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Endoglycosidase expression in pubocervical fascia is up-regulated in menopause patients with severe pelvic organs prolapse

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Introduction

Pelvic organs prolapse (POP) in women is very inconvenient age-dependent pathology. The incidence of women with POP in USA and other countries is reaching up to 50% in patients aged 80 years [1].

The understanding of POP's pathophysiology is important for prevention and treatment. Presumably POP deals with so called undifferentiated dysplasia of connective tissue (UCTD). UCTD is genetically determined change in the morphogenesis of connective tissue due to abnormalities of fibrillogenesis and non-fibrillar extracellular matrix (ECM).

In POP it was known the expression changes mainly focusing on the genes/proteins of collagen, elastin, matrix metalloproteinases (MMPs) and their tissue inhibitors [2].

Besides MMPs the ECM also contains at least one enzyme involved in connective tissue remodeling, which has an endoglycosidase activity and is named **heparanase** (HPSE).

1. Nygaard I. et al. (2008) Prevalence of symptomatic pelvic floor disorders in US women. JAMA. 300: 1311–1316.
2. Lim V.F. et al. (2014) Recent studies of genetic dysfunction in pelvic organ prolapse: The role of collagen defects. Aust N Z J Obstet Gynaecol. 54: 198–205.

The aim

of this work was to study HPSE expression
in pubocervical fascia of menopause women with POP

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Methods and Algorithms

This study was performed on 150 women 60,7±7,73 years old with POP, the diagnosis was based on the POP Quantification (POP-Q ISC, 1996).

The average duration of menopause was 10,9±9,1 years. Women with malignances and/or active/latent urinary tract infection were excluded. All 150 patients were suitable for POP-Q III-IV (Ba) and underwent primary scheduled treatment using vaginal access surgery; also in all cases the informed consent of the patient to the examination and treatment of POP was taken in accordance with the directives of the European Community (86/609 / EEC) and the Helsinki Declaration.

Patients were divided into 2 representative by POP's severe groups, depending on the UCTD degree.

The 1st group consisted of 48 women with mild degree of UCTD.

The 2nd group consisted of 102 women with moderate and severe degree of UCTD.

Phenotypic manifestations of UCTD were evaluated on the basis of physical examination and ultrasound study of the heart and pelvic organs, focusing on pathological renal mobility.

The minimal manifestations of UCTD included joint hypermobility, mild skin striae and/or myopia.

Their combination with signs of the «MASS phenotype» (mitral valve prolapse, additional heart chords, scoliosis, and so on) were interpreted as moderate and severe degree of UCTD [3].

3. Smolnova T.Yu. et al. (2003) The phenotypical symptom complex of connective tissue dysplasia in females. Clin. Med. 8: 42-47.

Methods and Algorithms

Surgical samples (5 per each group) of the pubocervical fascia were fixed in 10% buffered formalin solution.

For immunohistochemistry (IHC), 5-mkm sections of formalin-fixed, paraffin-embedded tissue sections were deparaffinized and antigens were retrieved in sodium citrate buffer (10 mM sodium citrate, 0,05% Tween-20) at 95-98°C for 20 min.

The rabbit polyclonal anti-HPSE («Abcam», cat. № ab85543,1:100) primary antibodies were used.

HPSE-immunostaining patterns were visualised using Histostain-Plus 3rd Gen IHC Detection Kit (ThermoFisher Scientific).

The sections were counterstained with Hematoxylin and observed by light microscopy using Axio Scope.A1 microscope with the camera AxioCam MRc5 and software ZEN blue for the quantitative analysis of 30 images per group with magnification 40x10 (Zeiss, Oberkochen, Germany).

Statistical processing of the results was performed using the statistical software application package STATISTICA v.6.0. A value of $p < 0,05$ was considered to indicate a statistically significant difference.

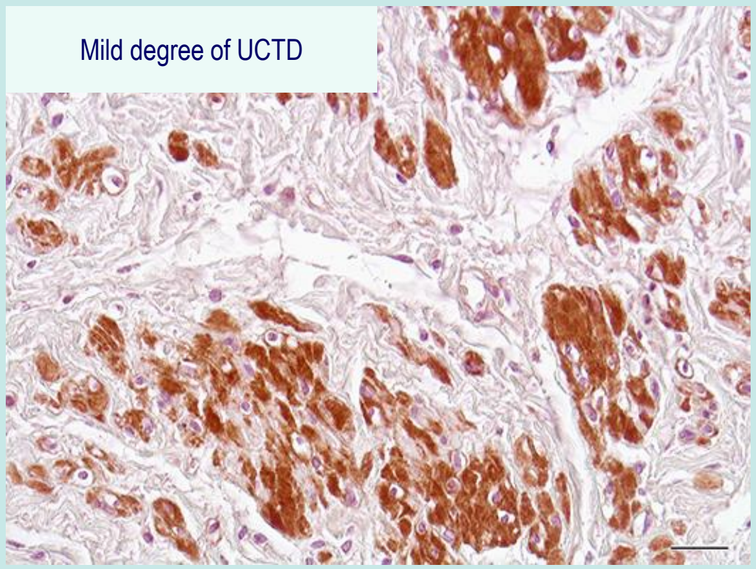
Results

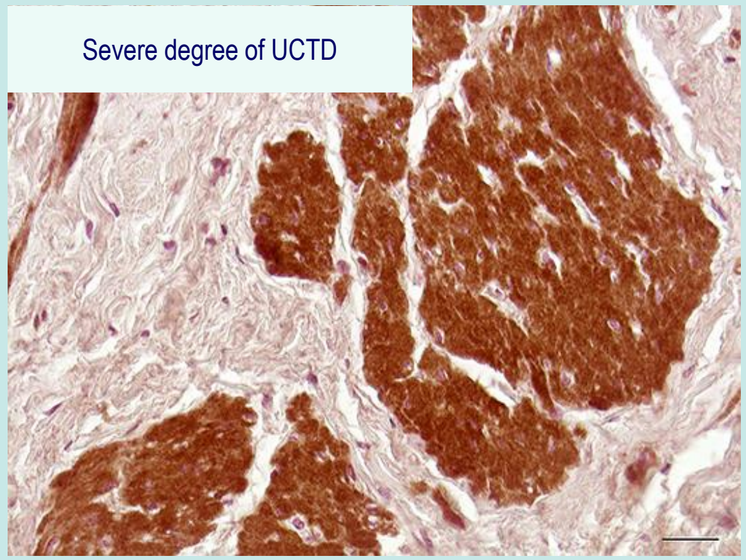
An analysis of the incidence of clinically polymorphic symptoms and diseases inherent to the UCTD in 150 women of 1st and 2nd groups have revealed the genital prolapses and hernia as universal UCTD manifestations in first-line relatives of 40% and 49% cases, respectively.

In women of the 2nd group, varicose veins occurred in 79,4% comparing 33,3% cases in the 1st group. And in contrast to the 1st group, in the 2nd group, the biliary dyskinesia was detected in 45% of women, and heart rhythm and conduction disturbances were revealed in 48% ones

The investigation a hypothesis on a potential negative effect of HPSE content on the POP pathogenesis, the dense fibrous connective tissue of pubocervical fascia was studied with emphasis on the intra- and extracellular endoglycosidase localization.

HPSE protein content was significantly up-regulated in cellular compartment of pubocervical fascia of the 2nd group with prominent UCTD degrees compared with the 1st one: the average area of total positive immunostaining products amounted as 2,4±0,6 mm^2 versus 1,3±0,5 mm^2 ($p < 0,01$).





Heparanase expression.
IHC. Rabbit anti-HPSE («Abcam», 1:100). Magn.x 400.

Conclusion

HPSE known as heparan sulfate cleaver and active participant in different pathological processes such as inflammation and tumorigenesis may affect the ECM properties of pubocervical fascia and should serve as new molecular marker of UCTD and POP.

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