

The presence and location of estrogen and progesterone receptors in the human pelvic cardinal ligaments

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Abstract: OBJECTIVE: To determine the presence, location and intensity of estrogen and progesterone receptors in the cardinal ligaments. PATIENTS AND METHODS: A prospective study was conducted by sampling the cardinal ligaments of 29 consecutive women undergoing hysterectomy. The presence and location of estrogen and progesterone receptors were assessed by an immunohistochemical staining technique. RESULTS: Estrogen receptors were detected in 25 samples (86.2%), while progesterone receptors were detected in all of them. The estrogen receptors staining intensity index (SII) was significantly higher in premenopausal than postmenopausal women (1.5 ± 0.7 vs. 0.9 ± 0.7 , $p < 0.02$). The percentage of progesterone receptors was significantly higher in the premenopausal group (88.1 ± 15.5 vs. 60.0 ± 31.1 , $p < 0.002$). There was no association between the presence of both receptors with parity, body mass index, or smoking. All receptors were located in the nuclei of the smooth muscle cells and the blood vessel. CONCLUSIONS: Our data suggest that the cardinal ligaments serve as a target for the effect of estrogen and progesterone.

Key words: Cardinal ligaments; Estrogen; Menopause; Progesterone; Receptor.

INTRODUCTION

Pelvic organ prolapse and urinary stress incontinence are major problems affecting quality of life in a vast number of women. The etiology is most probably multifactorial, with a major contribution of muscular denervation during vaginal delivery, genetic factors, and the aging process.¹⁻⁴ The higher prevalence of pelvic organ prolapse and urine incontinence in the postmenopausal period suggests that the hypo-estrogenic state might play an important role in their appearance.

To prove a role for sex hormones in pelvic support, one of the first steps would be to find evidence of sex hormone receptors in these tissues. The presence of estrogen and progesterone receptors has been already established in the sacrouterine ligaments and levator-ani muscles.⁶⁻⁹ However, a thorough search of the English literature yielded no such studies about the cardinal ligaments, which are the most important ligaments that support the uterus.

The aim of this study was to assess the presence and location of estrogen and progesterone receptors in the cardinal ligaments.

PATIENTS AND METHODS

All women undergoing abdominal or vaginal hysterectomy for fibroid uterus or uterine prolapse at the Assaf Harofe Medical Center Between June and September 2000 were enrolled in the study. All the women were consented before surgery. The exclusion criteria were: hormonal replacement therapy users (current or past users), patients who had a known or suspected malignant disease, cardinal ligaments distortion due to disease or adhesions, (endometriosis, pelvic inflammatory disease etc.) or patients who had previous pelvic surgery. A small piece of tissue from the cardinal ligaments was biopsied from the discarded hysterectomy specimen. No changes were done in the original operation for taking the biopsies. To ensure consistency, all biopsies were performed under the supervision of the main author (HK). All surgical specimens were sent as a routine for histological evaluation.

Tissue samples were fixed in 10% formaldehyde solution, processed by routine histological techniques, and embedded in paraffin. A five-micron section from each sample was stained with Hematoxylin & Eosin, and with monoclonal antibodies for immunohistochemistry for estrogen receptors (Zymed

Laboratories Inc., San Francisco, CA) and progesterone receptors (Zymed Laboratories Inc., San Francisco, CA).¹⁰ After antigen retrieval with 10 mmol citrate buffer (pH-7.6), the monoclonal antibodies were diluted 1:100. Indirect immuno-peroxidase staining was performed using the Avidin-Biotin peroxidase technique.^{11, 12} Counterstaining with Hematoxylin was performed to improve identification of cellular elements. Breast carcinoma, known to be positive for estrogen and progesterone receptors, was used as a positive control.

Histological evaluation of estrogen and progesterone receptors was done on all samples at the same time with Olympus BX light microscope (Olympus, Tokyo, Japan) under high power magnification x 40-eye piece by an experienced pathologist (RK) blind to the patient's characteristics. Estrogen receptors were calculated for each sample with the staining Intensity Index (SII) as follows¹⁰: (% weakly positive cells X 1) + (% moderately positive cells X 2) + (% strongly positive cells X 3) and the result was divided by 100. Progesterone receptor expression was calculated as the percent of stained cells.

All pertinent clinical and laboratory data including patients characteristics (age, parity, BMI, smoking, medical history) and estrogen and progesterone receptors evaluation were collected prospectively, recorded into a computerized database and was evaluated at the end of the study period. Menopause was defined as the absence of menstruation more than one year in women over the age of 45 years.

Statistical analysis was performed using the SPSS statistical software (Release 6.0, SPSS Inc.; Chicago, IL). Categorical variables were analyzed using chi-square test, and continuous variables were analyzed using Student's t-test. $P < 0.05$ was considered statistically significant. The mean \pm standard deviation (SD) was computed for continuous variables.

RESULTS

Twenty-nine patients were enrolled in the study. The mean age was 51.4 ± 10.0 years (range 35-74). Thirteen patients (44.8%) were postmenopausal with an average time from menopause of 13.8 ± 9.3 years (range 2-24). Only one patient was a smoker. Patient's characteristics are shown in Table I.

On histological examination, all specimens were consistent with the cardinal ligaments and were therefore

TABLE I – Patient's characteristics (n = 29).

Variables	Value	
Age* (yr)	51.4±10.0	(35-74)
Parity*	3.1±1.1	(1-5)
BMI (kg/ m ²)*	24.3±4.3	(18.2-32.6)
Menopausal status**		
Premenopausal	16	(55.2%)
Menopausal	13	(44.8%)
Time from menopause (years)*	14.7±8.5	(2-24)
Indication for operation**		
Fibroid uterus	16	(55.1%)
Uterine prolapse	13	(44.9%)

* Mean ± standard deviation with the range in parentheses.

** Number of patients with the percentage in parentheses

suitable for the study. All the samples contained connective tissue, blood vessels, and smooth muscle fibers. Seven of 29 samples (24.1%) contained nerve fibers (5/16 in the premenopausal group and 2/13 in the post menopausal group. The difference was not statistically significant. The estrogen and progesterone receptors were located in the nuclei of smooth muscle cells and the blood vessels muscular wall, but not in the connective or neural tissue.

Estrogen receptors with variable intensities were found in 26 (89.6%) samples (Table II). Of the three negative samples, two were of premenopausal women with fibroid uterus and the other was of postmenopausal women with uterine prolapse. The mean SII of the all group was 1.3 ± 0.7 . Dividing the study group to pre and postmenopausal women yielded a higher estrogen SII in the former (1.5 ± 0.7 vs. 0.9 ± 0.7 , $p < 0.02$). All samples contained progesterone receptors. Premenopausal women had a significantly higher mean percentage of progesterone receptors compared with postmenopausal women (88.1 ± 15.6 vs. 60.0 ± 31.1 , $p < 0.002$). There was not a statistically significant difference in estrogen or progesterone receptors in women with fibroid uterus compared with normal uterus (confirmed histologically).

DISCUSSION

The cardinal ligaments form a wide fibro-elastic tissue extending from each side of the cervix and upper vagina to the pelvic sidewalls. They are composed of various types of collagen, elastin, smooth muscle, autonomic nerves, fibroblasts and vascular structures.¹³ Along with the sacrouterine ligaments, endopelvic fascia, and levator ani muscles they play a major role in pelvic support and the prevention of uterine prolapse mainly presented in postmenopausal women. The mechanism of pelvic floor support, which is based on the anatomical and physiological qualities of the support tissues, needs to be elucidated before effective strategies can be formulated for the prevention and treatment of pelvic prolapse and urine incontinence. Some studies have suggested that the decreased estrogen level in this period, particularly in those who do not use hormonal replacement therapy plays a major role in these conditions.^{2, 14} A large randomized placebo controlled study¹⁵ found that treatment with raloxifene, a selective estrogen receptors modulator for the prevention of osteoporosis was associated with 50% reduction in the risk of pelvic floor surgery.

The existence of estrogen and progesterone receptors in the tissue is a major prerequisite for a hormonal effect on it. This has been previously proved in a variety of female genital tract structures, including the vagina, cervix, uterus, fallopian tubes, round ligaments, sacrouterine ligaments, levator ani muscles, and urethra.⁵⁻⁹ This study establishes the presence of estrogen and progesterone receptors in

TABLE II - Estrogen and progesterone receptors intensity according to the menopausal state.

Variables	Premenopausal N=16	Postmenopausal N=13	P Value*
Mean age (yr)	47.0 ± 5.0 (35-55)	65.4 ± 9.2 (52-74)	<0.002
Mean BMI (kg/ m ²)	26.0 ± 4.2 (18.2-33.8)	22.9 ± 4.2 (19.5-29.1)	NS
Estrogen receptors (SII)	1.5 ± 0.7 (0-2.4)	0.9 ± 0.7 (0-2.1)	P<0.02
Estrogen receptors (%)	68.1±32.7 (0-100)	38.1±29.7 (0-75)	P<0.001
Progesterone receptors (%)	88.7 ± 14.0 (20-100)	68.0 ± 32.7 (20-100)	P<0.002

All data is given in mean ± standard deviation with the range in parentheses.

SII = Staining Intensity Index

NS= Not significant

* = Student's t-test

the cardinal ligaments a main pelvic ligament that hold the uterus and upper vagina at their normal position. We specifically located these receptors to the nuclei of smooth muscle cells and blood vessel. These findings are consistent with the known embryonic origin of the ligaments from the mullerian ducts, and supported by the lack of effect of parity, BMI on there presence. The intensity of estrogen and progesterone receptors was significantly higher in premenopausal compared with postmenopausal women. However, a significant overlap exists between the intensity of these hormones receptors in premenopausal and postmenopausal women. We assume that the quantity of estrogen and progesterone receptors may be dependent on the presence of these hormones in the tissue. A hypo-estrogenic state may lead to a down regulation of these receptors in the estrogen and progesterone depended tissues in the body including in the support tissues in the pelvis. It may raise the question about the possible influence of early hormonal replacement therapy on pelvic organ prolapse and urine incontinence later in life. A large randomized study¹⁶ found that estrogen replacement therapy reduces the total vaginal collagen by increasing breakdown. The intermediate collagen was increased and the mature collagen was decreased. The type 1/3 collagen ratio was unchanged. The vaginal biopsies were taken after 6 months. Potentially, the replacement of mature old collagen by new immature collagen may be the transitional phase for increase in the collagen content. But we cannot exclude a paradoxical detrimental effect of exogenous estrogen on the pelvic floor.

Further studies are needed to evaluate the progesterone and estrogen receptors including estrogen receptors subtypes, which are of possible clinical importance in women with pelvic floor dysfunction, as well as the modulation of these receptors expression by endogenous and exogenous hormones.

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5 RETENTIONS

Efficacy of traditional Chinese medicine for the management of constipation: a systematic review. *Lin LW, Fu YT, Dunning T et al. Journal of alternative and complementary medicine. EPUBDATE: 2009-12-05.* In this Cochrane review of 137 controlled studies, traditional Chinese medicine interventions (Chinese herbal medicine, acupuncture) appear to be useful to manage constipation. Significant positive results were found in 15 high-quality studies. However there was heterogeneity in diagnostic procedures and interventions, hence the results should be interpreted cautiously.

Sexual abuse: a strong predictor of outcomes after colectomy for slow-transit constipation. *O'Brien S, Hyman N, Osler T, Rabinowitz T. Diseases of the Colon & Rectum. EPUBDATE: 2009-12-08.* Patients undergone subtotal colectomy and ileorectal anastomosis for slow-transit constipation at a university hospital from 1991 to 2006 were questioned about a history of anal and vaginal sexual abuse and 13 out of 15, all women, highly satisfied with the results of their surgery, came for assessment; 8 (62%) reported a history of sexual abuse, 7 (88%) both anal and vaginal. The history of sexual abuse resulted a strong predictor of more functional diagnoses, more pre-colectomy operations, and more post-colectomy medical care for abdominal complaints.

Botox treatment for vaginismus. *Pacik PT. Plastic and Reconstructive Surgery. EPUBDATE: 2009-12-03*

Stapled transanal rectal resection for obstructed defecation: a cautionary tale. *Titu LV, Riyad K, Carter H, Dixon AR. Diseases of the Colon & Rectum. EPUBDATE: 2009-12-08.* Stapled transanal rectal resection can be performed on a day-case basis with 77% of patient satisfaction. However major complications were seen in 7%; fecal urgency, reported by 46%, persisted at six months in 11%; 5% patients reported severe postoperative pain.

6 INCONTINENCES

Defects on endoanal ultrasound and anal incontinence after primary repair of fourth-degree anal sphincter rupture: a study of the anal sphincter complex and puborectal muscle. *Sakse A, Secher NJ, Ottesen M, Starck M. Ultrasound in obstetrics & gynecology. EPUBDATE: 2009-12-03.* In a 1-9-year follow-up period after primary suture of fourth-degree anal sphincter rupture, the frequency of anal incontinence was 67%. No clear association was seen between incontinence and sphincter defects detected on ultrasonography. There was an association between the angle of the puborectalis muscle and the extent of ultrasound defects.

Retention test in sacral nerve stimulation for fecal incontinence. *Michelsen HB, Maeda Y, Lundby L et al. Diseases of the Colon & Rectum. EPUBDATE: 2009-12-08.* Though sacral nerve stimulation is an established treatment for fecal incontinence, the mechanism of its action remains obscure. It does not alter the ability to retain rectal content and further studies are needed to investigate the reasons why it may be successful.

Randomized controlled trial shows biofeedback to be superior to pelvic floor exercises for fecal incontinence. *Heymen S, Scarlett Y, Jones K, Ringel Y, Drossman D, Whitehead WE. Diseases of the Colon & Rectum. EPUBDATE: 2009-12-08.* Biofeedback was more effective than pelvic floor exercises alone in producing adequate relief of fecal incontinence symptoms in patients for whom conservative medical management had failed.

Factors associated with percutaneous nerve evaluation and permanent sacral nerve modulation outcome in patients with fecal incontinence. *Govaert B, Melenhorst J, Nieman FH, Bols EM, van Gemert WG, Baeten CG. Diseases of the Colon & Rectum. EPUBDATE: 2009-12-08.* Older age, repeated procedures, and a defect in the external anal sphincter are factors that may indicate lower chances of success for test stimulation but do not exclude patients from sacral nerve modulation treatment. Although assessed in a selected patient group, no factors were predictive of the outcome of permanent stimulation.

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