

Candoso B¹, Meneses M J², Alves M³, Sousa M⁴, Oliveira P⁵

1. Oporto Hospital Center - Urogynecology Department at Maternal Infant North Centre, Porto, Portugal, **2.** Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal, **3.** CICS-UBI-Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal, **4.** Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Unit for Multidisciplinary Research in Biomedicine (UMIB), University of Porto, Porto, Portugal., **5.** Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Unit for Multidisciplinary Research in Biomedicine (UMIB), University of Porto, Porto, Portugal

DIFFERENCES IN COLLAGENOLYSIS IN FEMALE STRESS URINARY INCONTINENCE BY URETHRAL HIPERMOBILITY VERSUS INTRINSIC SPHINCTER DEFICIENCY

Hypothesis / aims of study

Stress Urinary Incontinence (SUI) is defined by the International Continence Society (ICS) as leakage resulting from increased abdominal pressure. From Petros and Ulmsten (Integral Theory) and DeLancey (Hammok hypothesis) this occurs from lack of support that can have an explanation in damage of the pelvic connective tissue, including biochemical changes. In the most recent Joint Report of International Urogynaecology Association (IUGA) and ICS in Terminology for female pelvic floor dysfunction, the diagnose of Intrinsic Sphincter Deficiency (ISD) is ignored. In an era where some surgeons think that the difference between SUI by Urethral Hypermobility (UH) and by ISD is only of historical importance, it is important to understand if there are significant biological differences between these two disorders. It is proven from scientific literature that in SUI and Pelvic Organ Prolapse the quantitative expression of extracellular matrix metalloproteinases (MMP) and their inhibitors (TIMP) plays an important role in collagen degradation of vaginal and pelvic floor tissue (1). Numerous studies have also proved that the quantity of collagen in endopelvic fascia or skin is significantly less in women with SUI compared with continent women, and that the difference is not due to collagen synthesis (2). From previous works we know that the activity of MMP-2 is dependent of TIMP-2 and that MMP-9 and MMP-1 are inhibited by TIMP-1. Alpha-2-macroglobulin (A2McG) has been described as an extracellular proteinase inhibitor with the unique ability to inhibit almost all known proteases (among which are comprehended the MMPs).

Study design, materials and methods

To study the mRNA expression of MMP-1, MMP-2, MMP-9, TIMP-1, TIMP-2 and A2McG, between two groups in a total of 30 patients. Each group had one or two criteria for SUI with Urethral Hypermobility (UH) or by Intrinsic Sphincter Deficiency (ISD). Our goal was to understand if there are any biological tissue differences between these two groups, as one was described as provoked by a deficient support of the urethra dependent of levator ani muscle, endopelvic fascia and its connection to tendinous arch and the other by a deficiency in urethral sphincter, dependent of smooth and striated muscle and venous plexus of urethral wall.

On outpatient clinics of Urogynaecology, we proceeded to the selection of 30 patients, 15 with Stress Urinary Incontinence, by UH and 15 with UI by ISD. Patients could present stress urinary incontinence or mixed incontinence, diagnosed after questionnaire, physical examination and urodynamic studies when justified. The first group presented UH with Abdominal Leak Point Pressure (ALPP) above 90 cm H₂O with Maximal Urethral Closure Pressure (MUCP) above 20 cm H₂O and the second group did not have UH and had ALPP below 60 cm H₂O or MUCP below 20 cm H₂O. After written informed consent was obtained, we collected a sample of about 1 cm of vaginal sub urethral mucosa that was transported in saline to the cellular biology laboratory. The mRNA expression levels of the three MMPs (MMP1, MMP2 and MMP9) and of the specific endogenous TIMPs (TIMP1, TIMP2 and A2McG) was evaluated in each biopsy specimen using the quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) technique. For that, extraction of total RNA (RNAT) was performed using the EZNA Total RNA Kit (Omega Biotek, Norcross, USA) and RNAT was reversely transcribed into cDNA. qRT-PCR was performed using specific exon-spanning primers, designed for the amplification of the target and housekeeping transcripts (the same that were used in RT-PCR). Amplification conditions comprised an initial denaturation step of 3 min at 95°C, followed by 35 or 40 runs of a 3 steps cycle: a denaturation step of 3 s at 95°C; an annealing step of 30 s with a specific temperature for each set of primers; and an extension step of 10 min at 72°C. Fold variation of the expression levels was calculated following the mathematical model proposed by Pfaffl (2001), using beta-2-microglobulin as housekeeping gene. The normality of data was evaluated using the Kolmogorov-Smirnov test. Statistical analysis was performed using the GraphPad Software (San Diego, CA, USA). Statistical differences between the experimental groups were assessed by unpaired two-tail Student's t test. Identification of the outliers was performed using the ROUT Method (Q = 5.0%). All experimental data are shown as Mean±SEM (n=15 for each condition) and p<0.05 was considered significant. Data correlation was evaluated by assuming a two-tailed Gaussian distribution, with a confidence interval of 95%.

Results

Concerning the expression of MMPs the main difference between the two samples was found in MMP-2 with a significant statistical difference (p=0,042), though MMP-1 and MMP-9 had also a higher expression in ISD. Expression of TIMP-1 was two times higher in ISD, TIMP-2 with an expression similar in both samples, A2McG was two times more elevated in UH, but it did not reach a statistically significant difference between the two groups. In Ratios, significant statistical differences were between MMP-9/TIMP-1 (p=0,0045) and MMP-9/A2McG (p=0,0072). The ratios between MMP-1/TIMP-1 and MMP-1/A2McG, were very similar and there was a tendency of higher ratio in ISD to MMP-2/TIMP-2 and MMP-2/A2McG.

Interpretation of results

As MMP-1 is a protease capable of cleavage of the triple helical collagen and MMP 2 and MMP-9, gelatinases that degrade the products from this cleavage, producing weaker and disorganized collagen, we conclude that in our sample, protease MMP-1 and collagenases MMP-2 and MMP-9 had a higher expression in ISD group although only MMP-2 reached a statistically significant difference. The expression of MMPs inhibitors like TIMP-1 were found more increased in the ISD group, trying to counteract the

initial cleavage, but its action to inhibit the expression of MMP-9 was clearly insufficient as we see in the ratio MMP-9 /TIMP-1 that is statistically significantly decreased in ISD. A2McG had a lower expression in ISD in all ratios, which means higher collagenolysis in this group. This was statistically significant in the inhibition of MMP-9.

Every MMP has a higher expression in ISD and TIMPs did not responded as expected to avoid collagenolysis. TIMP-2 has no rise for compensating the significant expression of MMP-2 in ISD. The ratio values between MMP-1/TIMP-1 were almost identical in both groups. To inhibition of collagenolysis happen we should have a higher ratio in ISD group. We also had a higher value in the ISD for MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios reaching a statistically significant difference, which also suggests more collagenolysis in ISD. All ratios between MMPs and A2McG, were higher in ISD group and the one with statistically significant difference was MMP-9/A2McG, which means that collagenolysis dependent from this inhibitor was also not prevented.

Concluding message

Our work suggests that what we thought about the aetiology of SUI by UH or ISD probably has no clear explanation in former theories. A higher collagenolysis is demonstrated in our sample for ISD, which probably means that we cannot blame the sphincter mechanism alone for this pathology. A greater sample would probably produce more statistically different results, and more studies are needed to validate or not this new way of understanding ISD

References

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It was approved by the Ethical Commission of the hospital and was conducted following the Declaration of Helsinki principles. Informed consent was obtained from every patient. **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** It was approved by the Ethical Commission of the hospital - Centro Hospitalar do Porto, Portugal **Helsinki:** Yes **Informed Consent:** Yes