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Changes in the quantity of collagen type I in women with genuine stress incontinence

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Abstract The aim of this study, was to examine changes in the quantity of collagen type I in the pubocervical fascia of women with genuine stress incontinence (GSI), with and without pelvic relaxation . Seventy-eight patients participated in the study and they were divided into three groups that were comparable with respect to their age and parity. All the patients underwent filling cystometry and patients with detrusor instability were excluded from the study. Biopsies were obtained from the pubocervical fascia. The presence of collagen type I was determined with an immunohistochemical technique. The X-test was used for statistical analysis and a $P < 0.05$ was considered statistically significant. Collagen type I was significantly reduced in patients with GSI irrespective of the presence or absence of genital prolapse. Thus we found that women with GSI had a significant reduction of collagen type I in the pubocervical fascia which consequently affects the tensile strength of the pubocervical fascia and the support provided to the bladder neck.

Key words Collagen Type I · Genuine stress incontinence · Genital prolapse

Introduction

Genuine stress incontinence (GSI) is believed to be caused by inadequate support of the bladder base and bladder neck or insufficiency of the urethral sphincter [1, 3]. The adequate function of the pubourethral ligaments, the pubococcygeal muscle and the connective tissue of the suburethral wall (pubocervical fascia) is important for urinary continence in women [6]. The main constituent of the connective tissue of the pubocervical fascia is collagen [9]. The quantity and organization of collagen fibers affects significantly the tensile strength of the pubocervical fascia and consequently the support that is provided to the bladder neck and bladder base. A reduction in total collagen content of pubocervical fascia in women with GSI has been found [4, 7] but which type of collagen is reduced mainly needs to be investigated further. The aim of the study was to evaluate changes in the quantity of type I collagen in patients with GSI as compared with continent women independent of the presence of pelvic relaxation.

Material and methods

Seventy-eight patients participated in the study. Their age ranged from 34 to 75 years old (mean 52.8 + 7.6) and their parity ranged from 1 to 4 deliveries (mean 2.3 + 1.15). Forty-one patients were postmenopausal and 37 were premenopausal. None of them was receiving or had received hormone replacement therapy in the past. All the patients underwent filling cystometry and patients with detrusor instability were excluded from the study. Approval from the ethical committee of our hospital and informed consent was obtained from all participants in the study. The patients were divided in to three groups according to the findings at clinical examination and urodynamic investigation, as follows: (1) 32 patients with GSI and genital prolapse in group I, 26 patients with genital prolapse but without GSI in group II and 20 patients with neither genital prolapse nor GSI in the control group. All the groups were comparable with respect to their age, parity (Table 1). Biopsies were obtained during surgery from the pubocervical fascia at the level of the bladder neck. The balloon of the Foley catheter that was inserted in to the bladder at the time of the operation was used as a guide for recognition of the bladder neck. The patients of group I and group II had stage I pelvic organ prolapse, according to the International Continence Society classification and the presence of genital prolapse was assessed during straining with the patient in lithotomy position. All biopsies were obtained

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by the same operator to ensure adequacy of the specimens. Immunohistochemical analysis was performed in the histopathology laboratory of our hospital by two histopathologists and the magnification used was 20×. The two examiners did not know to which patient each sample belonged and they did not know the findings of each other. We had five specimens from biopsies of pubocervical fascia where there was disagreement between two examiners about the results of the immunohistochemical analysis. These specimens were not included in the study. All the biopsy specimens were fixed in formalin and embedded in paraffin and subsequently were processed according to the immunohistochemistry protocol that is presented below. Minimal or no staining reaction was scored as (−) (Fig. 1), a weak reaction was scored as (+) (Fig. 2) and a strongly positive staining reaction was scored as (++) (Fig. 3). The data were analyzed and compared in relation to collagen I content in all three groups. The X test was used for statistical analysis and a $P < 0.05$ was considered statistically significant.

Table 1 Patients characteristics (*SUI* stress urinary incontinence, *BMI* body mass index)

	Patients with SUI ($n = 32$)	Patients with prolapse ($n = 26$)	Control group ($n = 20$)	*P*
Age (years)	54.6 + 6.2	58.2 + 9.1	51.8 + 7.5	0.75
Parity	2.2 + 1.2	2 + 0.82	2.1 + 0.2	0.84
BMI	28.27 + 2.8	25.2 + 3.5	24.9 + 3.1	0.001

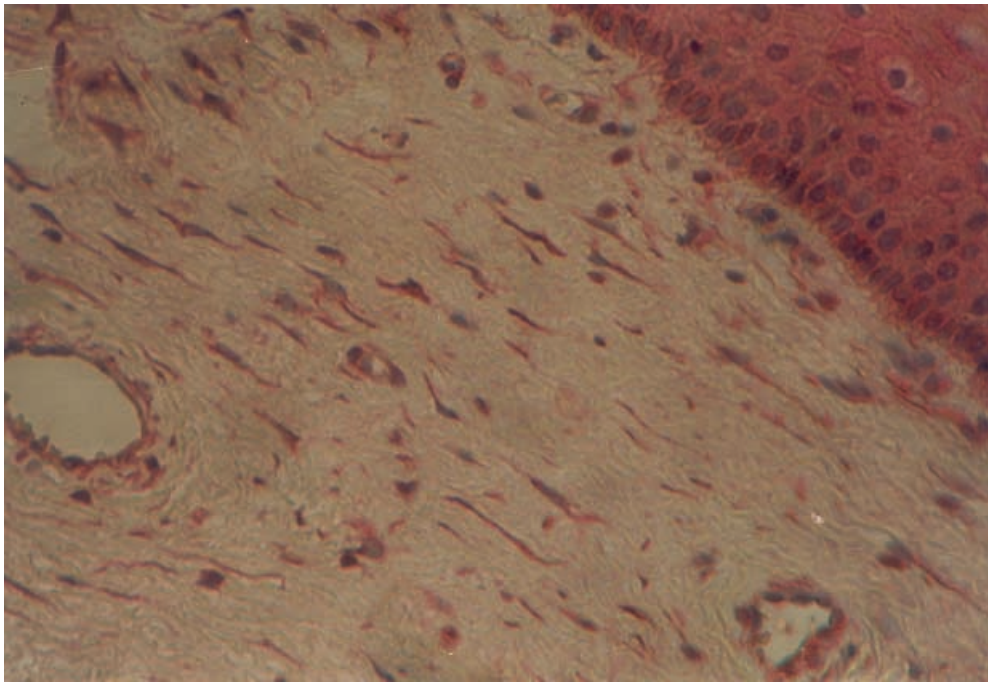

Fig. 1 Microscopic appearance of tissue specimen with minimal staining reaction for collagen Type I, graded as (−)

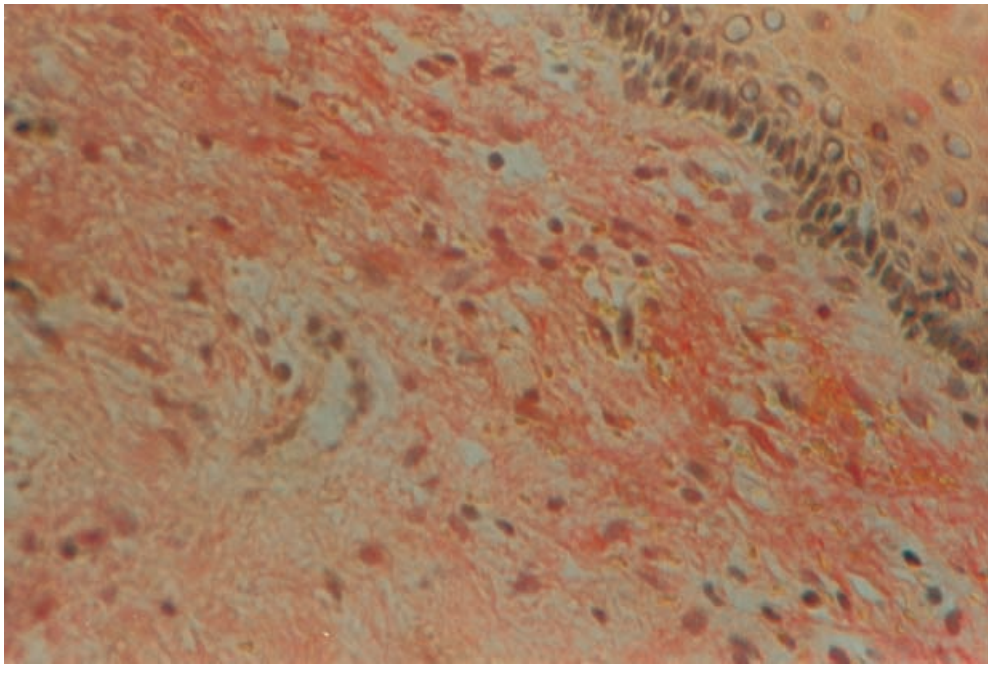

Fig. 2 Microscopic appearance of tissue specimen with weak staining reaction for collagen Type I, graded as (+)

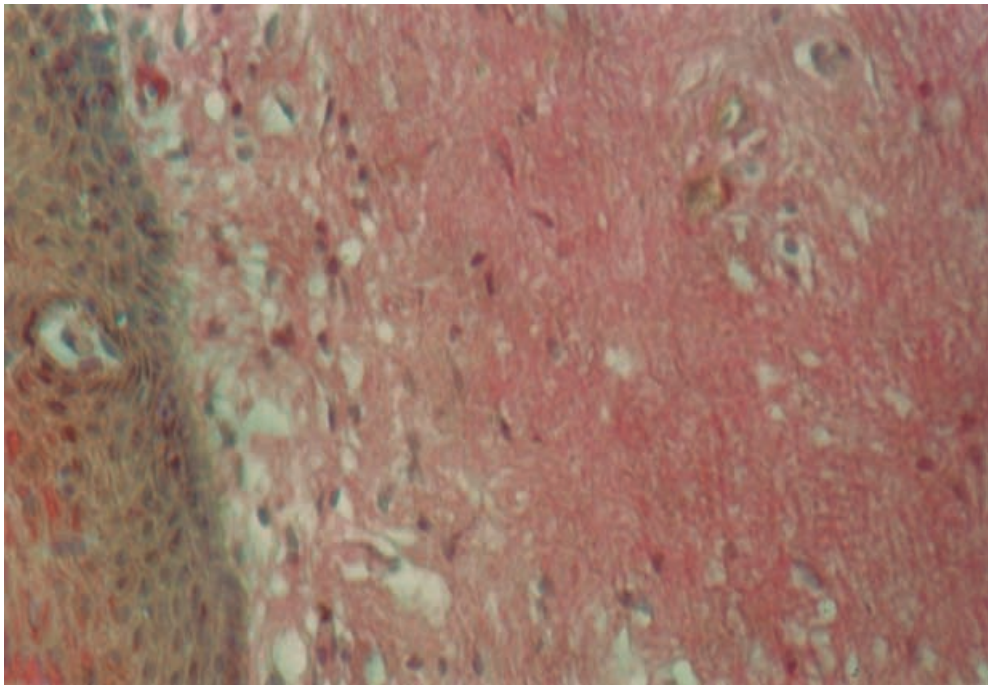

Fig. 3 Microscopic appearance of tissue specimen with strong staining reaction for collagen Type I, graded as (+ +)

Table 2 Findings of collagen type I in the pubocervical fascia of the three groups (*SUI* stress urinary incontinence)

Patients with SUI (n = 32)			Patients with prolapse (n = 26)			Control group (n = 20)		
–	+	+ +	–	+	+ +	–	+	+ +
17	6	9	2	> 10	14	–	3	17
> 53%,	19%	28%	8%	38%	54%	0	15%	85%
$P < 0.01$								

Immunohistochemistry (IHC) protocol

Briefly, four-m sections of tissue were placed on Fisher Plus slides (Fisher Scientific, Pittsburgh, Pa., USA). These were deparaffinized and rehydrated in graded alcohol incubations. Tissue sections were then digested in 0.1 mg/ml Proteinase K (Boehringer-Mannheim Corporation, Indianapolis, Ind., USA) in 0.6 M Tris (pH 7.5)/ 0.1% $CaCl_2$ and blocked with normal sheep serum. Primary antibodies were applied to tissue sections and allowed to incubate for 60 min. Optimal dilutions of primary antibody and digestion conditions were determined by a series of previous titration experiments. This was followed by sequential application of rabbit anti-mouse link antibody, alkaline phosphatase anti-alkaline phosphatase (APAAP) complex, and napthol/fast red substrate (Dako Corporation, Carpenteria, Calif., USA). Sections were then counterstained in Mayer's hematoxylin (Fisher Scientific) and mounted with aqueous mounting medium (Signet Laboratories, Dedham, Mass., USA). Finally, microscopic examination was performed.

Results

Collagen type I staining reactions were assessed under the microscope

In specimens from the pubocervical fascia, we found a significant reduction of collagen type I in about 53% patients with GSI and pelvic relaxation (group I) compared to those from groups 2 and 3 (Table 2). The quantity of collagen type I was significantly reduced in only 8% patients of group 2 as compared to group 3. A statistically significant relationship was found between BMI and the presence of SUI.

Discussion

Female urinary incontinence is a common problem that disables many women, especially after the menopause. Recent research has focused on functional changes of the ligaments of the pelvic floor and the condition of the fibrous connective tissue which is their main constituent – as factors that play a significant role in the development of stress urinary incontinence (SUI). Five types of collagen have been identified and classified using progressive roman numbers. Type I collagen is the most abundant in the skin, tendons, ligaments, bone etc., where it comprises 80–99% total collagen. Type III collagen is thought to contribute to the more elastic properties of tissues [5]. It has been found that in women with SUI there is a reduction of total collagen in the pubocervical fascia, with no change in collagenase activity [7, 8]. The content of collagen type III has been

found to be significantly reduced in the pelvic supportive tissue from patients with SUI, in comparison with tissue from patients with pelvic relaxation without SUI and tissue from control subjects [2]. In addition the production of collagen by the fibroblasts has been found reduced in patients with SUI by 30%, compared to the control group of healthy women. According to theses findings, it appears that in women with SUI there is a reduction of total collagen production by the fibroblasts while the collagenase activity remains unchanged.

In the present study, we found a significant reduction of collagen type I in about 53% patients with SUI, in comparison to women with pelvic relaxation without SUI and the control subjects. It seems that the reduction of collagen type I in both sites of biopsies is associated with the development of SUI. The possible explanatory mechanism could be that a reduction of collagen type I and collagen type III [2] is associated with inadequate support of the bladder neck, causing unequal transmission of the intra-abdominal pressure between bladder neck and urinary bladder, with a consequent development of SUI. When compared with the control group, there was a significant reduction of collagen type I in only 8% patients with prolapse but no SUI. This finding implies that the presence of SUI is associated with a reduction of collegen type I, irrespective of the presence or absence of genital prolapse and that genital prolapse is not significantly associated with a reduction in collagen type I. Furthermore, a statistically significant association was found between BMI and the development of SUI ($P < 0.001$).

Up to the present, only a few articles in the medical literature have suggested or confirmed the presumed role of changes in the connective tissue components, in the development of SUI. The present study is the first to report a significant reduction of collagen type I in women with SUI, irrespective of the presence or absence of genital prolapse. Furthermore, a reduction in collagen Type I is not found to have a significant association with the development of genital prolapse.

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