

LYSYL OXIDASE ENZYMES EXPRESSION IN VAGINAL TISSUE OF PREMENOPAUSAL WOMEN WITH PELVIC ORGAN PROLAPSE

Hypothesis / aims of study

The female reproductive organs are rich in elastic fibres that undergo massive remodelling throughout pregnancy and birth. Recent animal studies have shown that a failure to maintain elastic fibre homeostasis in mice causes pelvic floor disorders.¹ We hypothesize that faulty Elastin synthesis in pelvic floor tissue may play a role in the pathogenesis of pelvic organ prolapse (POP) in women. Lysyl oxidase (LOX) and Lysyl oxidase like LOXL1, LOXL2, LOXL3, LOXL4, Fibulin-5, Fibrillin-1, and Fibrillin-2 are proteins involved in elastin metabolism. We aimed to study the expression of these genes and proteins as well as their the *in situ* localization of LOX, LOXL1, LOXL3 and LOXL4 proteins in the vaginal tissue of patients with advanced POP and compare the findings to asymptomatic controls.

Study design, materials and methods

Pre-menopausal Caucasian patients affected by POP (≥grade 3 by POP-Q), and control patients (no POP) matched for age and BMI undergoing vaginal and abdominal hysterectomy respectively were recruited. Hospital Ethics Board Approval was obtained. During the surgical procedure and after informed consent, full thickness anterior vaginal epithelial tissue was obtained from the surgical cuff of POP patients and asymptomatic controls. The tissue samples were immediately placed in liquid nitrogen and stored at -80°C. Total RNA was extracted using TRIZOL. Real time PCR was performed to quantify mRNA level of each gene. Total protein was extracted using RIPA lyses buffer and Western immunoblot analysis was performed. Part of vaginal biopsy tissue samples were fixed in PFA and used for Immunohistochemical analysis.

Results

From January to December 2006, 12 patients and 21 controls met our selection criteria. Patients with POP, and asymptomatic controls had a mean parity of 2.6 (range 1-4), and 1.6 (range 0-4) respectively, (P=0.01). Positive family history of POP was significantly different in patients versus controls (P=0.01). All POP patients and controls included in our study were in the proliferative phase of the menstrual cycle as confirmed by endometrial histology. We also collected samples of asymptomatic controls in the secretory phase of the menstrual cycle. The expression of all genes we have studied was detected in vaginal samples. The mRNA expression of LOXL3 and LOXL4 were reduced by 50-60% (P=0.01 and P=0.04, respectively). Additionally, the expression of LOXL4 gene in the vaginal tissue of the control group was significantly higher in the proliferative phase of the menstrual cycle compared to the secretory phase (P=0.01), implicating a potential regulation of this gene by reproductive hormones.

Western immunoblot analysis of vaginal biopsy samples from both patient and control groups confirmed that all four members of LOX family proteins were expressed: LOX (47 kDa pro-form and 35 kDa active form), LOXL1 (47 kDa pro-form and 35 kDa active form), LOXL3 (83 kDa) and LOXL4 (84 kDa). The pro-form of LOX protein showed a statistically significant reduction in its expression (P= 0.01).

LOX, LOXL1, 3 and 4 proteins were detected by immunohistochemistry in all three layers of vaginal skin biopsies: (1) stratified squamous epithelium; (2) the lamina propria and (3) the muscularis layer from both patients with POP and asymptomatic controls. Importantly, in both groups we detected a numerous macrophages scattered throughout the vaginal biopsy samples which displayed a very strong immunostaining to LOXL-1.

Interpretation of results

The homeostasis of vaginal ECM relies upon coordinated biosynthesis and biodegradation. The LOX family of enzymes plays a crucial rule in ECM proteins assembly and their reduction may result in loss of tissue strength and resilience resulting in mechanical failure in the pelvic floor. The expression of LOXL1 enzyme by resident macrophages is a fascinating finding that may add to the functional diversity of these unique cells.

Concluding message

Patients with severe POP showed reduced expression of proteins regulating collagen and elastin biogenesis. Our finding raises the possibility that failure of ECM assembly and repair could underlie the etiology of POP in women

References:

- 1- Am J pathol (2006) 168; 519-28

Specify source of funding or grant	Department of Obstetrics & Gynecology, Mount Sinai Hospital, Toronto, Canada
Is this a clinical trial?	No
What were the subjects in the study?	HUMAN
Was this study approved by an ethics committee?	Yes
Specify Name of Ethics Committee	Research Ethics Board, Mount Sinai Hospital,
Was the Declaration of Helsinki followed?	Yes
Was informed consent obtained from the patients?	Yes