Alteration of Elastin Metabolism in Women With Pelvic Organ Prolapse

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Abbreviations and Acronyms

AAT = alpha-1-antitrypsin
ECM = extracellular matrix
FP = follicular phase
LOX = lysyl oxidase
LOXlike-1 = LOXL-1
LP = luteal phase
MMP = matrix metalloproteinase
NE = neutrophil elastase
PCR = polymerase chain reaction
POP = pelvic organ prolapse
SUI = stress urinary incontinence
TIMP = tissue inhibitor of metalloproteinase
USL = uterosacral ligament

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Purpose: Although there are many studies about the effects of vaginal birth, the effects of menopause on pelvic floor support have not been identified. We compared elastin metabolism in the uterosacral ligament of women with and without pelvic organ prolapse, and defined the menopausal regulation of this process.

Materials and Methods: The study group consisted of 35 women who underwent hysterectomy for pelvic organ prolapse. The control group consisted of 39 women without pelvic organ prolapse. A questionnaire was administered to assess age, parity, body mass index, and menopausal status. Expression levels of mRNA, and protein for neutrophil elastase, matrix metalloproteinase-2, and matrix metalloproteinase-9 were determined by real-time quantitative polymerase chain reaction and ELISA, respectively, using uterosacral ligament samples from each patient. Expression of alpha-1-antitrypsin, an inhibitor of neutrophil elastase, was also determined. ANOVA, the Kruskal-Wallis test and multivariate linear regression were used for statistical analysis.

Results: Expression of neutrophil elastase and matrix metalloproteinase-2 mRNA was higher in women with pelvic organ prolapse than in those without pelvic organ prolapse. Compared to before menopause, neutrophil elastase and matrix metalloproteinase-2 showed a significant decrease in postmenopausal women without pelvic organ prolapse, although these remained increased in postmenopausal women with pelvic organ prolapse. Alpha-1-antitrypsin was significantly less in postmenopausal women with pelvic organ prolapse than in postmenopausal women without pelvic organ prolapse. The activities of neutrophil elastase, matrix metalloproteinase-2 and matrix metalloproteinase-9 were increased in women with pelvic organ prolapse, and these trends were similar to neutrophil elastase and matrix metalloproteinase-2 expression even after adjustment for age, parity and menopausal status.

Conclusions: After menopause increased elastolytic protease has a significant role in the development of pelvic organ prolapse.

Key Words: pelvic organ prolapse, menopause, matrix metalloproteinase 2, matrix metalloproteinase 9

Pelvic organ prolapse is the herniation of the uterus, bladder, small bowel or rectum into the vaginal cavity, and occurs more commonly in postmenopausal women. The incidence of POP is nearly 35% and is expected to increase to 46% in the next 40 years.[1] Despite the high incidence of this disease, the pathogenesis remains poorly understood. Although vaginal parity, old

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age, obesity and menopause are consistently identified as risk factors, it does not develop in most women with these risk factors. The pathophysiology of POP is a multifactorial process, and prolapse develops in a genetically predisposed woman after experiencing a series of life events such as vaginal delivery and menopause.[2]

The pelvic organs rely on the tensile strength of the pelvic floor connective tissues for support. Elastin has a vital role in the extension, resilience and recoil of these tissues.[3] Several studies have reported that elastin is decreased in the USL or the vaginal wall in patients with POP[4,5] and that changes in the homeostasis of elastin may lead to the development of POP.

The most prominent proteases implicated in the proteolytic degradation of elastin are NE and MMP. NE is a 29-kDa serine protease stored in its active form in azurophil granules until release following neutrophil exposure to inflammatory stimuli. NE, inactivated by AAT, is best known for elastin degradation, although it can digest many types of protease inhibitors and several proteases.[6] MMP represents one of the most prominent proteases cleaved by NE. This counter-regulation between NE and MMPs is important as MMPs can cleave nearly all protein components of the ECM including elastin. MMPs are produced as zymogens containing a secretory signal sequence and a propeptide, which requires proteolytic cleavage for activation.[7] Knowledge about elastolytic proteases found in the USL in women with and without POP is limited.

The USLs are condensations of endopelvic fascia and provide the primary uterine support.[8] In this study we hypothesized that elastin homeostasis was disrupted in women with POP, secondary to increased activity of elastolytic proteases within the USL. Thus, we compared the mRNA expression and enzyme activity of NE, MMP-2, MMP-9 and AAT in the USL of women with and without POP. We also evaluated the effects of the menstrual cycle and menopausal status.

MATERIALS AND METHODS

This study was approved by the institutional review board at Severance Hospital, Yonsei University, Seoul, Korea and all women provided informed consent before participation. Patients were recruited and enrolled from the Department of Obstetrics and Gynecology, Severance Hospital, from May to December 2009. The study group consisted of 35 women who underwent hysterectomy for POP-Quantification stage III-IV. The control group consisted of 39 women with POP-Quantification stage 0 or I who underwent hysterectomy. Study exclusion criteria were previous pelvic surgery, connective tissue disease, history of endometriosis and history or presence of cancer. All patients were administered a standard questionnaire and a physical examination before surgery. The questionnaire assessed age, parity, body mass index, menopausal status, hormone replacement therapy status and prior gynecologic or incontinence surgery. Medical history included connective tissue disease, hypertension, diabetes mellitus, pulmonary disease and disc herniation.

Pelvic examinations were performed by 1 examiner. SUI was confirmed by urine leakage during urodynamic study. Classification of menstrual cycle and menopausal status was determined according to the last menstrual period and the histology of the endometrium. The endometrium was recorded as FP, LP or post-menopause. Postmenopausal was defined as no menses during the previous 12 months with the histology of an atrophic endometrium. Tissue samples were obtained immediately after the hysterectomy in the operation room, 0.5×1.0 cm excision of the USL, 0.5 cm from its insertion into the cervix, by scalpel, since previous study identified this location as the most important site for uterine support.[8] The specimens were immediately frozen in liquid nitrogen and stored at −80C.

Total RNA for real-time PCR was extracted from frozen tissue samples using the High Pure RNA Tissue Kit (Roche Diagnostics, Mannheim, Germany) according to manufacturer protocol. Reverse transcription was performed with 11 μl total RNA from each sample using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics). Real-time PCR was performed according to manufacturer protocol (Roche Diagnostics). The housekeeping proto-oncogene ABL (c-ABL) was amplified as a normalization control. Amplification conditions consisted of 95C for 10 minutes, followed by 45 cycles of 95C for 10 seconds, 60C for 30 seconds and 72C for 1 second performed simultaneously in capillary tubes. mRNA levels were measured using the LightCycler® 480 system (Roche Diagnostics) and calculated by the $\Delta\Delta$Ct method. Each test was repeated twice.

For activity measurement tissues were thawed on ice and minced. For NE, minced tissues were homogenized in 0.5 ml solubilization buffer (150 mM NaCl, 1% N-40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate, 4 mM EDTA, 50 mM Tris-HCl, 2 mM PMSF, pH 7.4), then transferred into small tubes and rotated at 4C overnight. NE activity was measured using the synthetic substrate Suc-Ala-Ala-Pro-Val p-nitroaniline, which is highly specific for NE.[9] The extracted substrate (1 mM) was incubated with the sample (200 μl) in 0.1 M Tris-HCl (pH 8.0) containing 0.5M NaCl in a final volume of 210 μl at 37C for 24 hours. The increase in the absorbance at 405 nm was obtained. We defined 1 unit of NE activity as the quantity of enzyme that liberated 1 mmol p-nitroaniline in 24 hours standardized with 1 mg protein.

For MMP, minced tissues were washed in phosphate buffered saline containing 2 mM N-methylamine until the supernatant was clear, and then homogenized in MMP-2,-9 assay buffer (AnaSpec EnzoLyte 520 MMP-2,-9 Assay Kit, AnaSpec, San Jose, California). The homogenates were clarified by centrifugation at 10,000 × gravity for 15 minutes at 4C and the concentrations were determined using a BCA protein assay. For MMP activity, pro-MMP-2,-9 enzyme was activated by incubating the tissue homogenates with 1 mM 4-aminophenylmercuric acetate in