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PROCOLLAGEN C PROTEINASE AND LOX ENZYMES EXPRESSION IN VAGINAL TISSUE OF WOMEN WITH AND WITHOUT PELVIC ORGAN PROLAPSE

Hypothesis /Aims of Study: The integrity of the pelvic floor support is maintained by complex interactions between muscles and connective tissue of the urethra, rectum and vaginal wall. Lysyl oxidases (LOX) family proteins and Procollagen C Proteinase (PCP) are essential for the collagen and elastin biogenesis, the two major proteins of the connective tissue. LOX are involved in the maturation of collagen and elastin by cross-linking their precursors: procollagen and tropoelastin. Deficiency in Lysyl oxidase-like-1 (LOXL1) led to weakness of vaginal wall and severe POP in mice shortly after vaginal birth [1]. It was reported earlier that the LOX, LOXL1 and LOXL3 family gene and protein expression was decreased in the vaginal tissue of premenopausal women with severe pelvic organ prolapse (POP) compared to controls [2]. The expression of LOX proteins in the pelvic floor tissues is influenced by ovarian hormones variation in young women. However, studies of LOX expression in postmenopausal women are still lacking. PCP is involved in both collagen and LOX maturation, activating and releasing immature forms into the active proteins. Besides being a biological control point for the regulation of extracellular collagen deposition, PCP has not studied in women with POP. Also, the influence of age and menopause in PCP content has not being addressed in vaginal tissue of women. We hypothesize that the expression of LOX family and PCP enzymes in vaginal tissue is (1) altered in patients with POP and (2) affected by age-related hormonal status. Studying vaginal tissue, we aim (1) to examine the gene and protein expression of LOX family enzymes (LOX, LOXL1-4) in postmenopausal women with advanced POP compared to controls; (2) to study PCP gene and protein expression in pre and postmenopausal patients with severe POP, compared to controls and (3) to analyze the gene expression of LOX family and PCP of premenopausal in comparison to postmenopausal healthy women.

Study Design, Material and Methods: Caucasian women undergoing total hysterectomy for benign conditions were recruited and divided in 4 groups: premenopausal women with advanced POP and controls, and postmenopausal women with POP and control. We considered stage ≥ 3 by POP-Q classification as advanced POP and stage 0 as controls. During the surgical procedure, 1 cm^2 of full thickness vaginal tissue was obtained from the surgical cuff. The samples were immediately frozen in liquid nitrogen and storage at -80^oC until analysis. Total RNA and protein were extracted using TRIZOL and RIPA Buffer, respectively. Real time PCR was performed to quantify mRNA levels and Western Immunoblot used for protein level analysis. We would need 15 patients in each group to have 80% power to detect differences in these genes using independent groups for testing. Mann-Whitney test ($p<0.05$) was used for statistical analysis.

Results: 39 premenopausal (26 patients and 13 controls), and 18 postmenopausal women (13 patients and 5 controls) were enrolled. Vaginal biopsy samples from patients and controls expressed all four members of LOX family proteins and PCP. LOXL2 and LOXL3 ($p<0.05$) genes were significantly down-regulated in 3-fold change in postmenopausal POP patients compared to controls, whereas LOX, LOXL1 ($p<0.05$) and LOXL4 were increased. However, LOXL1 protein expression was not different between patients and controls. In contrast to the gene expression, LOXL2 protein expression was significantly higher in postmenopausal patients compared to controls ($p<0.05$). PCP gene expression was significantly down-regulated in both pre and postmenopausal POP patients compared to controls ($p<0.001$), both in 5-fold change. Following the same trend as gene expression, PCP protein expression was found down-regulated in postmenopausal patients when compared to controls, difference not observed in the premenopausal groups. LOXL2 gene expression was significantly higher in postmenopausal controls ($p<0.05$), being up-regulated by 10-fold change after the menopause.

Interpretation of Results: Our results detected changes in gene and protein expression involved in the collagen and elastin metabolism in vaginal tissue of women with POP which allow us to propose that impaired expression of LOX and PCP enzymes may result in defective assembly of pelvic tissues and development of POP. Differentially from premenopausal groups, LOXL1 gene expression increased in postmenopausal patients compared to controls, which shows the influence of age and hormonal status in the vaginal connective tissue. As we expected, the PCP gene expression was significantly down-regulated in POP patients in both pre and postmenopausal groups which confirms our hypothesis that PCP is crucial for the maintenance of the pelvic floor support. We believe that PCP pathway is one biological control point for the regulation of extracellular collagen deposition and possible target for future therapeutic intervention in POP.

Concluding Message: Patients with severe POP showed altered expression of genes and proteins regulating collagen and elastin metabolism which can be associated with the POP development. Age-related hormonal status influences the expression of enzymes regulating the extracellular matrix biogenesis in vaginal tissue.

References

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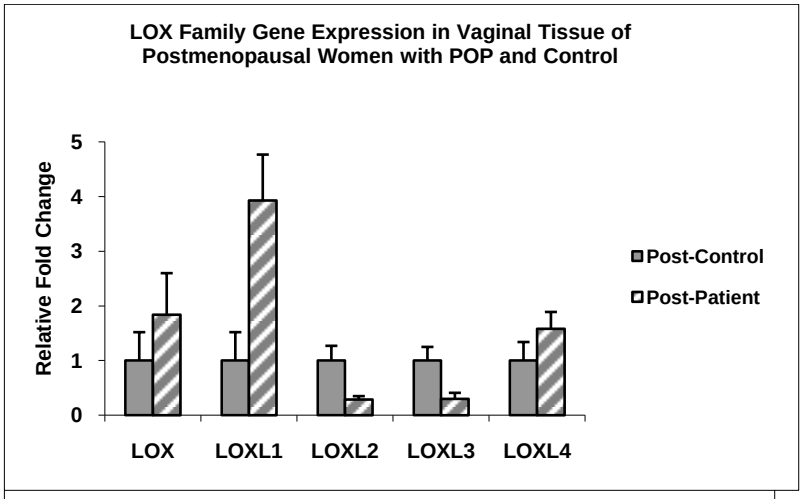

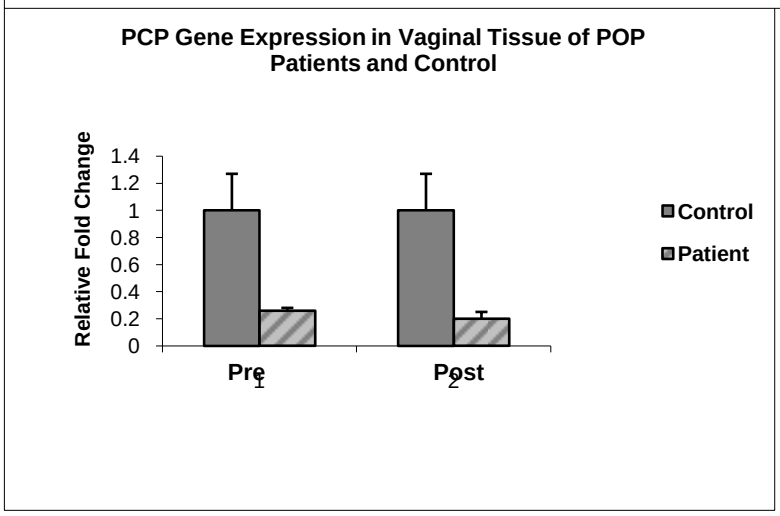

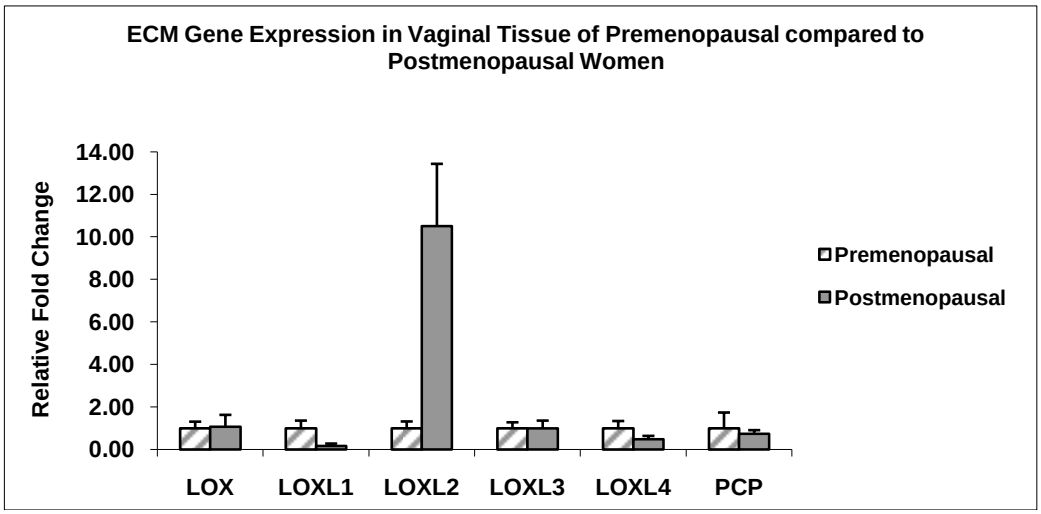

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Was informed consent obtained from the patients?	Yes