

The Role of Estrogen Receptor, Progesterone Receptor and p53 in Development of Stress Urinary Incontinence

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Recent studies have been reported the roles of the estrogen receptor (ER), progesterone receptor (PR) and p53 in the development of a pelvic organ prolapse (POP). The pathogenesis of stress urinary incontinence (SUI) is related to that of POP in the weakness of pelvic support. Therefore, this study was carried out to assess the relationship between ER, PR, p53 and the development of SUI, and to elucidate the biomolecular pathophysiology of SUI. The periurethral fascia was obtained from 6 menopausal patients diagnosed with SUI and 10 menopausal patients without SUI who visited the Department of Obstetrics and Gynecology, Severance Hospital, Seoul, Korea. The relative ER, PR and p53 protein levels in the periurethral fascia were obtained by western blot analysis and densitometry. A Mann-Whitney U test was used for statistical analysis ($p < 0.05$). The mean age (\pm SD) of the 16 patients was 59.0 ± 5.5 years (range, 50-74 years). The mean body mass index was 25.2 ± 2.7 kg/m² (21.8-30.8) and the average number of vaginal deliveries was 2.8 ± 1.9 (1.0-9.0). The ER level (0.33 ± 0.17 vs. 1.86 ± 0.83 , $p = 0.02$) and the p53 level (1.25 ± 0.67 vs. 4.71 ± 2.40 , $p = 0.01$) were lower in the experimental group. However, the PR level of the two groups were similar (0.64 ± 0.13 vs. 0.48 ± 0.33 , $p = 0.56$). The p53 and ER levels were significant lower in the study group. This suggests that p53 and ER might be important factors in the development of SUI. Further prospective studies about the association of ER, p53 and SUI will be needed to elucidate the molecular pathogenesis of SUI.

Key Words: Stress urinary incontinence, estrogen receptor, progesterone receptor, p53, pathogenesis

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INTRODUCTION

Stress urinary incontinence (SUI) occurs when urine is passed involuntarily when the abdominal pressure is increased, and its prevalence has been reported to range from 10 and 58.4%.¹⁻⁴ Recently, the prevalence of the disease has increased as a result of the aging society and the improvement in the quality of life.

The pathophysiology of SUI includes 1) urethral hypermobility and 2) a defect in the urethral sphincter. Urethral hypermobility means a disorder in the urethral obstruction in the body action due to attenuation in the pelvic floor supporting urethra, and it has been reported that 80-90% of patients with SUI have the disease as a result of hypermobility.⁵

The attenuation in the support of the pelvic floor serves as the main pathogenesis not only for SUI but also for pelvic organ prolapse (POP). Lang, et al.⁶ reported that patients with POP and SUI have lower expression levels of estrogen and the estrogen receptors of the serum and uterine ligament, and Yamamoto, et al.⁷ reported that the number of fibroblasts cultured in the cardinal ligament of POP patients were reduced in the p53 and p53 receptors. However, molecular biological studies on SUI are needed.

The aim of this study was to determine the roles of p53, the estrogen receptor, and the progesterone receptor in the onset of SUI to determine the molecular biological mechanism of the disease.

MATERIALS AND METHODS

Subjects

Of the menopausal patients who visited the Obstetrics and Gynecology Department of the Severance Hospital of Yonsei University between January 2002 and December 2002 and showed no physical findings of POP in a pelvic examination, 6 patients who had SUI as the main finding and underwent a sling operation under the definite diagnosis of SUI through a urodynamic study were enrolled in the experimental group, while 10 patients who visited the hospital as a result of a Bartholin's gland cyst without SUI were selected to be in the control group. The hospital's institutional review board approved this study. During surgery, 0.5 cm × 0.5 cm sized sampled the periurethral fascia in the inferior area of the bladder neck was taken.

A Western blot assay was performed for the estrogen receptor, the progesterone receptor, p53, and tubulin, which were expressed in the tissue of the periurethral fascia, the relative concentration of each protein was calculated using tubulin and densitometry. The results from the experimental and the control groups were compared.

Methods

Specimens

Five millimeters thick slices of the periurethral fascia were obtained from a part of the urethrovaginal junction. Tissue samples were collected either during the sling operation or during the Bartholin's gland cyst removal surgery. The samples were immediately fixed in 10% formal saline for 24 hours, embedded in paraffin wax, and 5 μm sections were mounted onto silane-coated slides and allowed to dry at 37°C for 48 hours. The sections were stained with hematoxylin and eosin (H&E) for a histological assessment of the ligamentous tissue. The local ethics committee approved the investigation protocol and all patients signed a consent form to allow the use of the tissues removed for research purposes.

Protein extraction

After PBS buffer (500 μl) had been added to the periurethral fascia obtained from the control group and experimental group, the tissue was ground and centrifuged at 5,000 rpm three times for five minutes. The washed tissue was placed into a lysis buffer 200 μl (NP-40 homogenization buffer, 1 mM PMSG, 1 μg/ml aprotinin & leupeptin) and was left on ice for 30 minutes. The tissue was then centrifuged at 13,000 rpm and 4°C for 10 minutes. In order to quantify the amount of protein in each sample, the absorbance was measured at 540 nm (UV-1601PC, Shimadzu). Bovine serum albumin (BSA) was used as the standard sample.

Western blot analysis

Using SDS-polyacrylamide gel electrophoresis (SDS-PAGE; 15%), 20 μg (from each group) or 40 μg of the protein was electrophoresed at 100V for 90 min. After electrophoresis, the gel was isolated, poured in a transfer buffer (48 mM Tris, 39 mM glycine, 20% methanol, 3.75 ml 10% SDS/L) for electroblotting, and left for 20 minutes. Meanwhile, the ImmobilonTM-P membrane (Milipore) for blotting was cut at the size of the gel, wet with methanol in order to give hygroscopicity, and poured in a transfer buffer. A semi-dry transfer system (Bio-Rad Trans-blot SD semidry transfer cell) was used for electroblotting, which was conducted at 15V for an hour. The membrane after blotting was sufficiently washed with tween-tris buffered saline (TTBS; 20 mM Tris-HCl pH 7.6, 137 mM NaCl, Tween-20 1 ml/L), and moved in a blocking buffer (5% skim milk in TTBS) in order to remove nonspecific reactions. The membrane was then reacted at room temperature for one hour. The membrane after blocking was reacted with a sufficiently diluted primary antibody overnight, washed with TTBS for 20 minutes three times to remove the primary antibody, and reacted with the secondary antibody (BD PharMingen Co., USA) at room temperature for one hour. The membrane was washed with TTBS for 20 minutes three times to remove the secondary antibody and TTBS. It was then colored using of an ECL kit (Amersham Pharmacia Co., England), and sensitized to a film.

Statistical analysis

After the relative concentration of the estrogen receptor, progesterone receptor, and p53 for tubulin in the periurethral fascia of each patient, the difference between the control and the experimental groups was compared using a Mann-Whitney U test with SPSS software windows version 10.0 (SPSS INC, Chicago, Ill, USA) ($p < 0.05$).

RESULTS

The mean age (\pm SD) of the 16 patients was 59.0 ± 5.5 years (range, 50 - 74 years). The mean body mass index was 25.2 ± 2.7 kg/m² (21.8 - 30.8) and the average number of vaginal deliveries was 2.8 ± 1.9 (1.0 - 9.0). The patients' characteristics in both groups are listed in Table 1.

In the control group, 6 out of 10 subjects (60%)

and 3 out of the 6 subjects (50%) in the experimental group were undergoing hormonal replacement therapy. The patient characteristics of the two groups were similar (see Table 1).

Western Blot was analysis for the estrogen receptor, progesterone receptor, p53, and tubulin protein expressed in the periurethral fascia, showed a shift in the relative concentrations using tubulin as a reference. When the concentrations of the estrogen receptor, the progesterone receptor, and p53 were compared, the expression level of the estrogen receptor (0.33 ± 0.17 vs. 1.86 ± 0.83 , $p=0.02$) and p53 (1.25 ± 0.67 vs. 4.71 ± 2.40 , $p=0.01$) were significantly lower in the experimental group than in the control group, the expression level of the progesterone receptor was similar in both groups (0.64 ± 0.13 vs. 0.48 ± 0.33 , $p=0.56$) (see Table 2, Fig. 1 and Fig. 2).

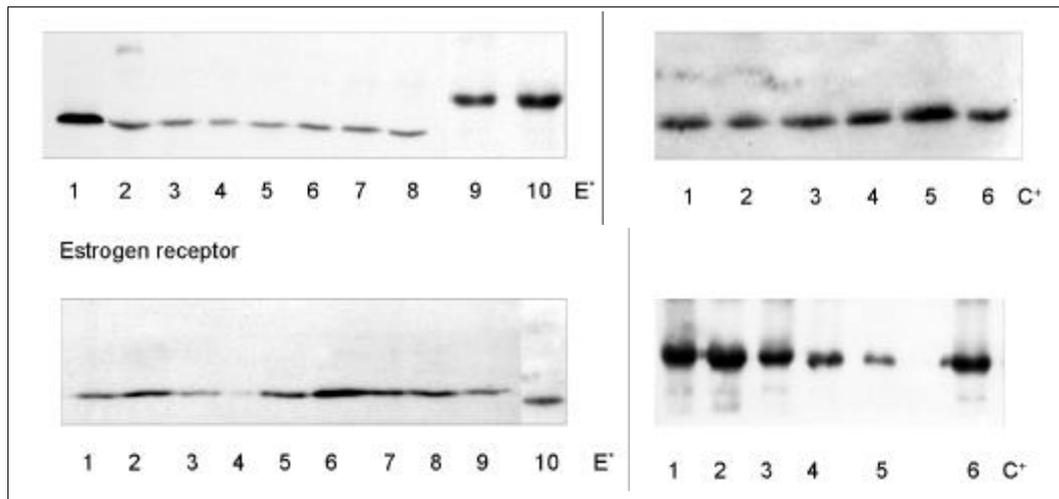


Fig. 1. Western blot analysis for ER and p53. p53; E*, Experimental group; C+, Control group.

Table 1. Patients' Characteristics

	Experimental group (N=6)	Control group (N=10)
Age (years)	60.2 ± 7.2	58.3 ± 4.5
No. of Vaginal delivery	3.3 ± 1.4	3.0 ± 0.7
BMI (Kg/m ²)	24.0 ± 1.7	25.9 ± 3.0
Menopause patients (%)	100% (4/4)	100% (10/10)
Patients receiving HRT (%)	50% (3/6)	60% (6/10)

BMI, Body Mass Index; HRT, Hormone replacement therapy.

Table 2. Mean ER, PR and p53 Value

	Experimental group	Control group	p-value
ER	0.33 ± 0.17 (N=6)	1.86 ± 0.83 (N=10)	0.02
PR	0.64 ± 0.13 (N=4)	0.48 ± 0.33 (N=5)	0.56
P53	1.25 ± 0.67 (N=6)	4.71 ± 2.40 (N=10)	0.01

ER, estrogen receptor; PR, progesterone receptor.

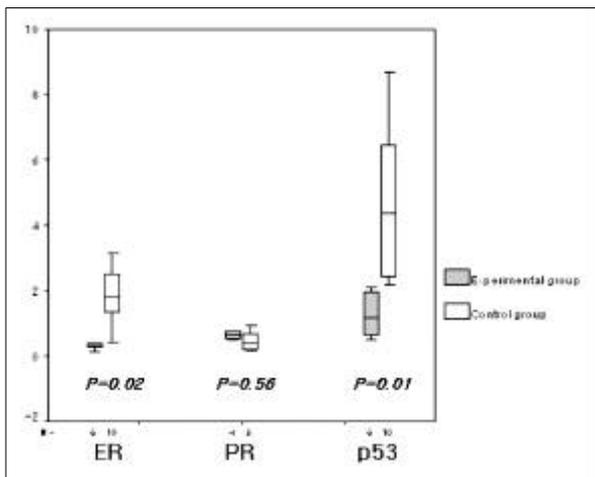


Fig. 2. The means and standard deviations of the western blot assay from the experimental and control groups.

DISCUSSION

The factors that affect the onset of SUI include pregnancy, birth history, menopause, age, nerve damage during pelvic surgery or a procedure associated with delivery, occupation, constipation, obesity, and chronic coughing.⁸ In this study, the patient characteristics in the two groups were similar.

Of the factors related to SUI, menopause serves as the important factor for the onset of SUI.⁹ for a possible mechanism for how menopause affects the onset of SUI, when estrogen is lost by menopause and the urogenital system including urinary mucous layer becomes atrophied, the submucous vascular plexus in the urethra is decreased and the sensitivity in the urinary unstriated muscle for α -adrenergic stimulation is lowered to exacerbate the SUI. Therefore, many studies have attempted to find a treatment for menopausal patients with SUI using hormonal

replacement therapy, and find the most effective hormonal replacement therapy for treating SUI patients.¹⁰⁻¹³ Accordingly, this study selected menopausal patients.

Such role of estrogen is expressed by the estrogen receptors distributed in the bladder trigone except the bladder dome, the proximal and distal parts of urethra, and the lower urinary tract such as the vagina.¹⁴ The estrogen receptor was first reported by Jensen et al. in 1962.¹⁵ Green et al. identified the estrogen receptor in the uterine tissue, and Mosselman et al. discovered that the estrogen receptor was different from the existing one. The previous receptor was called estrogen receptor- α while the new receptor was called estrogen receptor- β .^{16,17} Although the role of each receptor is unclear, it has been reported that the estrogen receptor- α might be more important in the control of reproduction than the estrogen receptor- β .¹⁸

In this study, the expression levels of the estrogen and progesterone receptors in the periurethral fascia of the patients with SUI, and the control group were compared using a western blot test. As shown in Table 1, the number of estrogen receptors in the experimental group were significantly lower, which corresponded to the report by Lang, et al. in which the expression of the estrogen receptor was decreased in the cardinal and uterosacral ligaments of the POP patients.⁶ Estrogen affects the target organ through the estrogen receptor, and in patients with SUI, the expression of the receptor was lower, and the affection of estrogen in the patients is believed to be lower than in the subjects in the control group. The reasons for the effect of hormonal replacement therapy on menopausal patients with SUI vary.^{12,13,19,20} It is possible that although the blood estrogen concentration is higher, the estrogen

receptors of the target organ are lower and the affection of estrogen on the target is also lower. While such results have been applied clinically, further studies on the effect of hormonal replacement therapy on the expression of the estrogen receptor of the patients with SUI are needed, which would be expected to reduced the unnecessary performance of this therapy.

Meanwhile, the progesterone receptor was detected in only 9 of the subjects (9/16), and such results corresponded to those of the other studies on the expression of progesterone receptor in the women's lower urinary tract.¹⁴ The results of the comparison between the five subjects in the control group and the four in the experimental group showed that there was no significant difference in the expression of the receptor between the two groups. Therefore, the role of progesterone in hormonal replacement therapy using combined agents is not believed to be important. Fantl, et al. and Grady et al. reported that hormonal replacement therapy using of combined agent for the treatment of urinary incontinence had no effect.^{12,21}

Strasser et al. reported that a reduction in the rhabdosphincter cells in the urethra as a result of apoptosis due to aging served as a cause of SUI.²² Yamamoto et al. reported that p53 was reduced in the cardinal ligament of the POP patients and because of the consequent proliferation of fibroblasts and the reduction in the elastin level, the supporting function of the uterine connective tissue was lost.⁷ In this study, the p53 was significantly lower in the patients with SUI than in the control group. Further studies will be needed to determine if such results can be applied to the periurethral fascia of SUI, which has a similar level of fibroblasts proliferation and reduction in elastin, as suggested as the mechanism of POP by Yamamoto et al.

In this study, the reduction in the estrogen receptor and p53 levels in the periurethral fascia was associated with SUI. However, it was difficult to conclude that menopause or hormonal replacement therapy served as the risk factor for SUI. This is because the number of the subjects was small. Further studies with a larger number of patients will be needed. In addition, further studies will be needed to identify the precise

course or mechanism for how the reduction in the levels of the p53 and estrogen receptor in the periurethral fascia serves as the cause of SUI and how the blood estrogen concentration and estrogen receptor level in the periurethral fascia are associated with the disease.

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