

## IMMUNOHISTOCHEMICAL ANALYSIS OF UTEROSACRAL LIGAMENTS IN PATIENTS WITH PELVIC ORGAN PROLAPSE

### Hypothesis / aims of study

The aim of this study is to compare the expression of collagen I, collagen III, collagen IV, fibronectin and laminin in the uterosacral ligaments of women with or without pelvic organ prolapse. Recent studies indicate that pelvic organ prolapse can, at least in part, be defined as a connective tissue disease. High rates of pelvic organ prolapse and urinary incontinence were found in patients suffering from connective tissue disorders (e.g. Marfan syndrome and Ehlers-Danlos syndrome). Moreover, there is some evidence that abnormalities of the connective tissue composition may contribute to the genesis of pelvic organ prolapse also in other patients. The uterosacral ligaments are an important part of the pelvic support system and establish the level 1 support of the cervix and the upper vagina. As such the extracellular matrix composition of the uterosacral ligaments could play an important role in pathogenesis of the pelvic organ prolapse.

### Study design, materials and methods

Forty patients participated in the present study. The first group consisted of twenty women with pelvic organ prolapse, as defined by descent of the cervix to, or beyond, the introitus. The second group comprised of twenty control females with no pelvic organ descent but suffering from any other benign disease of a genital tract (e.g. menorrhagia). Pelvic malignancies were excluded. Biopsies of the right and left uterosacral ligaments were obtained during abdominal, vaginal and laparoscopic hysterectomies. The biopsies were performed at the level of the cervical insertion of the uterosacral ligament to the uterus using a standardized technique. All biopsy specimens were fixed in formalin and embedded in paraffin. The standardized immunohistochemical protocols were used. A panel of extracellular matrix proteins (collagen I, collagen III, collagen IV, fibronectin and laminin) was evaluated by immunohistochemistry. Monoclonal antibodies (Medicorp Inc., Montreal, Canada) against collagen subtypes, monoclonal antibody against laminin (Dako, Glostrup, Denmark) and polyclonal antibody against fibronectin (Dako, Glostrup, Denmark) were used. All slides were separately examined by two experienced pathologists blinded to the clinical diagnosis. Minimal or no staining reaction was scored as ( - ) (Figure 1), a weak reaction was scored as ( + ) (Figure 2) and a strongly positive staining reaction was scored as ( ++ ) (Figure 3). The data were analyzed and the groups were compared. For statistical analysis, the exact Fisher test was used.

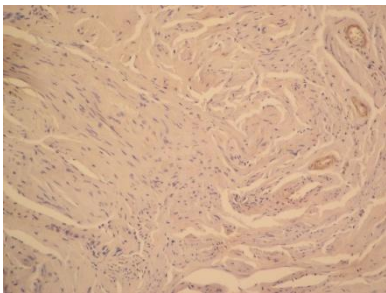


Figure 1: Negative reaction to fibronectin in extracellular matrix of uterosacral ligament (immunohistochemistry).

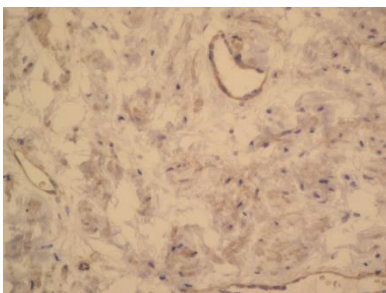


Figure 2: Weak reaction to collagen IV in extracellular matrix of uterosacral ligament (immunohistochemistry).

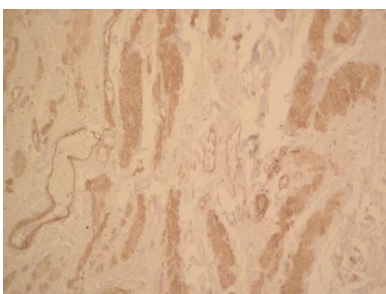


Figure 3: Strongly positive reaction to collagen IV in extracellular matrix of uterosacral ligament (immunohistochemistry).

## Results

The uterosacral ligaments of 40 women were analyzed. We included 20 women in the control group with a mean age of 51 years and 20 women in the pelvic organ group with a mean age of 61 years ( $p < 0.05$ ). There was no difference in parity, menopausal status and use of hormonal replacement therapy. We found no differences in collagen I and collagen III expressions. Both groups showed high expression of collagen III; expression of collagen I was scored as weak or strong but without any difference between the two groups. Consequently, we found no alteration in ratio between collagen I and collagen III. Importantly, women with pelvic organ prolapse revealed a significantly higher collagen IV expression than women in control group ( $p = 0.0097$ ; Figure 2, 3) as well as a higher expression of fibronectin but in this case the difference was not statistically significant ( $p = 0.114$ ). On the other hand, the expression of laminin was low in both groups. However, it was even lower in the pelvic organ prolapse group where all samples but two expressed no laminin but the statistically significant level was not reached ( $p = 0.096$ ) due to the size of the set. We revealed no significant differences in expression patterns between left and right uterosacral ligaments.

## Interpretation of results

The uterosacral ligaments of women suffering from pelvic organ prolapse show significantly higher collagen IV expression compared to control women. Fibronectin expression was also higher but not statistically significant. Generally low expression of laminin in uterosacral ligaments was even lower in the pelvic organ prolapse group. Expressions of collagen I and collagen III are constantly high irrespective of pelvic organ prolapse. This study contributes to the evidence on connective tissue alterations in pelvic organ prolapse. Further research in this field aimed to clarify the etiology and pathogenesis of pelvic organ prolapse is needed.

## Concluding message

Immunohistochemical analysis of the uterosacral ligaments in women with pelvic organ prolapse revealed the significantly higher expression of collagen IV; no alteration in ratio between collagen I and collagen III expression and generally low expression of laminin.

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<i>Is this a clinical trial?</i>	<b>No</b>
<i>What were the subjects in the study?</i>	<b>HUMAN</b>
<i>Was this study approved by an ethics committee?</i>	<b>Yes</b>
<i>Specify Name of Ethics Committee</i>	<b>Ethics Committee of the Teaching Hospital Na Bulovce, Budinova 2, Praha 8, Czech Republic</b>
<i>Was the Declaration of Helsinki followed?</i>	<b>Yes</b>
<i>Was informed consent obtained from the patients?</i>	<b>Yes</b>