The Alteration of Collagen Subtypes and Myofibroblasts may Account for Pelvic Organ Prolapse

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INTRODCUTION

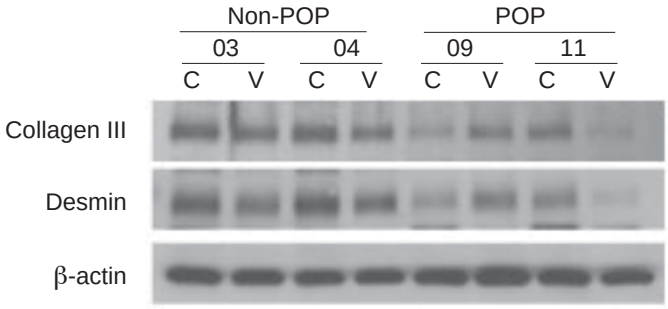
Pelvic organ prolapse (POP) is a prevalent and bothersome problem in women. POP with or without stress urinary incontinence may have a significant impact on the quality of life for affected women. The Women's Health Initiative reported the prevalence of some degree of POP in 41% of women aged 50-79 years, including cystocele in 34%, rectocele in 19%, and uterine prolapse in 14% [1]. The anterior vaginal wall is the main support tissue for the bladder base; while the uterosacral/cardinal ligament (USCL) complex is the main support for the uterus or vaginal stump. Pelvic connective tissue resilience decreases with vaginal delivery, menopause and uterine prolapse [2]. Many reports have identified changes in connective tissue status in women with POP. However, its underlying risk factors are heterogeneous defects within ligaments, fasciae, and muscles [3]. Myofibroblasts, also called activated fibroblasts, play an important role in extracellular matrix (ECM) remodeling during both physiologic and pathologic healing processes [4]. However, there is a paucity of information about the etiology and patho-physiology of POP. Therefore, the related fields are worthy of further exploration for the two key events for POP, i.e. abnormal synthesis and/ or degradation of ECM. We hypothesized that accelerated remodeling in patients with POP is caused by the biochemical changes of ECM (collagen and elastin), and stromal cell (myofibroblasts) activation.

MATERIALS AND METHODS

After informed consent from the patients and the approval of the Institutional Review Board (IRB), we conducted this study. Six women (mean 45.2 y/o, range 37- 49 y/o) who had received laparoscopic-assisted vaginal hysterectomy (LAVH) due to benign disease without clinical POP were recruited as our non-POP group. Six women with clinical POP-Q stage 3 to 4 (mean 67.3 y/o, range 51-77 y/o) were classified as our POP group. We quantitated the ECM components (collagens type I and III, elastin, desmin) with Western blot; and the amount of myofibroblasts [by counting α-smooth muscle actin (α-SMA)] with immuno-histochemistry (IHC) in the anterior vaginal wall and USCL complex in frozen tissue and formalin-fixed tissue on both the POP and non-POP group. Myofibroblasts are characterized by α-SMA overexpression which plays an important role in ECM remodeling during the healing process. The number of α-SMA-positive cells was used to represent myofibroblasts amount.

RESULTS

The cardinal ligament and the anterior vaginal wall of the non-POP cases were used as a reference. We found that POP women had decreased desmin amounts in the cardinal ligament (100% vs 45%) and anterior vaginal wall (72% vs 42%) when compared with non-POP women; POP women also had decreased collagen subtype III in the cardinal ligament (100% vs 40%) and anterior vaginal wall (87% vs 47%) (Fig. 1), the cardinal ligament in non-POP was used as the stan-



C: cardinal ligament; V: anterior vaginal wall

ECM protein collagen III and desmin in POP

ECM amount	Collagen III	Desmin
Cardinal lig.		
non-POP	100%	100%
POP	40%	45%
Vaginal wall		
non-POP	87%	72%
POP	47%	46%

Note: cardinal ligament in non-POP was used as reference

Fig. 1. Western blots showed that collagen III and desmin amounts were less in both the cardinal ligament and vaginal wall from pelvic organ prolapse (POP) women, when compared with those from non-POP women. ECM=extracellular matrix.

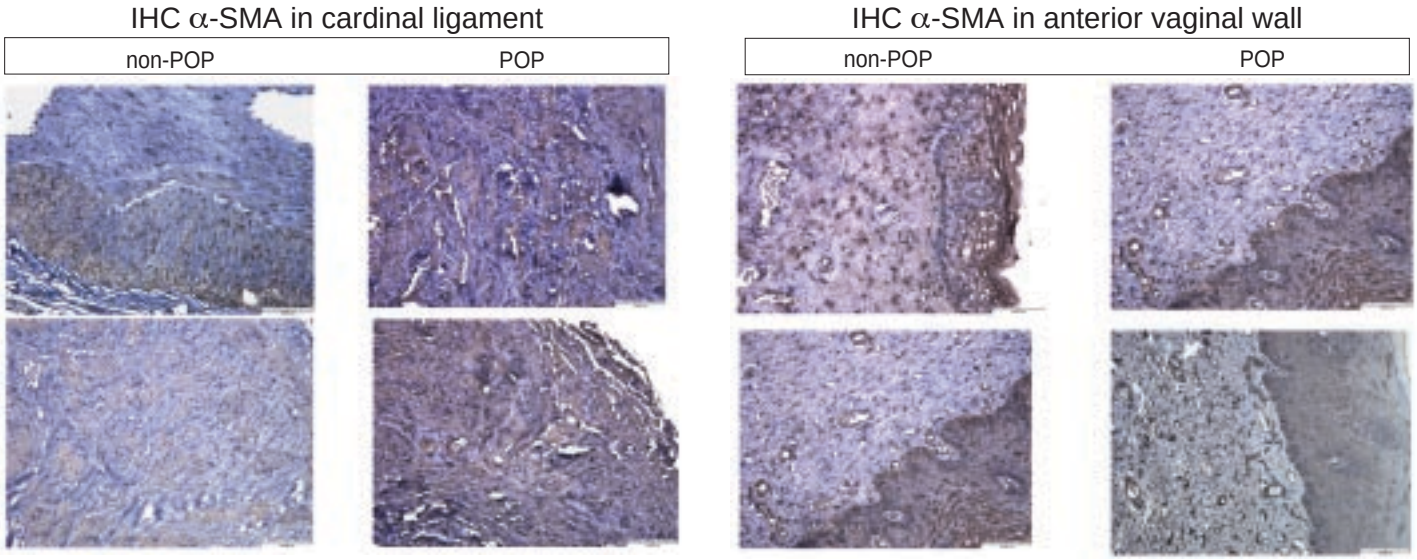


Fig. 2. Immuno-histochemistry (IHC) study, α-smooth muscle actin (α-SMA) represented the amount of myofibroblasts. Myofibroblasts in the cardinal ligament from pelvic organ prolapse (POP) women were higher than from non-POP women. The myofibroblasts in the vaginal wall were not different in either POP or non-POP women.

dard (100%). In the IHC study, the cardinal ligament and anterior vaginal wall were harvested from six women with clinical POP-Q stage 3 to 4 as a study group, and compared with six women receiving LAVH due to benign disease without clinical POP as the non-POP group. The myofibroblast amount in the cardinal ligament was seen to be higher in POP women by measuring α-SMA, as compared with non-POP women. It was not different in the vaginal wall in either POP or non-POP women (Fig. 2).

DISCUSSION AND CLINICAL IMPLICATION

The significance of the amount of collagen and subtypes on POP is still controversial because of different tissue types and different methodology. Our study revealed that collagen III amounts were less in both the cardinal ligament and vaginal wall from POP women, as compared with those from non-POP women. Whether those tissue alterations are primary or secondary to the pelvic floor disorder remains unknown. Our results were in concordance with the Lin et al report [5], but different from the Gabriel et al report [6]. Lin et al reported that type III was significantly less in the anterior vaginal wall; quantitative immunoreactivity of collagen I and III had significant positive correlations with ageing [5]. On the contrary, Gabriel et al reported type III expression in the uterosacral ligament was significantly more related to the presence of POP ($p<0.001$) rather than age or parity. The higher type III expression or the decrease of type I/ III ratio, might be a typical characteristic of POP patients' connective tissue. They explained that the higher collagen III amount in women with POP could be responsible for an increased tissue laxity in these patients [6].

The information of ECM component changes that occur in POP is useful to surgeons when reconstructing the pelvic floor with native tissue and/or synthetic mesh [7]. According to our previous work with matrigel multicellular co-culture system with or without different synthetic meshes embedding, the impaired recruitment and tube-formation ability of myofibroblasts and endothelial cells into the type III multifilamentous mesh, compared to the type I monofilamentous, macroporous mesh, may account, at least in part, for the usefulness as well as limitations of these meshes [8]. By using both frozen and formalin-fixed tissue in both the anterior vaginal wall and USCL complex for the comparative and correlative studies, the debatable issue can be solved. From this, we can potentially identify or develop the usefulness and feasibility of ideal prostheses. Gaining a better understanding of the complexities of the ECM-myofibroblast interaction will improve our prospects for developing more effective pelvic reconstructive surgery. The ideal synthetic mesh should offer a good environment for stromal cells, e.g. myofibroblasts and endothelial cells recruitment. The altered microenvironment of the POP itself is a powerful and insidious target in the pelvic reconstructive surgery [6].

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