Alteration of elastin metabolism in women with pelvic organ prolaspe

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Alteration of elastin metabolism in women with pelvic organ prolaspe

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<ABSTRACT>

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Objective: To compare elastin metabolism in the uterosacral ligament of women with and without pelvic organ prolapse (POP), and to define the menopausal regulation of this process.

Methods: The study group consisted of 35 women who underwent hysterectomy for advanced (stage III-IV) POP. The control group consisted of 39 women without POP. A questionnaire was administered to assess age, parity, body mass index, and menopausal status. Expression levels of the mRNA and protein for elastolytic proteases, including neutrophil elastase (NE), matrix metalloproteinase (MMP)-2, and MMP-9, were determined by real-time quantitative polymerase chain reaction and enzyme-linked immunosorbent assay, respectively, using uterosacral ligament samples from each patient. Expression of alpha-1-antitrypsin (AAT), an inhibitor of NE, was also determined. ANOVA, Kruskal-Wallis test, and multivariate linear regression were utilized for statistical analysis.

Results: Expression of NE and MMP-2 mRNA was higher in women with POP than without POP. Compared to premenopause, NE and MMP-2 showed a

significant decrease in postmenopausal women without POP, although these remained elevated in postmenopausal women with POP. AAT was significantly less in postmenopausal women with POP than postmenopausal women without POP. The activities of NE, MMP-2, and MMP-9 were increased in women with POP, and these trends were similar to NE and MMP-2 expression, even after adjustment for age, parity, and menopausal status.

Conclusion: After menopause, elevated elastolytic protease activity could play a significant role in the development of POP

Key words: pelvic organ prolapse, neutrophil elastase, matrix metalloproteinase-2, matrix metalloproteinase-9, menopause

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I. INTRODUCTION

Pelvic organ prolapse (POP) refers to the herniation of the uterus, bladder, small bowel, or rectum into the vaginal cavity, and occurs more commonly in post-menopausal women. The incidence of POP is nearly 35%[1] and is expected to increase to 46% in the next 40 years[2]. Despite the high incidence of this disease, the pathogenesis of POP remains poorly understood. Although vaginal parity, old age, obesity, and menopause are consistently identified as risk factors for POP, it does not develop in most women with these risk factors. The pathophysiology of POP is a multifactorial process, where a genetically predisposed woman develops prolapse after experiencing a series of life events, such as vaginal delivery and menopause[3].

The pelvic organs rely upon the tensile strength of the pelvic floor connective tissues for support. Elastin, the major fibrillar component of the pelvic floor connective tissues, plays a vital role in the extension, resilience, and recoil of these tissues[4]. Several studies have reported that these elastic fibers are decreased in the uterosacral ligaments or the vaginal wall in POP patients[5,6], and that changes in the homeostasis of elastic fibers may lead to the development of POP.

The most prominent proteases implicated in the proteolytic degradation of elastic fibers are neutrophil elastase (NE) and matrix metalloproteinase (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 alpha-1-antitrypsin (AAT), is best known for elastin degradation, although it can digest many types of protease inhibitors and several proteases[7,8]. 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 extracelluar matrix (ECM), including elastin. MMPs are produced as zymogens containing a secretory signal sequence and a propeptide, which requires proteolytic cleavage for activation[9]. Knowledge about elastolytic proteases found in the uterosacral ligament in women with and without POP is limited.

The uterosacral ligaments are condensations of endopelvic fascia and provide the primary uterine support[10]. In this study, we hypothesized that elastin homeostasis was disrupted in women with POP, secondary to increased activity of elastolytic proteases within the uterosacral ligament. To this end, we compared the metabolism of elastin within the uterosacral ligament in women with and without POP. We also evaluated the effect of the menstrual cycle[11] and menopausal status on elastolytic protease expression and activity. Finally, the gene expression and enzyme activity of NE, MMP-2, MMP-9 and AAT in uterosacral ligaments of women with and without POP were measured, and then separated for analysis according to the menstrual cycle and menopausal status.

II. MATERIALS AND METHODS

1. Subjects

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 Division of Urogynecology, the Department of Obstetrics and Gynecology at Yonsei University Hospital from May to December of 2009. The study group consisted of 35 women who underwent hysterectomy for Pelvic Organ Prolapse Quantification (POP-Q) stage III–IV POP. The control group consisted of 39 women with POP-Q stage 0 or I who underwent hysterectomy for endometrial pathology, benign ovarian tumor, or uterine myoma. Study exclusion criteria included 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, history of hormone replacement therapy, and prior gynecologic or incontinence surgery. Questions about connective tissue disease, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, and lumbar disc herniation were also reviewed.

POP was scored according to the International Continence Society's POP-Q system. Pelvic examinations were performed by one examiner. Classification of the menstrual cycle and menopausal status was determined according to the last menstrual period and the histology of the endometrium. The endometrium was recorded as either follicular phase (FP), luteal phase (LP), or post-menopausal (MENO). Postmenopausal status was defined as no menses over the previous 12 months with an atrophic endometrium. Tissue samples were obtained during the hysterectomy in the operation room. A 1.0 × 1.0cm biopsy of the uterosacral ligament, 1 cm from its insertion into the cervix, was taken immediately after the hysterectomy, since previous histological

examination identified this location as the most important site for uterine support[10]. The specimens were immediately frozen in liquid nitrogen and stored at -80°C.

2. Real time PCR

Total RNA for real-time polymerase chain reaction (PCR) was extracted from frozen tissue samples using the High Pure RNA Tissue Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocols. Reverse transcription was performed with 11 μl of total RNA from each sample using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics). NE, AAT, MMP-2, and MMP-9 mRNA levels were measured using the LightCycle 480 system (Roche Diagnostic). The housekeeping proto-oncogene ABL (c-ABL) was amplified as a normalization control. The specific primers used for NE, AAT, MMP-2, and MMP-9 are indicated in Table 1. Real-time PCR was performed in 20 μl reaction volumes containing 5 μl of cDNA, 0.5 M of each primer, 0.4 M of each probe, and LightCycle 480 Probe Master mix (Roche Diagnostics). Amplification conditions consisted of 95°C for 10 min, followed by 45 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 1 s were performed simultaneously in capillary tubes. Each test was repeated twice. Relative NE, AAT, MMP-2, and MMP-9 mRNA levels were calculated by the ΔΔCt method as described previously (12). The normalization formula target amount was $2^{-\Delta\Delta Ct}$, where ΔΔCt = [Ct (candidate gene) - Ct (c-ABL gene)] - [Ct (candidate gene calibrator) - Ct (c-ABL gene calibrator)].

3. Assay of NE activity

NE activity was measured using the synthetic substrate Suc-Ala-Ala-Pro-Val pNA, which is highly specific for NE, by the method described previously[13]. Briefly, a sample was incubated for 24 h at 37°C in 0.1 M Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl and 0.001 M of the substrate dissolved in

1-methyl-2-pyrrolidone. After incubation, pNA release was measured spectrophotometrically (DU7400 spectrophotometer) at 405 nm as an indicator of NE activity.

4. Assay of MMP-2, MMP-9 activity

To measure enzyme activity, tissues were thawed on ice, minced, and washed in PBS with 2 mM N-methylamine (an inhibitor of antielastase activity) until the supernatant was clear. Tissues were then homogenized in MMP-2, MMP-9 assay buffer (AnaSpec EnzoLyte 520 MMP2 Assay Kit; AnaSpec, San Jose, CA) containing 0.1% Triton-X 100 (95x volume:tissue wet weight). Thereafter, the homogenates were centrifuged at 10,000 g for 15 min at 48°C. The supernatant was used for determination of protease activity. Protein concentrations were determined using a BCA protein assay and standard curves of BSA in appropriate buffers. Pro-MMP-2, pro-MMP-9 enzyme was activated by incubating the tissue homogenates with 1 mM 4-aminophenylmercuric acetate (APMA) in MMP-2, MMP-9 assay buffer for 15 min at 37°C. Enzyme activity was determined using a fluorescently labeled peptide (FRET peptide) as substrate and a standard curve of purified MMP2, MMP-9 as recommended by the manufacturer (AnaSpec EnzoLyte 520 MMP-2, MMP-9 Assay Kit). Fluorescence intensity was measured at Ex/Em¼490 nm/520 nm every 5–10 min for 40–60 min.

5. Statistical analysis

All statistical analyses were performed by a statistician (Lim HS), who was blinded to the study aims, using SAS 9.2 statistical software (SAS Inc., Cary, NC, USA). Statistical tests were evaluated at the .05 significance level. Normality of the data was assessed using the Shapiro-Wilk test, which indicated that the PCR data did not follow a normal distribution. Therefore, PCR data was analyzed using the Mann-Whitney U and Kruskal-Wallis tests, where

appropriate. Post-hoc comparisons were made using the Mann-Whitney U test evaluated at the .013 significance level. Multivariate linear regression was used to evaluate the impact of age, parity, body mass index, stage of prolapse, and menopausal status on elastase levels. Models were developed using a forward stepwise selection procedure.

III. RESULTS

Seventy-five women were initially recruited for this study. One individual was eliminated based on prior hormone replacement therapy. Uterosacral ligament samples were collected from the remaining 74 patients during surgery, where 30% underwent laparoscopic hysterectomy, 27% an abdominal hysterectomy, and 43% a vaginal hysterectomy. Thirty-nine percent of the patients needed a colporrhaphy, and 4% required vaginal vault suspension. Postmenopausal women with POP were older (mean age 68.3 years, P=.010) and had higher parity (median parity 4, P=.012) than postmenopausal women without POP. Premenopausal women with and without POP had similar ages, parity, and body mass indices. Among the postmenopausal women with POP, the median stage of prolapse was stage IV. Among the 35 women with POP, 51% had POP without urinary incontinence (Table 2).

Real-time PCR was used to analyze the expression of elastolytic proteases in the uterosacral ligaments of women with and without POP. We first evaluated the impact of the menstrual cycle on gene expression of NE, MMP-2, MMP-9, and ATT in premenopausal women regardless of POP status. Elastolytic protease expression in the FP was higher than the LP in women without POP, though this trend was not statistically significant (Table 3).

We compared the expression of elastolytic proteases between with and without POP in pre-and post-menopausal women. As shown in Table 4 and Figure1, after menopause, women with POP had greater expression of NE and MMP-2(P<.0001), and less expression of AAT(P=.035) than without POP. We next compared between pre-and post-menopausal women in the presence or absence of POP. Among women without POP, NE and MMP-2 were decreased in the postmenopausal group compared to the premenopausal group. However, there was less decrease of NE and MMP-2 for pre-and post-menopause in women with POP. Similarly, activity of MMP-2, MMP-9 was decreased significantly after menopause in women without POP, compared to the

insignificant decrease in the postmenopausal women with POP (Figure 2). However, expression of MMP-9 was unchanged in all groups, suggesting that this enzyme activity was regulated during the post-translational processing. AAT was significantly decreased in postmenopausal women with POP as compared to postmenopausal women without POP, indicating a disruption in NE homeostasis(western blot data not shown). Furthermore, after adjustment for age, parity, and menopausal effect, NE and MMP-2 remained significantly increased in women with POP. Together, these data suggest that homeostasis of elastolytic protease plays a significant role in the development of POP.

Table1. Primer and probe sequences

Gene			sequence
NE	Primer	Sense	5’-AGTTTGTAAACTGGATCGACTCTATC
		Antisense	5’-GCTGGAGAGTGTGGGTGTG
	Probe		CAACGCTCCGAGGACAACCCCTGT
AAT	Primer	Sense	5’-TACTGGAACCTATGATCTGAAGAGC
		Antisense	5’-CTCGTCGATGGTCAGCACAG
	Probe		CTGGGTCAACTGGGCATCACTAAGGTCTTC
MMP-2	Primer	Sense	5’-AGAAGATGCAGAAGTTCTTTGGAC
		Antisense	5’-CTTGCGAGGGAAGAAGTTGTAG
	Probe		TTGACCAGAATACCATCGAGACCATGCGG
MMP-9	Primer	Sense	5’-CCTCGCCCTGAACCTGAGC
		Antisense	5’-CTCTGAGGGGTGGACAGTGG
	Probe		TCCAACCACCACCACACCGCAGC
C-abl	Primer	Sense	5’-GCCGAGTTGGTTCATCATCATTC
		Antisense	5’-GTCGTAGTTGGGGGACACAC
	Probe		CGGGCTCATCACCACGCTCCATTATCC

NE: neutrophil elastase, MMP: matrix metalloproteinase, AAT: α1-antitrypsin

Table 2. Demographics of patients included in multiple linear regression modeling

	Without POP (n=39)	With POP (n=35)	Overall *P**	Post Hoc *P*†
Premenopause, n (%)	22 (56)	6 (17)		
Postmenopause, n (%)	17 (44)	29 (83)		
Age			<.0001	
Premenopause	46.7 ± 3.6	44.3 ± 8.3		1.000
Postmenopause	61.3 ± 8.0	68.0 ± 7.3		.010
Parity			<.0001	
Premenopause	2 (1-2)	2 (1-3)		1.000
Postmenopause	2 (2-3)	4 (3-5)		.012
BMI			.782	
Premenopause	23.2 (20.6-25.3)	23.7 (23.3-25.8)		
Postmenopause	24.3 (22.5-25.8)	24.4 (21.6-26.8)		
POP-Q stage			.112	
Premenopause	NA	3 (3-3)		
Postmenopause	NA	4 (3-4)		
SUI			<.0001	
Premenopause, n(%)	0	3 (50)		
Postmenopause, n(%)	0	14 (48)		

POP: pelvic organ prolapse; BMI: body mass index; NA: not available; SUI: stress urinary incontinence

* Overall P value from ANOVA test for age, χ^2 test for SUI, Kruskal-Wallis test for others

† *P* value from post hoc analysis using Mann-Whitney U test comparing no POP and POP in same menopausal status, *P* values were evaluated at the .025 significance level, using the Bonferroni method.

Table 3. Expression of elastolytic protease mRNA in the uterosacral ligament in each group of premenopausal women according to menstrual phase

Premenopause	Wihtout POP (n=22)	*P*	With POP (n=6)	*P*	All (n=28)	*P*
FP	15		4		19	
LP	7		2		9	
NE		.631		.865		.835
FP	3.80 ± 2.59		4.04 ± 3.43		3.55 ± 2.72	
LP	3.45 ± 0.71		4.52 ± 1.71		3.70 ± 0.99	
MMP-2		.586		.419		.681
FP	3.82 ± 2.70		4.92 ± 3.73		3.73 ± 2.90	
LP	3.41 ± 0.71		6.79 ± 1.29		4.16 ± 1.68	
MMP-9		.376		.811		.391
FP	2.08 ± 2.71		1.58 ± 1.31		1.88 ± 2.37	
LP	1.11 ± 0.91		1.33 ± 0.28		1.16 ± 0.80	
ATT		.375		.738		.401
FP	1.12 ± 0.36		0.61 ± 0.28		0.96 ± 1.18	
LP	0.64 ± 0.32		0.53 ± 0.10		0.62 ±0.28	

POP: pelvic organ prolapse; FP: follicular phase; LP: luteal phase; NE: neutrophil elastase; MMP: matrix metalloproteinase; AAT: α1-antitrypsin
Data presented as mean ± standard deviation.
P value from Student t-test.

Table 4. Expression of elastolytic proteases mRNA in the uterosacral ligament in women with and without POP

	Without POP (n=39)	With POP (n=35)	*P*	Overall *P* *	Post Hoc *P*†
Premenopause, n (%)	22 (56)	6 (17)			
Postmenopause, n (%)	17 (44)	29 (83)			
NE	2.03(0.81-3.32)	2.31(1.56-4.06)	.049		
				<.0001	
Premenopause	3.22 (2.35-4.41)	3.57 (1.82-5.74)			.806
Postmenopause	0.81 (0.57-1.49)	2.14 (1.51-3.95)			<.0001
Post Hoc *P*‡	<.0001	.235			
MMP-2	2.06(0.82-3.22)	2.35(1.75-5.85)	.024		
				<.0001	
Premenopause	3.10 (2.32-4.38)	6.37 (1.99-7.70)			.283
Postmenopause	0.82 (0.55-1.49)	2.14 (1.65-3.62)			<.0001
Post Hoc *P*‡	<.0001	.112			
MMP-9	1.19(0.65-1.91)	0.94(0.49-1.72)	.298		
				.482	
Premenopause	0.91 (0.65-1.91)	1.32 (0.60-2.07)			.836
Postmenopause	1.33 (0.65-2.00)	0.90 (0.45-1.48)			.126
Post Hoc *P*‡	.604	.312			
AAT	0.72(0.46-1.16)	0.50(0.32-0.73)	.036		
				.119	
Premenopause	0.58 (0.46-0.87)	0.56 (0.46-0.75)			.656
Postmenopause	1.00 (0.44-2.90)	0.50 (0.32-0.71)			.035
Post Hoc *P*‡	.168	.583			

POP: pelvic organ prolapse; NE: neutrophil elastase; MMP: matrix metalloproteinase; AAT: α1-antitrypsin

P value, * Overall *P* value from Kruskal-Wallis test.

†*P* value from post hoc analysis using Mann-Whitney U test comparing without POP group and the POP group, *P* values were evaluated at the .013 significance level, using the Bonferroni method

‡*P* value from post hoc analysis using Mann-Whitney U test comparing the premenopausal group and the postmenopausal group, *P* values were evaluated at the .013 significance level, using the Bonferroni method

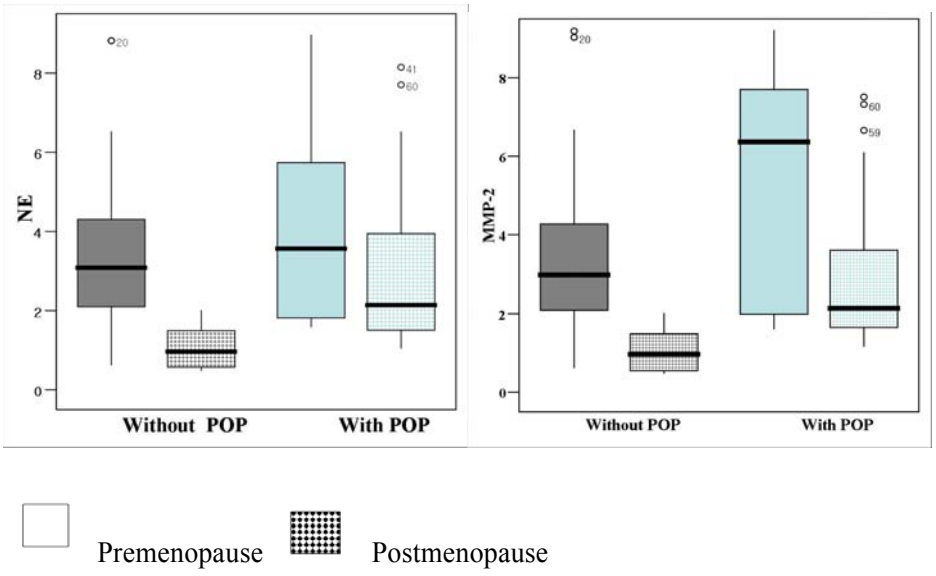

Figure1. Expression of NE, MMP-2 in uterosacral ligaments in women with and without POP combined with premenopause and postmenopause

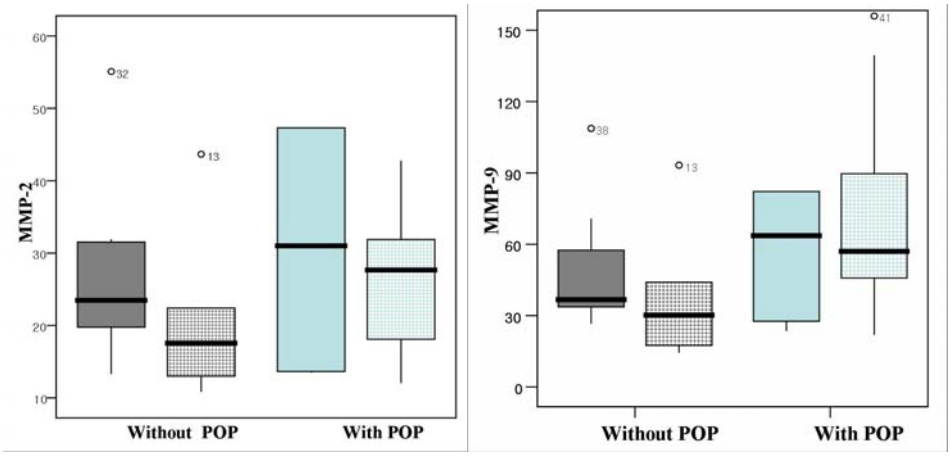

Figure 2. MMP-2, MMP-9 activity in uterosacral ligaments in pre- and post-menopausal women with and without POP

IV. DISCUSSION

The uterosacral ligament and its connective tissues provide support for the pelvic organs[10], and these connective tissues are composed of an ECM rich in collagen and elastic fibers. In this investigation, we focused on the uterosacral ligament and its role in the development of POP. Traditionally, research on the pathophysiology of POP has focused on altered connective tissue and faulty collagen metabolism[14, 15], though recent studies have begun to focus on the role of elastic fiber homeostasis in this process[16-21]. The main components of elastic fibers, which provide recoil to tissues, are elastin and microfibrils. Microfibrils facilitate alignment of elastin monomers prior to cross-linking by lysyl oxidase (LOX). This assembly process, with elaborate spatial and temporal regulation, is complex. Damaged elastic fibers are improperly organized and can malfunction, leading to tissue stiffness[16]. Recently, pregnant female mice with genes involved in defective synthesis (LOX like-1, LOXL-1) or assembly (fibulin-5, Fbln5) of elastic fibers developed spontaneous prolapse shortly after delivery[17,18]. However, defective LOXL-1 mice developed prolapse, Jung et al showed that LOXL-1 levels were two-times higher in POP patients, and increased LOXL-1 levels correlated with advanced POP stage[19]. Rather than focusing synthesis, several studies observed increased degradation of elastin in POP patients[20,21].

The most important finding of this study was the increased elastolytic activity of proteases NE, MMP-2 and MMP-9 in the uterosacral ligaments of women with POP, especially in postmenopause. The majority of studies investigating the role of MMPs in POP biology have focused on the synthesis of the pro-MMP or MMP mRNA. For example, Jackson et al observed that MMP-2 and MMP-9 mRNA were significantly higher in prolapsed tissue than in normal tissue[26]. Likewise, an increase in pro-MMP-2 in POP was confirmed by Gabriel and Phillips[15,27], and Moalli et al observed increased expression of MMP-9 in women with POP[14]. However, MMP-2 and MMP-9 are produced as

zymogens containing a secretory signal sequence and a propeptide requiring proteolytic cleavage for activation. Regulation of the post-translational processing of inactive proenzymes to mature active proteases is a crucial component in the homeostasis of MMP[9], which could play a role in POP. Increased NE activity induced both MMP-2, -9 activities in many disease. NE exposure produced greater amount of active MMP-2 which is decreased in NE knockout mice[22]. MMP-2 activation by NE was blocked by the elastase inhibitor AAT, but not by a MMP inhibitor[23]. Activated MMP-9 by NE recognizes AAT as a degradative substrate, thus indirectly enhance NE activity [24, 25]. But gene expression was different. Increased NE activity did not change MMP-9 gene expression. Unlike MMP-9, MMP-2 gene expression was increased. NE activation increased in gene expression of MMP-2, but MMP-9 gene expression was unchanged[22]. In this study, though MMP-9 gene expression profile remained unchanged, increased activity of MMP-9 was observed in POP patients, which suggest that the increased elastolytic protease gene expression does not always correlate with protease activity.

Vaginal birth and old age are two major risk factors for developing POP. Although there are many studies about the effects of vaginal birth on the incidence of POP[1,18], the effects of age and postmenopausal status on pelvic floor support mechanisms have not been identified. We observed decreased activity of the elastolytic proteases NE, MMP-2, and MMP-9 after menopause. Since MMP expression has been shown to fluctuate in the endometrium, vaginal tissue, and parametrium during the menstrual cycle[28,29], our findings support the theory that elastic fiber homeostasis can be affected by hormonal conditions. Prior studies detected an elevation in both the proactive and active forms of MMP-2 and MMP-9 in women treated with estradiol, which corresponded with a decrease in total collagen content in these women[30]. A combination of upregulation of MMPs and suppression of tissue inhibitor of metalloproteinases (TIMP) by estrogen resulted in increased breakdown of the

ECM[31]. These findings suggest that estrogen increases the connective tissue degradation of the pelvic floor by elastolytic proteases, despite the fact that estrogen deficiency is a known risk factor for POP[32]. Although estrogen deficiency after menopause decreases elastolytic protease activity, women with POP exhibit less of a decrease, which may have lead to POP in the first place.

Previous studies compared ECM homeostasis in premenopausal women with and without POP, and in premenopausal women with POP under various hormonal states; none of these studies were performed in postmenopausal women without POP. We demonstrated that the relative increase in elastolytic protease activity in premenopausal women with POP compared to premenopausal women without POP was not significant; however, this difference was significant in postmenopausal women.

Published reports indicate connective tissue differences in the elastolytic protease in vaginal tissue of POP women [14,26,33]. The uterosacral ligaments are condensations of endopelvic fascia which provide the primary support of pelvic organ, especially uterus in place[10]. The pelvic floor are generally subdivided into three levels of support: the uterosacral and cardinal ligaments (level I), paravaginal connective tissues suspending the lateral vaginal walls to the arcus tendineous and fascia of the levator ani (level II), and the perineal membrane and perineal body (level III)[34]. However, studies using vaginal tissues from women with or without POP reveal information only about the tissue differences at the time of surgery and little about the pathogenesis of prolapse. Prolonged stretch, mechanical stress, and hypoxia within the vaginal wall may produce secondary effects that contribute to the progressive deterioration of pelvic organ support. These factors may be unrelated to the primary pathogenesis of pelvic floor dysfunction[18].

The strengths of this study include the large sample size, the standardized method for tissue collection and processing, the multiple parameters for elastin metabolism analysis, and the blinding of the individuals

performing the assays. Postmenopausal women without POP were utilized as a control group and were compared to postmenopausal women with POP.

Together, our data support the idea that increased elastolytic protease activity can lead to the overall degradation of the ECM in the connective tissues of the pelvic floor and to the development of POP. Furthermore, disruption of the balance between ECM degradation and repair in these connective tissues after menopause may also contribute to the failure of pelvic organ support in women.

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V. CONCLUSION

Increased elastolytic protease activity may lead to overall degradation of the ECM in connective tissues of the pelvic floor and POP. Disruption of the balance between matrix degradation and repair in connective tissues after menopause may also lead to failure of pelvic organ support in women

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< ABSTRACT(IN KOREAN)>

골반장기탈출증에서 탄력섬유 대사의 변화

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문 여 정

목적: 골반 장기 탈출증의 원인으로 결체조직 대사의 이상이 중요하며 우리는 결체조직중 탄력 섬유를 분해하는 것으로 알려져 있는 Neutrophil elastase (NE), Matrix metalloproteinase (MMP)-2, MMP-9와 NE를 억제하는 것으로 알려져 있는 alpha-1 antitrypsin(AAT)의 발현 및 활성도에서 폐경이 미치는 영향에 대해서 연구해보고자 한다.

연구방법: 상기 연구는 연세대학교 세브란스 병원 임상 시험 심사위원회에서 심의를 완료 후 진행하였다. 2009년 5월부터 12월까지 연세 의료원 산부인과에서 골반 장기 탈출증 3기- 4기로 전자궁 적출술을 받은 환자 35명을 환자군으로 모집하였다. 같은 기간동안 골반 장기 탈출증 및 요실금이 없으며 양성 병변으로 전자궁 적출술을 받은 환자 39명을 대조군으로 하였다. 환자군 및 대조군은 각각 폐경 여부에 따라 폐경전 및 후로 나누어 분석하였다. 환자 연령, 출산력, 키, 몸무게등 기본 정보에 대한 조사도 시행하였다. NE, MMP-2, MMP-9, AAT의 유전자 발현을 real time PCR로 확인하였으며 NE, MMP-2, MMP-9 의 activity를 ELISA로 확인하였다. 통계 분석은 ANOVA test, Kruskal-Wallis test, multivariate linear regression을 사용하였다

결과: 유전자 발현 결과 NE, MMP-2는 골반장기 탈출증 환자에서 대조군보다 높게 발현되었다. 대조군에서는 폐경전에 비하여 폐경후에 급격한 발현 감소가 나타나는 반면 환자군에서는 폐경전에 비하여 폐경후에서 지속적으로 발현이 높게 나타났다. NE, MMP-2, MMP-9의 활성도는 환자군에서 대조군보다 높게 나타나며 발현과 같은 양상으로 환자군에서 폐경이후에도 높은 활성도를 보였다.
결론: 탄력 섬유 분해 효소는 골반 장기 탈출증 환자의 자궁 천골 인대에서 높게 발현이 되며 이는 폐경 이후에도 지속적으로 높게 유지가 된 소견을 관찰하였다.

핵심 되는 말: 골반 장기 탈출증, neutrophil elastase, matrix metalloproteinase-2, matrix metalloproteinase-9, 폐경