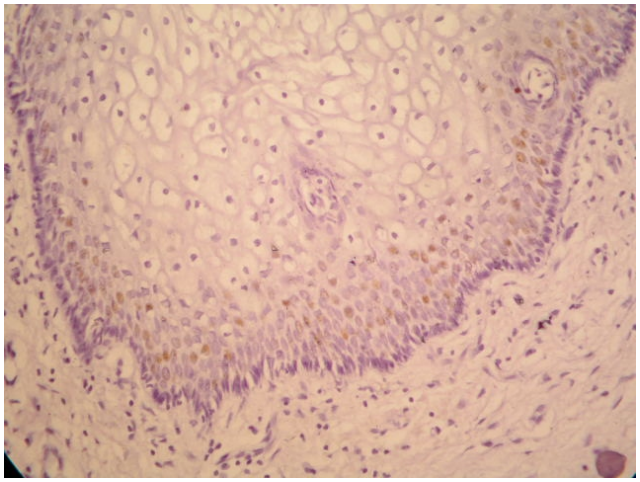
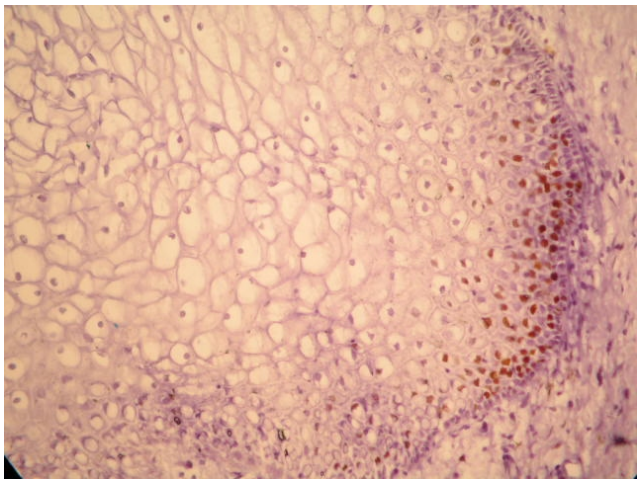


Figure 3.
PR positive immunohistochemical staining in epithelium cell (x 200)



Quantification
The sections were assessed for the distribution of nuclear staining. The staining penetration was assessed by counting epithelium cells in a randomized field (Figure 2, 3). The nuclear staining cells were counted by one blinded pathologist.

Statistical analysis
Statistical analyses were performed by the independent-samples T-test and One way ANOVA with SPSS software Windows version 11.5 (SPSS INC, Chicago, Ill, USA). *P*-values less than 0.05 were accepted as statistically significant.

Results
The two groups are compared according to the parameters shown in Table 1. Although the parameters such as gravidity, parity, number of vaginal delivery and number of pads used daily were similar in both groups, there was significant difference in age between the two groups (p=0.0001).

Under light microscopy, the specimens taken from the anterior vaginal wall were examined. Histopathological features were epithelial hyperplasia, subepithelial fibrosis and lymphoplasmocyt infiltration. The histopathological characteristics of the groups are seen in Table 2. The expressions of ER and PR were significantly higher in vaginal walls of fertile group than in menopausal group (p=0.016 and p=0.038 respectively).

ER expression had a significant association with age and PR expression in all patients. Also PR expression was found to be associated with age and ER expression similarly. However ER or PR expression had no association with other parameters such as epithelial hyperplasia, subepithelial fibrosis, lymphoplasmocyst infiltration, urge incontinence and POP (Table 2).

The overall success rate was 86 % (36 of 42 patients) after sling procedure. Success was defined as completely dry or leakage = 1/week [10]. Six patients (14 %) did not show symptomatic recovery after three months from the operation. Three of these patients were in fertile and the remaining three were in menopausal group. Four patients in this group had persistent mixed incontinence and two patients had persistent stress incontinence. No patient in this group had previously been exposed to anti-incontinence surgery. Histopathological examination of biopsy materials taken from these patients revealed that there was no ER or PR expression on anterior vaginal wall.

Discussion
The reasons for the onset of SUI vary. Pregnancy, birth, age, menopause, pelvic surgery, delivery procedures, constipation, chronic coughing and obesity are the

most factors that have been reported [11]. Oestrogen induces epithelial proliferation, stratification and cornification in vaginal epithelium [12]. The possible effects of menopause on SUI due to loss of oestrogen are; becoming of urinary mucous layer atrophied, decreasing of the submucosal vascular plexus in the urethra and lowering of the sensitivity in the urinary unstriated muscle for á- adrenergic stimulation [1]. The lost of oestrogen also is reflected physiologically in tissues with ERs. As the anterior vaginal wall is an important support of the incontinence mechanism, ER expressions in that tissue will be thought to be an appropriate indicator of HRT in the management of SUI. The effect of HRT in the treatment of SUI is not clear. However there have been some studies questioning the uses of HRT in incontinent menopausal women [13-14].

Fu et al have examined ER-á and ER-â expressions in anterior vaginal walls of 14 fertile women and of 18 menopausal women with SUI [15]. In this study, the authors reported a significantly higher expression of ER-á in vaginal walls of fertile women who were not using contraceptives than in menopausal SUI women not on HRT. Also they reported that ER-á was regulated by HRT in menopausal SUI women and contraceptives had no effect on the expression of ER in fertile SUI women. Although in another study, Lang et al [3] reported that there was no difference in ER values before and after menopause, the ER expression rate was significantly different between fertile and menopausal women in our study (p=0.016). Additionally we observed that; the expressions of ER and PR have associations with age independent from fertility or menopause.

PR is known to be expressed in vaginal tissue and the expression of PR is reported to increase when the fibroblasts of pelvic supportive tissue is exposed to 17 *â*-estradiol [16]. PR expression also showed a significant difference (p=0.038) between fertile and menopausal women like ER in our study. Although these two parameters, ER and PR, were significantly associated with each other and age, there was no association with histopathological parameters, urge incontinence and POP.

Bai et al compared the concentrations of ER and PR in the uterosacral ligaments between women with and without POP in their study [1]. Uterosacral ligaments were taken from 20 patients with stage III and stage IV POP and 24 patients who had no prolapse during surgeries. They have reported that ER and PR had been expressed significantly lower in patients who had POP than in those who did not. In our study, we did not find any association between ER and PR expressions and POP. This condition might be due to our patient selection. We only included the patients who had grade I POP different from Bai et al. Besides, the region where the ER and PR expressions examined was different also. In our study we preferred anterior vaginal wall instead of uterosacral ligaments. There may be regional differences on expressing receptors in POP. And at least the number of cases might be insufficient in Bai's and in our study.

In another study Zhu et al investigated ER in pelvic floor tissues which were obtained from 31 patients categorized into three groups: POP, SUI and control groups [17]. This study included postmenopausal women and a significant decrease of ER in the pelvic floor tissues of SUI group was reported. The authors suggest that this decrease of ER in pelvic tissues might be related to the occurrence of SUI. Also they suggest that hormone replacement therapy might not be effective in SUI.

The absence of control group in our study can be considered as a lack of study but in our study we aimed to compare the differences of ER and PR expressions in menopausal and fertile women with SUI. Besides, in our opinion, taking biopsy specimens from healthy women might be inappropriate due to ethics.

Our study's main finding is the failure of surgical treatment of SUI in women without ER and PR expression in anterior vaginal wall. As we know from the literature, no other studies had reported a relation like we found in our study. Although this relation is first reported, further studies with large number of patients are needed to investigate the relation between ER and PR receptors in anterior vaginal wall and the success of surgical treatments like pubovaginal or mid-urethral sling procedures. The better understanding of this possible association may lead clinicians to devýse better surgical treatment modalities for SUI.

References

1. Bai SW, Jung YW, Kwon HS, Yoon JM, Shin JS, Kim SK et al. The role of oestrogen receptor, progesterone receptor and p53 in development of stress urinary incontinence. *Yonsei Med J,* 2004; 45: 885-890
2. Menefee SA, Wall LL. Incontinence, Prolapse, and disorders of the Pelvic Floor. In: Jonathan SB, editor. *Novak's Gynecology.* 13th ed. Philadelphia: Lipincott Williams & Wilkins, 2002; 665-680
3. Lang JH, Zhu L, Sun ZJ, Chen SJ. Estrogen levels and oestrogen receptors in patients with stress urinary incontinence and pelvic organ prolapse. *Int J Gynecol Obstet,* 2003; 80: 35-39
4. Petros PE, Ulmsten U. Urethral pressure increase on effort originates from within the urethra, and continence from musculovaginal closure. *Neurourol Urodyn,* 1995; 14: 337-46

5. Winters JC, Cespedes RD, Vanlangendonck R. Abdominal sacral colpopexy and abdominal enterocele repair in the management of vaginal vault prolapse. *Urology,* 2000; 56: 55-63
6. Fatton B, Jacquetin B. Pelvic and perineal sequelae of delivery. *Rev Prat,* 1999; 49: 160-66
7. Bernier F, Jenkins P. The role of vaginal estrogen in the treatment of urogenital dysfunction in postmenopausal women. *Urol Nurs,* 1997; 17: 92-5
8. de Aloysio D, Altieri P, Penacchioni P, Mauloni M, Bottiglioni F. Premenopause-dependent changes. *Gynecol Obstet Invest,* 1996; 42:120-7
9. Bump RC, Mattiasson A, Bo K, Brubaker LP, DeLancey JO, Klarskov P et al. The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction. *Am J Obstet Gynecol,* 1996; 175:10-17
10. Kobashi KC, Govier F. The completely dry rate: a critical re-evaluation of the outcomes of slings. *Neurourol Urodyn,* 2005; 24: 602-5
11. Ballenger Ph, Rischmann P. Female Urinary Incontinence; An overview of a report presented to the French urological association. *Eur Urol,* 1999; 36:165-174
12. Buchanan DL, Kurita T, Taylor JA, Lubahn DB, Cunha GR, Cooke PS. Role of stromal and epithelial oestrogen receptors in vaginal epithelial proliferation, stratification and cornification. *Endocrinology,* 1998; 139: 4345-4352
13. Jackson S, Brookes S, Abrams P. The effect of oestrogen supplementation on post-menopausal urinary stress incontinence: a double blind placebo-controlled trial. *Br J Obstet Gynecol,* 1999; 106: 711-718
14. Grady D, Brown JS, Vittinghoff E, Applegate W, Varner E, Snyder T. Postmenopausal hormones and incontinence: the Heart and Estrogen/Progestin Replacement Study. *Obstet Gynecol,* 1999; 97: 116-120
15. Fu X, Rezapour M, Wu X, Li L, Sjögren C. Expression of oestrogen receptor- á and -â in anterior vaginal walls of genuine stress incontinent women. *Int Urogynecol J,* 2003; 14: 276-281
16. Moalli PA, Klingensmith WL, Meyn LA, Zyczynski HM. Regulation of matrix metalloproteinase expression by oestrogen in fibroblasts that are derived from the pelvic floor. *Am J Obstet Gynecol,* 2002; 187(1): 72-79
17. Zhu L, Lang J, Feng R, Chen J, Wong F. Estrogen receptor in pelvic floor tissues in patients with stress urinary incontinence. *Int Urogynecol J,* 2004; 15: 340-343