

## PROTEOMIC ANALYSIS OF PUBOCERVICAL FASCIA OF WOMEN WITH URODYNAMIC STRESS INCONTINENCE AND PROLAPSE COMPARED TO ASYMPTOMATIC CONTROLS.

### Hypothesis / aims of study

Pelvic Organ Prolapse (POP) and Stress Urinary Incontinence (SUI) are multifactorial diseases that negatively affect the quality of life of millions of women. The pathophysiology of these diseases is not fully elucidated. All previously examined tissue specimens from women with POP were examined with techniques that measured differences of expression of specific proteins directly (immunofluorescence microscopy, radioimmunoassay) or indirectly (quantitative real time PCR mRNA assessment). Gene studies of tissues from women with POP detected a number of candidate genes that could lead to altered pelvic support. [1] Whole genome microarrays were later used to identify genes involved with elastin metabolism in vaginal tissue from women with stress urinary incontinence without clinical POP. [2] Microarray analysis of 32878 genes from uterosacral and round ligament samples from women with POP and controls identified genes committed to Immunity and cellular Defense. [3] Gene profile of a specific tissue isn't the same as protein profile. Proteomic analysis will help us define if genes identified by microarray analysis are also translated to functional proteins. To our knowledge, this is the first study in humans that uses the advanced methods of proteomics to investigate and compare the pattern of protein expression of pubocervical fascia in women with USI with or without POP versus asymptomatic controls.

### Study design, materials and methods

**Materials.** In the present study, we used a combined approach, based on two-dimensional electrophoresis (2DE) and mass spectrometry (MS), in order to compare the protein composition of pubocervical fascia from four women with USI combined with POP and three asymptomatic controls. They all had urodynamic studies to assess the functional status of the lower urinary tract (LUT) and an assessment of their prolapse with the use of POPQ. Control women were admitted to have a hysterectomy for benign gynaecological conditions. Ethical approval was obtained and all participants gave consent for the study. Specimens of the pubocervical fascia were obtained at surgery and were placed in liquid nitrogen for transportation to the laboratory and were stored at -80°C until usage.

### Methods

1. Two-Dimensional Gel Electrophoresis (2DE).
2. Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS).
3. Western Blot Analysis.

### Results

The most significant difference in protein expression between patient and control groups was obtained for an actin-binding protein, transgelin that was 36,2 times upregulated in patient group. Besides transgelin two additional proteins related to actin and myosin were found to be overexpressed in patient group. More specifically, smooth muscle gamma-actin was 3,2-fold and myosin light polypeptide 6 was 4,4-fold upregulated. Furthermore, the precursor of alpha-1 antitrypsin was 4,3 times overexpressed in women with USI/POP. We identified also five proteins that were more than two-fold overexpressed. More specifically, the precursor of alpha-1-acid glycoprotein 1 was 4,1-, Galectin-1 was 5,7- and beta subunit of haemoglobin was 6,6- fold upregulated in patient group. We also identified two spots corresponding to transgelin isoforms to be 3,8 and 7,5 times downregulated in patient group and two spots corresponding to the precursor of serum albumin isoforms to be 34- and 3,98-fold overexpressed in patient group. Finally, two protein spots were found to be present only in gels obtained from patients and were absent in gels obtained from controls. Type I keratin cytoskeletal 10 (CK10) and two spots that correspond to transgelin isoforms were only present in patient group.

### Interpretation of results

It is possible that the overexpression of cytoskeletal proteins, like smooth muscle gamma-actin, could be a mechanism to balance the loss of support in women with USI/POP, since cytoskeleton is known to be responsible for cell stability and support.

Transgelin binds actin leading to actin gelation. Recently, transgelin has been found to be a repressor of MMP-9 expression. One of the physiological roles of MMP-9 is the cleavage of the extracellular matrix components, such as elastin and collagen type IV and proteoglycans. The excess of transgelin in these women could lead to the inhibition of MMP-9 expression, resulting in suppression of the cleavage of the extracellular matrix proteins that happens in prolapsed tissues. Thus, transgelin overexpression could represent a defence mechanism against the prolapse process and could also depict the transition of myofibroblasts to contractile SMCs that could enhance tissue stability for pelvic support. Although transgelin expression was found to be upregulated, none of the metalloproteinases (MMPs) showed any differences in their expression. The physiological role of alpha-1 antitrypsin is to inhibit elastase to cleave elastin, a component of the connective tissue. Elastin allows the tissue to stretch and return to its original shape without energy input. The accumulation of the precursor molecule probably shows that there is a defect in the mechanism that converts it into the mature alpha-1 antitrypsin enzyme which could result in the activation of elastase that causes degradation of elastin of the connective tissue and loss of pelvic organ support.

Another actin- and myosin-related protein that was found to be upregulated in our study is myosin light chain polypeptide 6. Myosin proteins are composed of both heavy and light chains and are essential part of skeletal muscles involved in muscular contraction. Although it is known that myosin heavy-chains play a role in the speed of contraction, the role of myosin light chains, overexpressed 4,4 times in patients of our study, is not clear yet.

Galectin-1 is a lectin that causes apoptosis interacting either with cell surface glycoproteins or with intracellular proteins. It is possible that overexpression of Galectin-1 is apoptotic for the cells of pubocervical fascia leading to degradation of the tissue and thus the loss of pelvic organ support.

Apart from the proteins that were found to be upregulated in patient group, we also found three protein spots that seem to be present only in patients. Two of them correspond to transgelin isoforms and one spot was identified as Type I Keratin cytoskeletal 10 (CK10), a component of the intermediate filaments of the cytoskeleton. This is in contrast to a previous study that showed that

another keratin gene (acidic keratin I) was underexpressed in patients with POP compared with controls. However, there is a great variety of Keratin proteins and it is possible that in pubocervical fascia which we used in our study the expression of other types of Keratins are downregulated. Therefore, in the future we can use Western blot analysis to compare the expression of different types of Keratins in tissues isolated from both patients and controls investigating possible tissue specificity of these proteins.

#### Concluding message

To our knowledge this pilot study is the first study in humans to estimate changes in protein expression related to pelvic organ prolapse. The differentially expressed proteins identified in pubocervical fascia from women with POP/USI could be related to the pathophysiology of POP. Nevertheless, follow-up experiments in a larger group of patients are necessary in order to verify and evaluate the relevance of our findings. Functionality assays of SMCs isolated from pubocervical fascia could also help elucidate the interplay between MMPs and their repressors.

#### References

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