Urol Res (2003) 31: 61–65
DOI 10.1007/s00240-002-0293-y

ORIGINAL PAPER

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Differences in the quantity of elastic fibres and collagen type I and type III in endopelvic fascia between women with stress urinary incontinence and controls

Received: 8 April 2002 / Accepted: 14 November 2002 / Published online: 2 April 2003

Abstract The aim of this study was to evaluate whether differences in the quantity of elastic fibres, collagen type I and collagen type III in the endopelvic fascia occur with female stress urinary incontinence (SUI). A total of 54 patients participated in the study. They were divided into two groups (continent and incontinent) that were comparable with respect to age and parity. All patients underwent gynaecologic surgical procedures and biopsies from the endopelvic fascia were obtained. Histological slides were stained with haematoxylin and eosin and Masson trichrome or Weigert's techniques and immunohistochemistry for either collagen type I or type III were performed. The elastic fibres constituted $3.81 \pm 0.6\%$ and $5.93 \pm 0.92\%$ of the cross-sectional area of the endopelvic fascia in incontinent and control groups of patients, respectively. Collagen type I and type III were not significantly reduced in patients with SUI. Our results suggest that the quantity of elastic and collagen fibres in the endopelvic fascia does not play a significant role in continence.

Keywords Collagen · Connective tissue · Elastic fibres · Stress urinary incontinence

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Introduction

The prevalence of urinary incontinence increases with age and is a common problem that affects at least 14% of women over 30 years of age [12]. Stress urinary incontinence (SUI) is a condition believed to be caused mainly by poor anatomic support to the bladder base and bladder neck or insufficiency of the urethral sphincter [18]. Mechanically, a poorly supported urethra cannot be squeezed or bent backwards once abdominal pressure is applied on the bladder [2].

An earlier studies found that the optimal function of the pubourethral ligaments, the pubococcygeal muscles, and the connective tissue of the suburethral vaginal wall is important for urinary continence in women [10, 13]. The main constituent of the connective tissue in the ligaments and the suburethral-vaginal wall is collagen. Collagens of type I and type III are the predominant components in this kind of connective tissue. The quantity and organisation of collagen fibres significantly affect the tensile strength of the endopelvic fascia and consequently the support that is provided to the bladder neck and bladder base. It has been reported that total collagen reduction of the paravaginal fascia is associated with the development of SUI [16, 12], while other investigators have found that the collagen concentration seems to be higher in women with SUI [8]. Elastic fibres are also present in structures of the urinary tract but have been studied less extensively. Unattached elastic fibres possess rubber like properties, they can stretch easily and rapidly, and can return to their prestressed state with minimal loss of energy, thereby providing elasticity and stretchability to tissue. The fibroelastic tissue has been assumed by some authors to be a major factor in the generation of SUI [4].

The aim of the present study was to evaluate whether changes in the quantity of elastic fibres, collagen type I and collagen type III in the endopelvic fascia occur with female SUI.

Materials and methods

A total of 54 patients participated in the study. Their ages ranged from 27 to 52 years (mean 43.3 ± 5.2). All had had two deliveries except two patients (one with one and one with three deliveries). Patients with previous operations for the management of urinary incontinence or anterior vaginal prolapse were excluded from the study. Approval from the ethics committee of Slovenia and informed consent from all participants was obtained. In the study group, SUI was confirmed by a 1 h pad test and urodynamic measurement performed by ICS standard [1]. Patients that had obvious leakage of urine on cough provocation were classified as stress-incontinent. In this group, 30 patients with a mean age of 42.8 years (range 27–52) were included. In the control group there were 24 continent patients with a mean age of 43.9 years (range 30–52) with a negative history of incontinence. Urinary incontinence in this group of patients was excluded by a 1 h pad test. All of the control patients were admitted to hospital for pelvic surgery due to benign gynaecologic disorders such as minor uterine myomas. Patients from both groups were comparable with respect of their age and parity. All of the patients were without clinically determined paravaginal defects but the study group had significantly greater mobility of the bladder neck than the control group.

During the surgical procedures, Retzius spaces were prepared and biopsies were obtained from the endopelvic fascia at the level of the bladder neck from both groups of patients. The bladder neck was determined by the Foly catheter balloon position. Tissue samples obtained were immediately immersion fixed in neutral buffered formalin for 24 h before processing and embedding in paraffin wax. From each paraffin block, five consecutive 4 μm thick sections were cut. Three of the sections were prepared for histochemical staining. One of them was stained with haematoxylin and eosin, the second with Masson's trichrome technique, the third for elastic fibre analysis with Weigert van Gieson staining.

Two sections from each tissue sample were stained immunohistochemically with anticollagen I and anticollagen III antibodies. Briefly, 4 μm thick sections were deparaffinized and hydrated. The sections were soaked in 3% hydrogen peroxide for 30 min at room temperature, and then incubated in blocking buffer. Immunohistochemistry was performed using polyclonal rabbit anti-human collagen type I or polyclonal rabbit anti-human collagen type III antibodies (supplied by Chemicon International, Calif.) The samples were incubated with primary antibody dilutions 1:200 overnight at 4°C. Immunoperoxidase detection was employed using the ABC method (DAKO, Denmark). The antibody-binding sites were visualized by incubation with a diaminobenzidine-H_2O_2 solution using a diaminobenzidine kit and finally counterstained with haematoxylin. A human skin tissue sample was used as a positive control. The specificity of the reaction was determined using non-immune serum instead of primary antibody as the negative control.

The collagen type I and collagen type III analysis was performed by two examiners using the modified scoring system introduced by Liapis and co-workers [11, 12]. The two examiners did not know to which patient the samples belonged and they did not know each others findings. A minimal staining reaction was scored as (+), a medium staining reaction as (++) and a strongly positive staining reaction as (+++). The data were analysed and compared in relation to the collagen type I and collagen type III content in both groups.

The elastic fibres were analysed morphometrically by the point counting method and the percentage area of elastin stain in the tissue sections was determined. Because connective tissue in the endopelvic fascia represents an isotropic non-random structure with preferential orientation of the fibres, use of the integrating grid according to Merz and Schenk, with its semicircular sampling lines, was prefered. Elastic fibre arrangement and distribution were also described. Data are expressed as the mean ± SD.

The Student's t-test and χ^2 test were applied for statistical analysis and $P < 0.05$ was regarded as statistically significant.

Results

Histologically, the tissue samples consisted of smooth muscle, elastic and collagen fibres and blood vessels. Masson's trichrome technique, which highlights the extracellular matrix (i.e. collagen and elastin as blue and smooth muscle as red), showed that the majority of the samples consisted of connective tissue. The connective tissue of the endopelvic fascia consisted mainly of collagen fibres with scattered fibrocytes. Some smooth

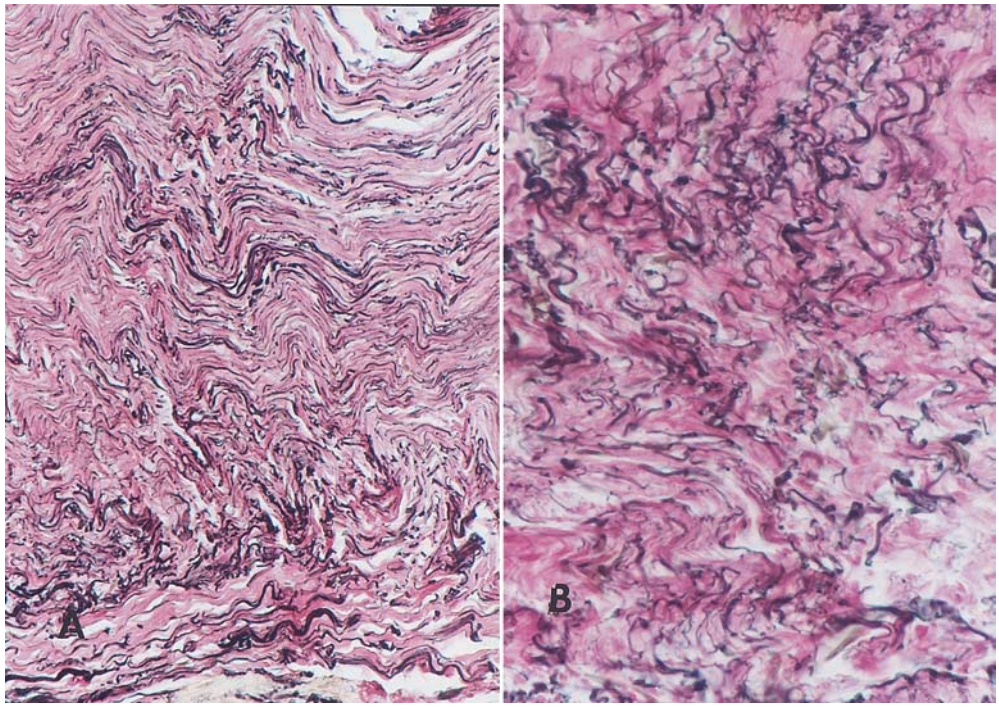

Fig. 1 Longitudinally arranged elastic fibres in the endocervical fascia of **A** a patient with stress urinary incontinence and **B** a continent patient. Their rippled appearance is clearly demonstrated (Weigert van Gieson staining; ×160 and ×240)

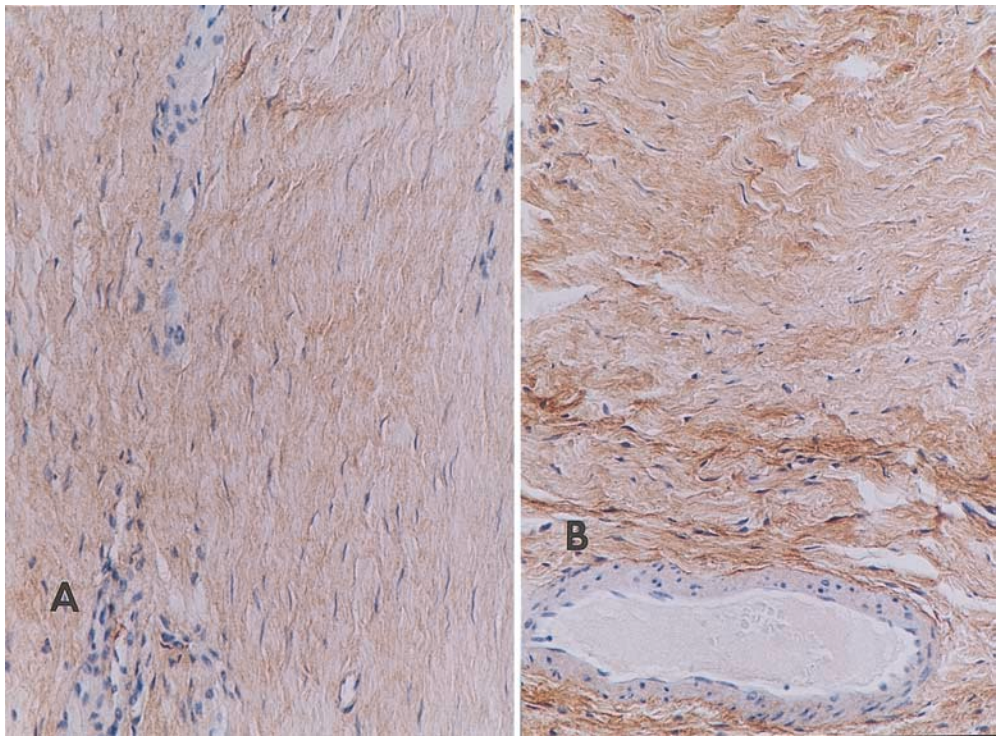

Fig. 2 Microscopic appearance of a tissue specimen from the endocervical fascia with **A** minimal (graded as +) and **B** medium (graded as ++) staining reaction for collagen type I (immunohistochemistry; ×160)

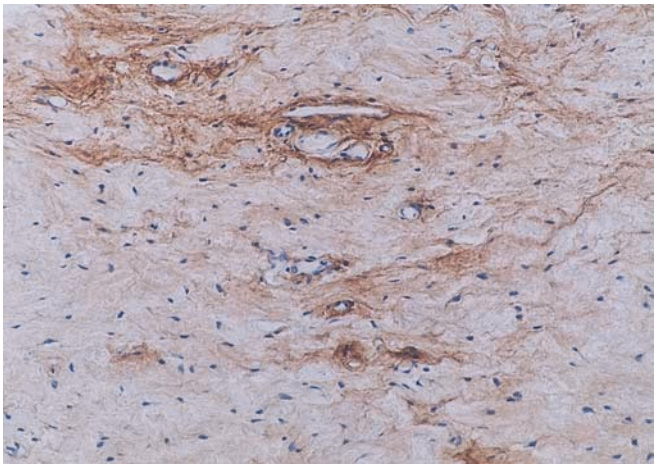

Fig. 3 Weak staining for collagen type III alternates with parts showing a distinct but focal increase in staining intensity around blood vessels (immunohistochemistry; ×160)

muscle cells, as well as some sparse skeletal muscle fibres, were also found. Light microscopic examination of the Weigert van Gieson stained slides showed that the elastic fibres were sparse in the endopelvic fascia. These fibres were very fine, displaying a longitudinal orientation. Individual elastic fibres were tightly coiled and rippled in appearance (Fig. 1). Elastic fibres were also present in blood vessel walls.

Immunohistochemically stained slides showed a fine, evenly distributed meshwork of collagen in the endopelvic fascia (Fig. 2A, B). In some samples, an overall increase in staining intensity for both type I and type III collagen was found, but with an uneven distribution. Indeed, weak staining areas alternated with parts showing a distinct but focal increase in collagen intensity staining around the blood vessels (Fig. 3). Since all tissue sections were immunohistochemically stained simultaneously and the dilutions of the primary antibodies were the same, the general opinion was that the collagen type III staining reaction in the endopelvic fascia was stronger than for collagen type I. Collagen type I and collagen type III staining reactions for SUI and continent groups of patients are shown in Table 1. There were no significant differences in the quantity of collagen type I and collagen type III between the SUI and control groups. Morphometric analysis of the elastic fibres demonstrated that they constituted, on average, $3.81 \pm 0.6\%$ and $5.93 \pm 0.92\%$ of the cross-sectional area of the endopelvic fascia in incontinent and control groups of patients, respectively (Fig. 4). There were 12 (40%) patients with less than 5% of the elastic fibre area in the incontinent group and 16 (66%) in the control group. The difference between the groups was not significant.

Discussion

Although female urinary incontinence is a common symptom that disables many women, especially after menopause, its aetiology is still unclear. Most probably, the aetiology of the disease is multifactorial. The proximal urethra is a mobile structure, and voluntary control of its position is an integral part of the initiation of urination and continence. The changes in urethral pressure that occur during a cough, and microtransducer

Table 1 Collagen type I and collagen type III staining reaction frequency in the endocervical fascia in stress urinary incontinent (SUI) and continent (control)groups of patients

	Collagen type I staining reaction			Collagen type III staining reaction		
	1+	2+	3+	1+	2+	3+
SUI	40.0%	36.7%	23.3%	30%	50%	20%
Control	45.8%	41.7%	12.5%	33.3%	29.2%	37.5%

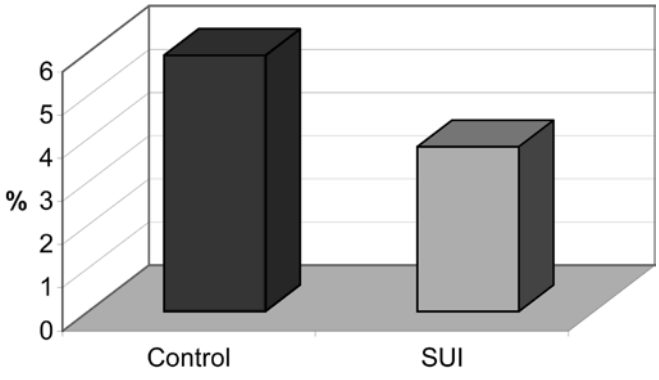

Fig. 4 Percentages of the elastic fibre area in the endocervical fascia in stress urinary incontinent (*SUI*) and continent (*control*) groups of patients

measurements of urethral pressures at rest, suggest that structures extrinsic to the urethra and vesical neck influence the sphincter function [6]. Many authors believe that a main cause of SUI involves defects in the urogenital suspensory apparatus, resulting in increased mobility of the urethra and bladder base [10, 16]. The important components are the levator ani muscles. These muscles lie lateral to the arcus tendineus fascia pelvis, a band of endopelvic fascia stretched between the pubic bone and the ischial spine. A combination of fascia and the anterior vaginal wall forms a layer in which the bladder and vesical neck rest. Although this fascia support is usually thought to be a passive rather than an active mechanism, the connection between the fascia and the levator ani muscle is an important element of this system [6].

Recent research has focused on functional changes in the ligaments of the pelvic floor and the condition of their main constituent, the fibrous connective tissue, as important factors for the preservation of urinary continence [7, 13]. Connective tissue consists mainly of elastin and collagen fibres which, if it becomes weak, causes weakness of the muscular support due to the attachments that permit the muscles to apply their action becoming unreliable. The present investigation was undertaken to test whether differences in the quantity of elastic and collagen fibres in the endopelvic fascia may be an aetiological factor in stress urinary incontinence in women.

Sayer et al. showed that collagen was the main component of the endopelvic fascia connecting the striated muscles of the pelvic floor to the urethra [15]. 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 comprise 80–99% of the total collagen. Type III collagen has the same distribution as type I but the ratio between the two varies. Type III is thought to contribute to the more elastic properties of tissue. Collagen subtypes, their location, total amount and the architecture of the fibrillar network are of functional importance. The architecture in terms of density of the fibrillar network is assumed to be reflected by the intensity of the immunohistochemical staining of collagen. There are conflicting opinions as to the importance of the collagen content in the urogenital suspensory apparatus.

Falconer et al. suggested that women with urinary stress incontinence have an altered connective tissue metabolism causing decreased collagen production [8]. Versi et al. have shown a decreased amount of type I collagen in incontinent women [17]. On the other hand, Bergman et al. showed that collagen type III content was significantly reduced in specimens from patients with SUI [2]. However, this study was based on a biochemical assay of paraurethral and the skin tissue samples and therefore did not allow the determination of the exact localization of collagen in the tissue. Fitzgerald et al. analysed collagen in biopsy samples from incontinent and continent women with electron microscopy and found that collagen fibril diameter did not vary with continence status [9]. Another study using immunostaining techniques for collagen type I showed a decrease in the staining intensity for collagen type I in incontinent patients [11]. The results of our study did not show any statistically significant differences in the quantity of collagen type I and collagen type III between SUI and control groups of patients.

The patients in Liapis et al.'s study were older (with a mean age of 54.5 years) and more than half of them were post-menopausal [11]. In our study, we excluded all the post-menopausal women and only two patients were over 50 years of age. This could explain the differences between our results and the results from the Liapis et al. study [11]. It is known that the incidence of SUI increases after menopause. On the other hand, it is also known that collagen metabolism appears to be oestrogen dependent. Recently, Copas et al. reported that cells in the endocervical fascia showed nuclear oestrogen, progesterone and androgen receptor expression to varying degrees [3].

In another study by Liapis et al., patients were divided into three groups: patients with SUI and genital prolaps, patients with SUI without prolaps and patients with neither SUI nor genital prolaps [12]. They found that collagen type III was significantly reduced in the group of patients with SUI and genital prolaps. On the other hand, there was no difference between the groups without

genital prolaps. In our study, we analysed only patients without genital prolaps, so our results for collagene type III are in agreement with those of Liapis et al. [12].

Mature elastic fibres are composed of two very different components, an amorphous protein called elastin and a microfibrilar structure, the former being the more abundant and giving rise to the characteristic wavy appearance of the fibres when viewed under a microscope [14]. For elastic fibre analysis, we used Weigert's staining. The quantification of elastic fibres in our case was not a measure of the actual intensity of the elastin staining but a measure of the percentage occupation of whole cross-sectional areas by elastic fibres. As all tissue sections used were stained simultaneously, it was considered feasible to compare the relative amounts of stained elastic fibres between sections from different patients. The differences between incontinent and continent groups of patients in our study did not reach statistical significance.

Urinary control involves both the ability to remain dry during a cough and sneeze and normal voiding. This control requires several functional components, both of the lower urinary tract, and of the surrounding tissue and pelvic floor. Smooth muscle, collagen and elastin fibres of the vaginal wall and paraurethral tissue directly interdigitate with the muscle fibres of the most medial portion of the levator ani in the region of the proximal urethra [5]. According to our results, the composition of the endopelvic fascia alone does not play a specific role in voiding and continence but further studies and analysis of all components of continence mechanisms are needed to understand and to cure SUI. The essence of the urethral support concept is its multifaceted nature. No single structure provides urethral support; rather support is provided by the coordinated action of fasciae and muscles under neural control acting as an integrated unit.

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