UROGYNECOLOGY

Changes in tissue composition of the vaginal wall of premenopausal women with prolapse

Manon H. Kerkhof, MD; Alejandra M. Ruiz-Zapata, MSc; Herman Bril, MD, PhD; Maaike C. G. Bleeker, MD, PhD; Jeroen A. M. Belien, PhD; Reinout Stoop, PhD; Marco N. Helder, PhD

OBJECTIVE: The objective of this study was to compare histological and biochemical features of the (normal) precervical anterior vaginal wall and the prolapsed anterior vaginal wall of women with pelvic organ prolapse (POP). These data were compared to tissue of the precervical anterior vaginal wall of age-matched controls without POP to identify possible intrinsic and acquired effects.

STUDY DESIGN: Biopsies were collected from the apex of the anterior vaginal cuff after hysterectomy from a control group of 13 premenopausal women undergoing hysterectomy for benign gynecological diseases, and a case group of 13 premenopausal women undergoing prolapse surgery (cystocele POP-Quantification stage $\geq$2). In women with POP an additional full-thickness vaginal wall sample was taken from the POP site during anterior colporrhaphy. Histomorphometric and biochemical analysis were performed for different components of the extracellular matrix.

RESULTS: There were no differences between case and control group in the precervical vaginal wall tissue with respect to the different components of the extracellular matrix and the biochemical parameters. However, there was a tendency toward a higher amount of collagen III and elastin, and a significant increase of smooth muscle cells and pyridinoline collagen cross-links in the POP site compared to the non-POP site of the same POP patient.

CONCLUSION: Our findings suggest that the changes seen in connective tissue in the anterior vaginal wall of women with POP are the effect, rather than the cause, of POP.

Key words: collagen, cross-linking, elastin, pelvic organ prolapse, smooth muscle cells

Cite this article as: Kerkhof MH, Ruiz-Zapata AM, Bril H, et al. Changes in tissue composition of the vaginal wall of premenopausal women with prolapse. Am J Obstet Gynecol 2013;210:168.e1-9.

Pelvic organ prolapse (POP) is a global health problem, affecting adult women of all ages. It decreases their quality of life considerably.[1,2] POP is one of the most common reasons for gynecological surgery in women after the fertile period. The failure rate of surgery is relatively high: an estimated 30% of women require reoperation.[2] Despite this, little is known about the underlying pathophysiology of POP.[3,4]

For decades, it has been speculated that POP occurs due to a structural defect in the vagina and its supportive tissues. Such defects could be a decrease in collagen content and quality, differences in collagen subtypes, changes in the amount and quality of elastin, and the density of smooth muscle cells (SMCs).[3,5] Due to different biopsy sites, lack of clarity as to which layers of the vaginal wall are actually being analyzed, and very heterogeneous study populations, data are conflicting. Moreover, by comparing tissue that is prolapsed to one that is not prolapsed, it is impossible to distinguish between causes and effects of POP. In other words, it is impossible to distinguish whether the observed changes in the anterior vaginal wall are the result of an intrinsic (genetic) or an acquired (environmental) effect.

The goal of this study was to identify the changes in the nonprolapsed anterior vaginal wall that occur in premenopausal women with POP, compared to age-matched women without POP using histologically defined samples. The second goal was to determine whether the observed changes are caused by the prolapse, by comparing the histological and biochemical features of the anterior vaginal wall at the site of the POP with the same features at the non-POP site within the same patient. This approach helps us to identify possible changes in the connective tissue of the vaginal wall due to the increased pressure and

From the Departments of Obstetrics and Gynecology (Dr Kerkhof) and Pathology (Dr Bril), Kennemer Gasthuis Hospital, Haarlem; Departments of Orthopedics, Netherlands Institute for Regenerative Medicine (Mrs Ruiz-Zapata and Dr Helder), and Pathology (Drs Bleeker and Belien), MOVE Research Institute, VU University Medical Center, Amsterdam; and Department of Metabolic Health Research, TNO, Leiden (Dr Stoop), The Netherlands. Dr Kerkhof is currently with the Department of Obstetrics and Gynecology, VU University Medical Center.

Received July 6, 2013; revised Sept. 15, 2013; accepted Oct. 29, 2013.

The authors report no conflict of interest.

Presented at the 37th annual meeting of the International Urogynecological Association, Brisbane, Australia, Sept. 4-8, 2012.

Reprints: Manon H. Kerkhof, MD, Department of Obstetrics and Gynecology, VU University Medical Center, Rijnlaan 1, 2105XH Haarlem, The Netherlands. mhkerkhof@freeler.nl.

0002-9378/$36.00 • • http://dx.doi.org/10.1016/j.ajog.2013.10.881

stretch of the prolapsing pelvic organs on the vaginal wall. We hypothesize that the precervical anterior vaginal wall (non-POP site) of women with POP is comparable to the precervical anterior vaginal wall of controls, thus allowing us to consider this precervical tissue in POP patients as a proper control site. We expect to find differences in the histological and biochemical features at the POP site compared to the non-POP site within the same woman with prolapse. By testing the hypothesis that the changes seen in the connective tissue of the anterior vaginal wall are an acquired rather than an intrinsic effect causing POP, we hope to provide useful information that should help to develop new approaches in reconstructive pelvic surgery.

MATERIALS AND METHODS

Patient population

Upon medical ethical committee approval and acquired informed consent, biopsies were collected from 26 Caucasian premenopausal women at the Department of Obstetrics and Gynecology of the Kennemer Gasthuis, Haarlem, The Netherlands, from March 2009 through March 2011. Eligible women were divided into 2 groups: a control group of 13 women undergoing abdominal or laparoscopic hysterectomy for benign gynecological diseases with no sign of POP during gynecological examination, and a case group of 13 patients undergoing vaginal hysterectomy and reconstructive pelvic surgery of the anterior vaginal compartment because of a cystocele, POP-Quantification (POP-Q) stage $\geq$2. Signs of POP were recorded during the pelvic examination by the same urogynecologist (M.H.K.) and were described according to the International Continence Society POP-Q.[6] Groups were matched for age, parity, smoking, and use of oral contraceptives. Premenopausal status was defined as having a regular period over the preceding 12 months. Women on oral contraception were asked to temporarily stop for 3 months to see whether a spontaneous regular menstrual cycle would occur. Endometrial biopsies were obtained from all women defining the stage of the menstrual cycle at time of operation. Exclusion criteria included the use of progestin-only hormone regimen, a history of pelvic surgery, pelvic malignancy or connective tissue disease affecting collagen or elastin remodeling, adhesions or scarring at the biopsy site, surgeons judgment that a biopsy may harm the patient, a history or presence of endometriosis, morbid obesity (body mass index >35 kg/m^2), diabetes, chronic inflammatory disease, chronic infections, steroid use, and inability to provide informed consent. Standardized demographic and pertinent clinical information was recorded prospectively and stored in a dedicated database.

Tissue acquisition and preparation

The site of tissue collection was standardized because of potential differences in composition of the extracellular matrix (ECM) throughout the vagina.[7] After removal of the uterus in the controls, full-thickness samples of the anterior vaginal wall were obtained from the vaginal cuff at the anterior midline portion of the vaginal wall. In women with POP, an additional full-thickness anterior vaginal wall (midline) sample was taken from the POP site during anterior colporrhaphy (point Ba POP-Q).

To minimize harm in the control subjects, only anterior vaginal wall tissue from the vaginal cuff was retrieved. The minimum size of the biopsy was 0.5 $\times$ 1.0 cm^2. All biopsies were large enough to perform (immuno-)histochemical as well as biochemical analysis. Biopsies were immediately passed off the surgical field and divided into 2 parts. For biochemical analysis, the biopsies were washed in phosphate-buffered saline solution and stored at -80°C until further processing. For analysis by microscopy, the collected tissue was fixed in neutral-buffered formalin for 24 hours, dehydrated, and processed into paraffin blocks. Serial 3-μm sections were cut from the paraffin blocks, mounted on slides, and stored until further processing.

All measurements were performed without knowledge of sample identity.

(Immuno-)histochemistry

Hematoxylin-eosin staining was performed to verify that the collected samples represented the vaginal wall containing the epithelial layer, connective tissue, and muscularis. To detect the amount of elastin in the connective tissue, the Lawson elastic van Gieson stain kit, purchased from Klinipath (Duiven, The Netherlands), was used. As positive controls, tissues from lung, appendix, and liver were used.

Monoclonal antibodies against desmin and CD31 were used to identify SMCs and blood vessels, respectively. Sections were also immunostained for the ECM proteins collagen I, III, and IV (Table 1).

Morphometric analysis

To quantify SMCs, elastin, and microvessels we performed a morphometric analysis. Complete slides were scanned with a digital Mirax slide scanner system (3DHistech Ltd, Budapest, Hungary) equipped with a $\times$20 objective with a numerical aperture of 0.75 and a Sony DFW-X710 Fire Wire 1/3, type progressive scan CCD camera pixel size 4.65 $\times$ 4.65 um (3DHistech Ltd) (pixel size 4.65 $\times$ 4.65 μm). The actual scan resolution of all pictures at $\times$20 was 0.23 μm. After scanning, representative areas of both the muscularis layer and the lamina propria layer between 0.55-0.64 mm^2 were randomly annotated by hand using Panoramic Viewer software (3DHistech Ltd). Resulting annotations were exported in the tagged image file format (tiff) image format. A computerized morphometric analysis of the desmin-, elastic van Gieson–, and CD31-stained slides was executed, using ImageJ 1.44p software (http://rsbweb.nih.gov/ij/) with a modified macro from Hadi et al.[8] Analysis was performed for the lamina propria and muscularis layer separately. The amount of SMC was expressed as the total area of desmin-positive cells vs the total tissue area. The amount of elastin was expressed by total area of fibers vs total tissue area. The CD31 staining was used to quantify the amount of microvessels per area as well as the amount of nuclei per area.

Collagen staining was quantified in a blinded fashion using a 4-grade scoring