

# Expression of Matrix Metalloproteinase-1 in Uterosacral Ligaments Tissue of Women with Genital Prolapse

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## ABSTRACT

*Collagen metabolism is altered in the pelvic organ tissues of women with genital prolapse. The aim of this study was to compare collagen metabolism by measuring matrix metalloproteinase – 1 (MMP – 1) expression in uterosacral ligament tissues of postmenopausal women with and without genital prolapse. Uterosacral ligament tissues were obtained at the time of abdominal or vaginal surgery from twenty-four patients with pelvic organ prolapse (POP) and 21 women who underwent gynecologic surgery for benign indications. The tissue samples were analyzed by immunohistochemistry. There were no differences in age, BMI and parity between two groups. The patients with genital prolapse demonstrated significantly higher occurrences of MMP – 1 expression compared to controls. These findings indicate that increased MMP – 1 expression in uterosacral ligaments is associated with genital prolapse. Our data are consistent with the theory that increased collagen breakdown may play an important role in the onset and development of pelvic organ prolapse (POP).*

**Key words:** MMP – 1, uterosacral ligament, genital prolapse

## Introduction

The female urogenital system is supported by pelvic muscles, ligaments, and the bony pelvis, all of which are constantly subjected to stresses and trauma including childbirth. Pelvic organ prolapse (POP) is one of the greatest morbidities influencing the quality of life, and it is common in both developed and developing countries. It is generally believed that POP has multifactorial aetiology, but it is still an enigma that some women develop POP while others with similar risk factors do not. Vaginal childbirth and aging are risk factors, and weakening of the pelvic support structures is a major aspect of the pathology<sup>1–7</sup>. There is some evidence that abnormalities of the connective tissue composition may contribute to the genesis of POP<sup>8–12</sup>. Collagen in the extracellular matrix of pelvic supporting tissues provides strength and mechanical stability. The colla-

gen fibres are responsible for the tensile strength of the tissue, and of those type I is the most common and strongest<sup>11</sup>. The matrix metalloproteinases (MMPs) are a family of structurally related proteins which degrade extracellular matrix<sup>13–15</sup>. Interstitial collagen, type I, the most abundant connective tissue protein, are cleaved by the interstitial collagenase MMP – 1<sup>15</sup>. Thus, MMP – 1 may be critically important to the loss of strength of fibrous collagen and subsequent loss of tissue integrity<sup>13,14</sup>. Increased collagen degradation occurs with, and may be etiologic for POP. The uterosacral ligaments are an important part of the pelvic support system<sup>8,14,16</sup>. The aim of this study was to investigate collagen metabolism by measuring MMP – 1 expression in uterosacral ligaments tissue of women with and without POP.

## Subjects and Methods

Forty-five women took part in this study and they were divided into two groups based on the presence or absence of POP. There were twenty-four women with POP and 21 control subjects. All the patients were post-menopausal and admitted to the hospital for surgery. Women with pelvic or other malignancies, previous pelvic inflammatory disease or pelvic surgery, endometriosis, diabetes, and women with hormone replacement therapy were excluded from the analysis. Biopsies of the left uterosacral ligaments were taken at the time of abdominal or vaginal surgery from 24 women with POP and twenty-one women who underwent gynecologic surgery for benign indications. The evaluation included medical history and gynecologic examination. Signs of POP were recorded during the pelvic examination, particularly speculum and bimanual examination, and were described according to the modified pelvic organ prolapse quantification system<sup>17</sup>. Body mass index (BMI, kg/m<sup>2</sup>) was calculated for every woman. The study was approved by the Ethic Committee of University Hospital Center Split. Informed consent of the subjects was obtained for excision of uterosacral ligaments tissue used in this study.

All biopsy specimens were fixed in formalin and embedded in paraffin, and 3–5 µm sections were mounted onto glass slides and allowed to dry at 37°C for 12 hours.

Immunohistochemistry for MMP-1 was performed on formaline fixed and paraffin embedded sections. Immunohistochemical staining was performed by the hemalaun-eosin method.

A goat monoclonal antibody against activated MMP-1 (dilution 1:10, anti-human MMP-1, AF 901; R&D systems, Minneapolis, USA) was used as the primary antibody.

For immunohistochemistry, specimens were deparaffinized, rehydrated, and then heated in a microwave oven in ethylen – diamin – tetraacetic acid (EDTA).

Each sample was cooled, and washed with phosphate-buffered saline (PBS) and distilled water, and then incubated with H<sub>2</sub>O<sub>2</sub> for blocking endogenous peroxidase during 15 minutes.

All tissue specimens and slides were examined independently by experienced pathologist. The examiner was

blinded to the clinical diagnosis. A score was applied to classify the staining intensity as weak (1+) moderate (2+) or strong (3+).

Statistical analysis was performed with Statistica SPSS version 13.0 (SPSS Chicago, IL) using the Mann-Whitney, T-test and  $\chi^2$ -test. The p-values <0.05 were considered statistically significant.

## Results

Forty-five women participated in the study and their uterosacral ligaments were analyzed. They were 24 women with genital prolapse and twenty-one controls without prolapse. Subject characteristics are listed in Table 1. The mean age of women with genital prolapse was 56.6 years with an average parity of 2.4, and 56.3 years with a mean parity of 2.1 for the controls. The mean BMI of examinees was 25.1, and 24.4 of controls. There were no differences in age, BMI and parity among women with and without POP (Table 1).

Immunohistochemistry was performed to determine the expression of MMP-1. In the POP group 5 samples showed weak, 7 samples moderate, and 12 samples were highly positive for MMP-1. In the non-POP group, 13 samples showed weak, five samples moderate, whereas 3 showed strong MMP-1 expression. The statistical analysis revealed that MMP-1 expression in the POP group was significantly higher than that in the non-POP group ( $p < 0.05$ ) (Table 2).

## Discussion

It has been speculated, that the vagina, pelvic floor, ligaments and supportive tissues in patients with prolapse have a decrease in collagen content<sup>8,10,11,14,16,18</sup>. Thus, in many studies collagen has been analyzed in biopsy specimens from vaginal or supportive tissues procured at the time of repair in patients with prolapse, or time of hysterectomy in patients without prolapse, with conflicting results<sup>8,10,11,14,16,18–25</sup>. The difficulty in quantitating collagen is inherent in its supermolecular structure<sup>19,26</sup>. Indeed, this highly stable protein is virtually impossible to solubilize in most physiological buffers<sup>19</sup>. A

**TABLE 1**  
ANTHROPOMETRIC AND CLINICAL PROFILES OF EXAMINEES

	Controls (N)	POP (N)	p-value
Age (years, $\bar{X} \pm SD$ )	56.3 ± 3.7	56.6 ± 3.2	NS*
BMI ( $\bar{X} \pm SD$ )	24.4 ± 2.5	25.1 ± 2.2	NS*
Parity ( $\bar{X} \pm SD$ )	2.1 ± 1.1	2.4 ± 1.4	NS**

\*t-test

\*\*Mann-Whitney test

NS = not significant

POP – Pelvic organ prolapse

**TABLE 2**  
MMP-1 IMMUNOHISTOCHEMICAL EXPRESSION IN  
UTEROSACRAL LIGAMENTS IN WOMEN WITH AND WITHOUT  
POP

	Score	Controls (N)	POP (N)	p
MMP-1	1+	13	5	<0.05
	2+	5	7	
	3+	3	12	
* $\chi^2$ -test	9.129			

MMP-1 – Matrix metalloproteinase-1

POP – Pelvic organ prolapse

The distribution of MMP-1 expression is given in Table 2

balance of matrix synthesis and degradation are important for tissue integrity<sup>16,19</sup>. Degradation depends on the activity of matrix metalloproteinases. The matrix metalloproteinases (MMPs) are a family of structurally related proteins which degrade extracellular matrix<sup>11,13–16,19,26</sup>. Interstitial collagen, type I, the most abundant connective tissue protein, is cleaved by the interstitial collagenase MMP – 1<sup>14,15</sup>. To overcome these limitations in quantitating collagen, a group of authors have investigated collagen metabolism by measuring MMP – 1 expression<sup>13,14,16</sup>.

In the present study we investigated MMP-1 expression in uterosacral ligaments tissue of women with and without POP. Our study showed that the uterosacral ligaments of the prolapsed uterus are characterized by a higher expression of MMP – 1 compared with women without prolapse. It was suggested that genital prolapse might be due to abnormality in collagen synthesis or an imbalance between synthesis and degradation. It is also known that collagen imparts a great mechanical strength to connective tissues. The mechanical strength of uterosacral ligaments may also depend on the extracellular matrix degradation by expression of MMPs. Therefore, a higher MMP – 1 expression is generally sign of collagen degradation and may be characteristic of tissue

laxity. The results of Chen et al. are similar<sup>14</sup>. Phillips et al. found an increase in proMMP-2 expression but no difference in active MMP-2 or proMMP-9 expressions using zymography in full-thickness vagina in patients with POP<sup>16</sup>. Gabriel et al. found increased active MMP-2, but not MMP-1, expression in the uterosacral ligament by immunohistochemistry in patients with POP<sup>13</sup>. The difference in these results most likely reflects the disparate tissues targeted for analysis and different methods of protein quantification. Nevertheless all this data confirm that remodeling of connective tissue in patients with prolapse is accelerated compared to control subjects. Our findings are consistent with the theory that increased collagen breakdown may play an important role in the onset and development of genital prolapse. It is difficult to be certain that the higher MMP – 1 expression in the prolapsed uterosacral ligaments observed in our study is the cause of prolapse. Many more studies are needed to improve our understanding of this disease.

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## **IMUNOHISTOKEMIJSKA EKSPRESIJA MATRIKSMETALOPROTEINAZE-1 U UTEROSAKRALNIM LIGAMENTIMA ŽENA S GENITALNIM PROLAPSOM**

### **S A Ž E T A K**

Metabolizam kolagena promijenjen je u žena s prolapsom genitalnih organa. Cilj istraživanja bio je usporediti metabolizam kolagena mjerenjem matriks metaloproteinaze – 1 (MMP – 1) u tkivu uterosakralnih ligamenata poslijemeno-pauzalnim ženama sa i bez genitalnog prolapsa. Tkivo uterosakralnih ligamenata uzeto je tijekom abdominalne ili vaginalne operacije u 24 bolesnice sa genitalnim prolapsom i dvadesetjednoj ženi koja je operirana poradi benignih indikacija. Uzorci tkiva analizirani su imunohistokemijski. Nije nađena značajna razlika u životnoj dobi, indeksu tjelesne mase niti broju poroda između ispitivanih skupina. U bolesnica s genitalnim prolapsom uočena je statistički značajno viša izražajnost MMP – 1 u usporedbi s kontrolama. Nalazi ukazuju da je povišena izražajnost MMP – 1 u uterosakralnim ligamentima povezana s genitalnim prolapsom. Naši podaci sukladni su s teorijom kako povećana razgradnja kolagena može igrati važnu ulogu u nastanku i razvoju prolapsa zdjeličnih organa.