Clinical study on collagen and stress urinary incontinence

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Summary

Objective: To investigate the histological characteristics in uterine ligaments of stress urinary incontinence (SUI) and pelvic organ prolapse (POP); to detect the alteration of collagen ultrastructure in uterine ligaments that contribute to SUI and POP; to study the relationship between collagen alteration and SUI and POP.

Methods: The cardinal ligament (Car lig.) and uterosacral ligaments (U-S lig.) samples were obtained from 73 subjects suffering from SUI, or normal controls who underwent hysterecotomy. Collagen ultrastructure was examined with transmission electron microscopy (TEM).

Results: 1) The smooth muscle fascicles in the patients with SUI and POP were thinner. Arrangement of smooth muscle fascicles was in disorder. 2) The collagen constituting the ligaments was active in metabolism. The mean collagen fibril diameters in the SUI and POP groups were about 25% larger than that in the control groups ($p \leq 0.01$). 3) Histological characteristics of the Car lig. and the U-S lig. were similar.

Conclusions: 1) The Car lig. and U-S lig. are homologous. Abnormalities in arrangement of smooth muscles and the collagen ultrastructure were obviously seen in SUI and POP. 2) The collagen fibril diameter in the SUI and POP subgroups was significantly larger than that in the control groups. Those ligaments are probably less elastic and more likely to break. Predominance of collagen degradation during tissue repair may contribute to POP and SUI.

Key words: Stress urinary incontinence; Etiology; Collagen.

Introduction

As the average life-span increases, much more attention has been paid to postmenopausal diseases. The incidence of stress urinary incontinence (SUI) is higher in middle and older-aged women. It has become an important sociological dilemma, as well as a health problem. However, the etiology and pathophysiology of SUI is still uncertain. The incidences of SUI and pelvic organ prolapse (POP) increase after menopause. Recent reports have shown an alteration in collagen biochemistry and ultrastructure in the patients with SUI or POP. It suggests an association between abnormalities in collagen and SUI, and POP. This study aimed to investigate the relationship between SUI and collagen.

Materials and methods

1. Patients

Seventy-three women were recruited into the study and divided into two groups: premenopausal and postmenopausal groups. The premenopausal (19) includes a normal control subgroup (12) and POP subgroup (7). The postmenopausal group (54) includes a normal control subgroup (20), POP subgroup (18) and SUI subgroup (16). None of the women had taken any hormonal drugs in the last three months before the study. Urinary infection or estrogen-related diseases (endometriosis, myoma, and functional ovarian tumor) were eliminated by gynecological examinations. SUI was defined objectively by medical history, gynecologic examination, stress test and urodynamic examination. In the control group none of the women showed any sign of POP or urinary incontinence.

Revised manuscript accepted for publication May 22, 2002

2. Sampling and Preparation of tissue

A specimen of 500 mg of tissue was obtained from the cardinal and uterosacral ligaments (Car. lig. and U-S lig.), respectively, during total abdominal hysterectomy (TAH) or total vaginal hysterectomy (TVH). A small piece of tissue 1-2 mm^3 was immediately cut from the Car lig. and U-S lig. and immersed in 2.5% glutaraldehyde for transmission electron microscope (TEM) examination. The remaining tissue was placed in 10% formalin for light microscope examination.

TEM samples: The specimen was fixed in 2.5% glutaraldehyde for more than two hours and in 1% osmium tetroxide for two hours, and later dehydrated step by step in increasing ascending concentrations of alcohol. It was then embedded in Epon and cut on an ultramicrotome. Contrast staining was carried out with uranyl acetate and lead citrate. Light microscopy samples: H.E. routine staining and Masson special staining were performed for all the paraffin-embedded specimens.

Statistics: Statistical analyses were performed by independent-samples T-test, one-way ANOVA and bivariate analysis; p values < 0.05 were considered statistically significant.

Results

The two subgroups in the premenopausal group were comparable in age, body mass, gestation and parity, and no significant difference was found between the two subgroups (Table 1). Also the three subgroups in the postmenopausal group were comparable and there was no significant difference among the three subgroups (Table 2).

Light microscopy: In the POP and SUI subgroups, whether before or after menopause, smooth muscles were arranged in disorder and muscle fascicles were slimmer,

Table 1. — *Comparison of the two subgroups in the premenopausal group.*

Subgroup	Cases (n)	Age (y)	Parity	Weight index (Kg/m²)	Menstual period (follicular/luteral)
POP	7	45.29±2.43	1.29±0.49	25.38±3.47	3/4
Control	12	42.58±6.58	1.33±0.49	24.26±4.16	5/7
p value		p > 0.05	p > 0.05	p > 0.05	

Table 2. — *Comparison of the three subgroups in the postmenopausal group.*

Subgroup	Cases (n)	Age (y)	Parity	Weight index (Kg/m²)	Period of postmenopause (y)
POP	18	64.22±5.41	3.44±1.65	26.17±3.00	14.83±8.01
SUI	16	63.81±7.09	2.81±1.38	24.84±2.24	12.88±8.38
Control	20	62.10±6.41	2.30±1.08	24.43±3.38	10.75±6.58
p value		p > 0.05	p > 0.05	p > 0.05	p > 0.05

whereas, smooth muscles were arranged in order and muscle fascicles were thicker in the control subgroups of the premenopausal or postmenopausal groups.

TEM: Active fibroblasts were more frequently found in the POP and SUI subgroups than in the control subgroups, whether before or after menopause (Figure 1). Muscular fibroblasts were found in the POP and SUI subgroups (Figure 2), but not found in the control subgroups. At a magnification of 20,000, the diameters of 90 fibrils were measured in each specimen. The average diameter of the fibril is shown in Table 3.

The mean collagen fibril diameter in the SUI and POP subgroups was about 25% larger than that in the control subgroups ($p < 0.01$) for both the premenopausal and postmenopausal groups.

Historical characteristics of the Car lig. and U-S lig. were similar. The fibril diameter of the Car lig. and U-S lig. in the POP, SUI and control subgroups were also similar ($p > 0.05$).

Table 3. — *Diameter of the fibrils in uterine ligments (nm).*

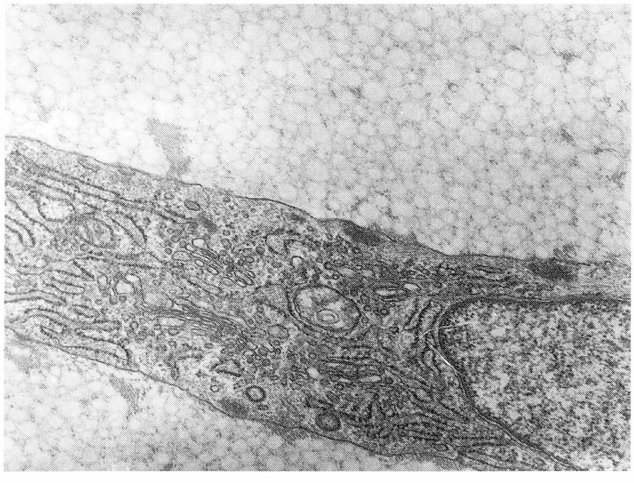
	premenopause		postmenopause		
Subgroups	Control (n:12)	POP (n:7)	Control (n:20)	POP (n:18)	SUI (n:16)
Car lig.	49.21±4.89 a	72.96±7.97 a'	59.69±8.00 c	76.38±9.60 c'	80.02±9.54 c"
U-S lig.	49.18±4.75 b	73.63±8.08' b'	59.33±8.57 d	76.72±9.34 d'	81.89±9.18 d"
	a: a' $p < 0.01$		c: c' $p < 0.01$		d: d' $p < 0.01$
	b: b' $p < 0.01$		c: c" $p < 0.01$		d: d" $p < 0.01$
			c': c" $p > 0.05$		d': d" $p > 0.05$

Discussion

Ligaments are made up of smooth muscle, fibroblasts, fibre, vessels and nerves. We found the smooth muscle fascicles in the uterine ligaments of SUI and POP subgroups to be thinner and the arrangement in disorder, while those in the control subgroups were thicker and arranged in order. Whether these changes are the pathological causes of SUI and POP or just the pathological appearance after the occurences of SUI and POP is still unknown.

Jackson *et al.* [1] reported the uterine ligaments of POP and SUI to be in an active repair situation. Our study showed more active fibroblasts in the TEM samples of the SUI and POP subgroups than those of the control subgroups, and the variations in fibril diameters were more obvious. It implies that collagen of SUI and POP is possible in the repairing state of active metabolism. This is coincident with Jackson *et al's* report. It is assumed that in POP the increased degradation of collagen leads to a decrease in the mechanical strength of the pelvic tissues, though during tissue reparation synthesis of new collagen is also enhanced. However, newly formed collagen is not stable and is liable to be degraded. Collagenolytic activity seems to be the main reason for the cause of weakness in the pelvic tissues in POP.

In the pelvic floor, the connective tissue is mainly composed of type I and type III collagen. Type I collagen is made



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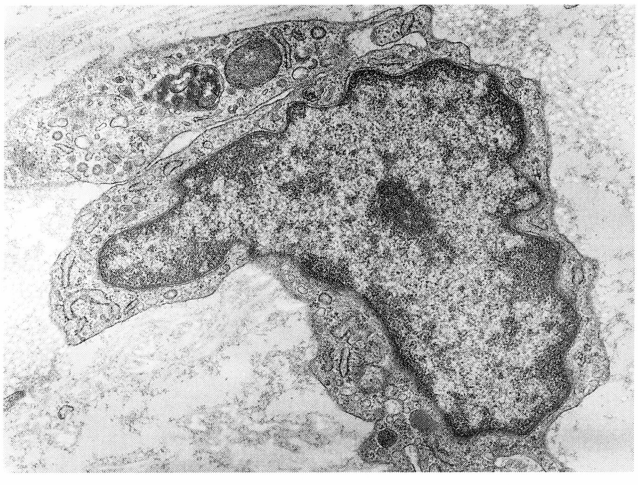

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Figure 1. — Active fibroblast of the SUI examined by TEM (x 15,000).
Figure 2. — Muscular fibroblast of the SUI examined by TEM (x 10,000).

up: of a larger diameter of fibrils than that of type III collagen. Type I collagen is more rigid, and type III collagen is thought to contribute more to the elasticity of tissues. Bergman *et al.* [2] found the amount of type III collagen of SUI was significantly reduced in perineal skin, uterosacral ligaments and the round ligaments of the uterus. In our study, the collagen fibril diameter was significantly larger in the SUI and POP subgroups than those in the control subgroup. It is possible that POP and SUI patients have less type III collagen, and that the collagen fibrils are thicker and less elastic. This collagen is easier to break and offers less support under stress. As a result, collagen is active in reparation during which both synthesis and degradation of collagen increase, but degradation is dominant and it leads to a reduced amount of collagen. Consequently, POP and SUI occur.

In each group the histological discoveries in the Car lig. and the U-S lig. under light microscopy and TEM were similar. Correlation analysis indicated the Car lig. and U-S lig. were significantly correlated in terms of fibril diameters. Thus the Car lig. and U-S lig. can be regarded as homologous, which is in conformity with the theory of uterosacral-cardinal ligament complex.

In a word, the etiology and pathogenesis of USI and POP are complex, involving a lot of factors. Much research needs to be done.

References

[1] Jackson S. R., Avery N. C., Tarlton J. F.: "Changes in metabolism of collagen in genitourinary prolapse". *Lancet*, 1996, *347*, 1658.
[2] Bergman A., Elia G., Cheung D., *et al.*: "Biochemical composition of collagen in continent and stress urinary incontinent women". *Gynecol. Obstet. Invest*, 1994, *37*, 48.

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