GYNECOLOGY

A systematic evaluation of collagen cross-links in the human cervix

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OBJECTIVE: The mechanical strength of the cervix relies on the cross-linking of the tissue's collagen network. Clinically, the internal os is functionally distinct from the external os. We sought to detect specific collagen cross-links in human cervical tissue and determine whether cross-link profiles were similar at the internal and external os.

STUDY DESIGN: Transverse slices of cervical tissue were obtained at the internal and external os from 13 nonpregnant, premenopausal women undergoing a benign hysterectomy. To understand how cross-links were distributed throughout the entire cervix and at the internal and external os, biopsies were obtained from 3 circumferential zones in 4 quadrants from each slice. Biopsies were pulverized, lyophilized, reduced with sodium borohydride, hydrolyzed with hydrochloric acid, and reconstituted in heptafluorobutyric acid buffer. Hydroxyproline was measured by ultraperformance liquid chromatography-electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS), converted to total collagen, and normalized by dry weight. Collagen cross-links pyridinoline (PYD), deoxypyridinoline (DPD), dihydroxylysinonorleucine (DHLNL), and the nonenzymatic advanced glycation end product pentosidine (PEN) were measured by UPLC-ESI-MS/MS and reported as cross-link density ratio (cross-link/total collagen). Generalized estimated equation analysis was used to compare results between the internal and external os and to compare quadrants and zones within slices from the internal and external os to determine if cross-link profiles were similar.

RESULTS: A total of 592 samples from 13 patients were analyzed. Collagen cross-links are detectable in the human cervix by UPLC-ESI-MS/MS. When comparing all samples from the internal and external os, similar levels of collagen content, PYD, DHLNL, and DPD were found, but PEN density was higher at the external os (0.005 vs 0.004, $P = .001$). When comparing all internal os samples, significant heterogeneity was found in collagen content and cross-link densities across zones and quadrants. The external os exhibited heterogeneity only across zones.

CONCLUSION: Collagen cross-links (PYD, DPD, DHLNL, and PEN) are detectable by UPLC-ESI-MS/MS in the human cervix. The internal os exhibits significant collagen cross-link heterogeneity compared with the external os. Further studies are needed to evaluate how collagen cross-link heterogeneity correlates to the mechanical strength and function of the human cervix.

Key words: cervix, collagen, cross-linking, preterm labor

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During pregnancy, the cervix undergoes dramatic changes to allow for successful parturition.[1] Ultrasound studies have shown that cervical ripening at term starts with dilation of the internal os and then progressive funneling toward the external os until total effacement is achieved.[2] A similar sequence of cervical changes occurs in pregnancies complicated by cervical insufficiency.[3] During a sterile vaginal examination, it is also common to note that the external os is open, whereas the internal os remains closed.

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Although cervical tissue architecture was traditionally thought to be homogenous, these clinical findings fuel the following questions: is the internal os functionally distinct from the external os? If so, are there differences in the cervical tissue properties at the level of the internal os that influences its mechanical performance? The answers to these questions remain elusive because studies evaluating cervical tissue strength at the internal and external os are lacking.

Human cervical tissue is a hydrated connective soft tissue composed mainly of a collagen-rich extracellular matrix (ECM).[4-8] Similar to other load-bearing tissues in the body (eg, bone, tendon), the mechanical strength of cervical ECM relies in part on the type and degree of collagen cross-linking in its collagen network.[9] Collagen cross-links stabilize collagen molecules, a vital requirement to building strong, organized collagen networks. During collagen cross-link formation, immature cross-links such as dihydroxylysinonorleucine (DHLNL) are formed between a telopeptide residue and a helical residue. Subsequently, another telopeptide residue is formed between 3 collagen molecules resulting in mature cross-links, deoxypyridinoline (DPD) and pyridinoline (PYD).[10]

Previously the degree of collagen cross-linking was indirectly determined by measuring collagen extractability, in which increased extractability correlated to decreased collagen cross-linking. Studies that used this method showed that collagen extractability significantly increases in a normal mouse cervix during pregnancy.[11] Myers et al[8] demonstrated similar results on human nonpregnant and term pregnant tissue samples. Recently Akins et al[12] measured mature collagen cross-links in mouse cervical tissue by reversed-phase, high-performance liquid chromatography and found cervical ripening in rodents is characterized by a decrease in PYD and DPD.

Given that (1) mature collagen cross-links provide stability and thus strength to the ECM, (2) collagen cross-link profiles are altered in cervical remodeling/softening, and (3) the internal os appears to soften or weaken first during parturition and premature cervical remodeling, the goal of this study was to determine whether specific collagen cross-links are detectable in the human cervix and whether the internal os has a different collagen cross-link profile compared with the external os.

Materials and Methods

This study was approved by the Columbia University Medical Center Institutional Review Board (institutional review board no. AAAI0337). Nonpregnant, premenopausal women undergoing a total hysterectomy for benign indications were identified and consented to participate. Women were excluded if they were older than 50 years of age, had an abnormal Papanicolaou smear, or had prior cervical surgery. Demographic information (age, race, body mass index, obstetric history) and indication for procedure were collected. Uterine weight was obtained from pathology reports (Table 1).

Immediately following the hysterectomy, 2-3 mm transverse slices of the cervix were obtained at the level of the internal and external os. Anterior/posterior orientation of the tissue was maintained. One slice from the internal and external os was frozen and stored at −80°C until collagen content and cross-links analysis were performed. An

TABLE 1
Patient demographics

Patient number	Age, y	Race	BMI	Parity	Obstetric history	Type of hysterectomy	Uterine plus cervix weight, g
1	44	H	32.6	0	None	TAH	2994
2	49	H	23.3	0	None	TAH	1150
3	46	AA	34.9	0	None	TAH	6100
4	40	AA	37.9	0	None	TAH	1005
5	49	C	28.3	1	NSVD	TRH	267
6	48	H	27.8	1	NSVD	TLH	113
7	42	C	25.3	1	NSVD	TRH	225
8	41	AA	25.1	1	NSVD	TLH	192
9	42	C	28.1	1	NSVD	TLH	75
10	49	C	29.9	2	NSVD × 2	TVH	175
11	44	H	21.2	4	NSVD × 4	TLH	223
12	46	H	30.9	4	NSVD × 3, CD × 1	TRH	178
13	48	H	30.2	5	NSVD × 5	LAVH	250

AA, African-American; *BMI*, body mass index; *C*, Caucasian; *CD*, cesarean delivery; *H*, Hispanic; *LAVH*, laparoscopic-assisted vaginal hysterectomy; *NSVD*, normal spontaneous vaginal delivery; *TAH*, total abdominal hysterectomy; *TLH*, total laparoscopic hysterectomy; *TRH*, total robotic hysterectomy; *TVH*, total vaginal hysterectomy.

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