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Age-related structural changes of the urethral plate in hypospadias

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Abstract *Purpose:* To describe age-related changes in the extracellular matrix (ECM) of the human urethral plate in patients operated on for hypospadias, specifically describing histological features and determining the differences in the major components of the ECM, and thus providing an evaluation of the quality and wound healing potential of the urethral tissue.
Patients and methods: Urethral plate samples were obtained from 16 patients who underwent hypospadias repair (6 months—53 years of age), not previously submitted to any surgery. As a control group, male urethras were obtained from five fetuses. ECM structural characterization was performed by Hematoxylin and Eosin, Masson's trichrome, Weigert's resorcin-fuchsin, and Sirius red. The concentration of total collagen was determined by a hydroxyproline assay.
Results: Urethral plates were lined with squamous epithelium. Most urethral plate samples showed well-vascularized connective tissue and typical vascular sinusoids surrounded by an ECM with smooth muscle cells, collagen, elastic fibers and fibroblasts. ECM of the older urethral plates was characterized by abundant collagen content (types I and III), scarce elastic fibers, low cellular density, and no vascular sinusoids. Total collagen concentration increased significantly with aging ($r = 0.798$; $p = 0.006$).
Conclusions: Urethral plates of hypospadias present important age-related structural changes. These changes may play a role in urethral healing following hypospadias repair, although this subject deserves more investigation.

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Introduction

Hypospadias is one of the most common anomalies of male genitalia, with an incidence of 1 in 250 male births [1]. The developmental changes of the human male urethra are a complex event and the cause of hypospadias remains unknown [2,3].

Urethral plate is the strip of tissue that extends distally from the hypospadiac meatus to near the tip of the glans, and several surgical repairs for hypospadias involving this structure have been described with a low complication rate [4,5]. Although similar procedures are used for pediatric urethral reconstruction, hypospadias repair in adolescents and adults has a higher complication rate (e.g., fistula) and the outcomes may be less satisfactory [6–9]. These data suggest that the wound healing of the male urethra, as other human tissues, can be impaired by aging [10].

The extracellular matrix (ECM) plays a crucial role in the development and healing process. Modern concepts changed the view of ECM function from passive physical support to a clear capacity for transducing signals with a significant structural influence on the molecular roles, and far from being an inert scaffold around cells it actively orchestrates the key steps of wound healing [11]. Furthermore, a greater production of pro-inflammatory cytokines expressed by fibroblasts derived from foreskin has been described with aging, and this may partially explain the higher complication rate of hypospadias repair in older boys [12]. An understanding of age-related changes in the structural microenvironment of the urethra also constitutes an important factor in any discussion of wound healing and aging. However, to the best of our knowledge, there are no data on ECM composition of the urethral plate with aging. Thus, we aimed to describe age-related structural changes of the urethral plate, specifically describing histological features and determining the differences in the major components of the ECM (i.e. collagen and elastic system fibers), in patients who were operated on for hypospadias.

Patients and methods

After approval by the local ethical committee on human research, we evaluated urethral plates from 16 patients who underwent surgical repair for hypospadias, ranging in age from 6 months to 53 years (median 15 years): Six prepubertal children, Tanner 1, ranging from 6 months to 11 years old; five pubertal, Tanner 2-4, ranging from 13 to 17 years; and five post pubertal, Tanner 5, ranging from 21 to 53 years. We classified adolescence according to Tanner criteria.

Urethral meatus was distal penile in 14 patients, mid penile in 1, and proximal penile in 1. No patient was operated on for penile abnormality previously. Chordee was found in ten (62.5%) patients and nine of them were successfully repaired by degloving the penile skin. The most common surgical procedure was tubularized incised urethral plate (TIP repair) ($n = 15$). Samples were obtained during surgical repair from a midline elliptical incision in the mid third segment of the urethral plate, in the position of the TIP incision, and were immediately immersed in 4% paraformaldehyde in phosphate buffered saline. Therefore, the size of the samples varied depending on the size of the urethral plate. Biopsies from younger boys, mostly in cases of distal hypospadias, resulted in small tissue samples ranging from 5 to 10 mm. Collagen quantitation consumes small samples completely since it is a biochemical analysis. So, due to the small size of the urethral samples at younger ages, in some cases it was not possible to use the same sample for histological and biochemical analysis. Thus, three samples were analyzed entirely and only for estimation of the total collagen concentration and six only for histological staining. Consequently, we evaluated the histological staining of 13 urethral plates and the collagen concentration of 10 urethral plates.

As a control group, we selected 5 fresh, macroscopically normal male urethras obtained from human fetuses. Considering hypospadias as an interruption of urethral development, we believed that would be the most adequate control group. The fetuses were well preserved and none had any kind of detectable congenital malformation. Fetuses were dated on the basis of fetal heel-toe length and were estimated ranging from 16 to 28 gestational weeks [13]. After dissection, the penis was removed, cross-sectioned at its midshaft and immediately fixed overnight in 4% paraformaldehyde in phosphate buffered saline at 4 °C.

All samples were embedded in paraffin and serially sectioned (5 μm). To verify the integrity of the specimens, from each sample, one section was stained with hematoxylin and eosin. This initial analysis showed that all samples were well preserved and fixed. Staining histological sections were performed with hematoxylin and eosin, Masson's trichrome, Weigert's resorcin-fuchsin, and Sirius red. With Sirius red under polarized light, collagen type I shows thick, strongly birefringent, yellow or red fibers. Collagen type III appears as thin, weakly birefringent, greenish fibers [14]. All histological samples were examined by the same pathologist in a blind fashion.

As an indication of total collagen concentration, a quantitative determination of hydroxyproline was made as previously described [15].

The association between the concentration of total collagen and age was evaluated by linear regression analysis, followed by the t test for the correlation coefficient. Differences among groups were determined by Kruskal–Wallis test, followed by the Mann–Whitney rank-sum test. Results are expressed as the mean ± one standard deviation of the mean. P values less than 0.05 were considered statistically significant.

Results

The subepithelial tissue of the fetal urethra was characterized by active mesenchymal proliferation and well vascularized tissue presenting vascular sinusoids that contains a large amount of collagen (Fig. 1A) and elastic fibers (Fig. 3A).

Initial descriptive analysis of sections stained with hematoxylin and eosin showed that all urethral plates were lined with squamous epithelium, with no difference throughout the specimen. In the vast majority of the urethral plate samples, well-vascularized connective tissue

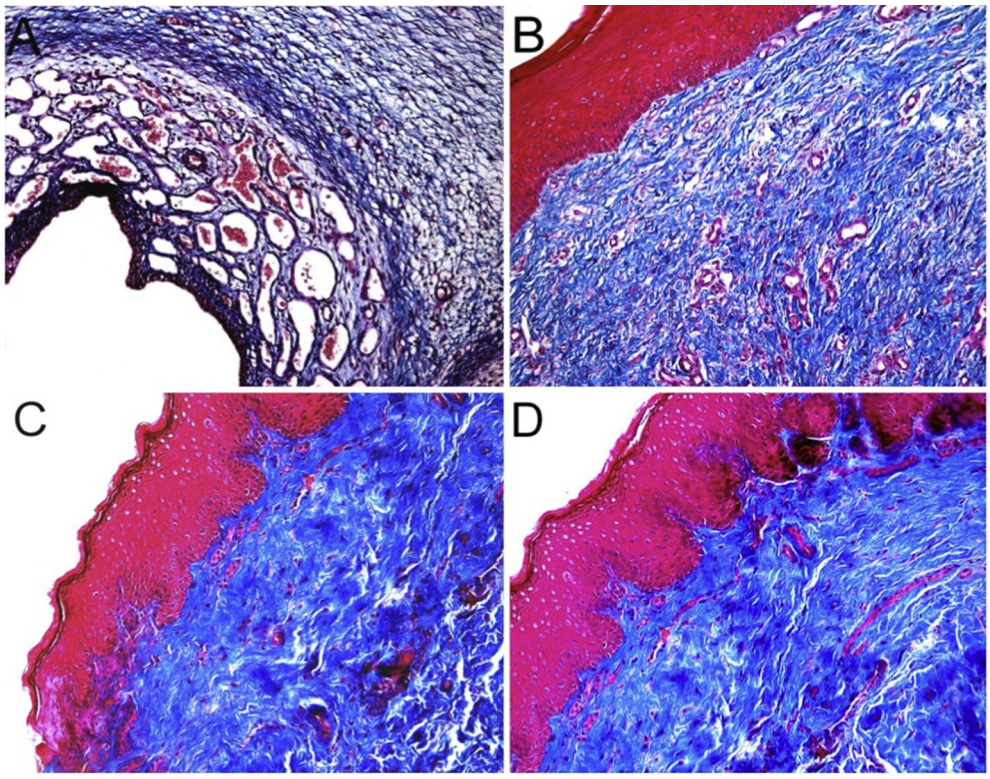

Figure 1 Distribution of the collagen fibers and smooth muscle cells in the spongy urethra of a human fetus and in urethral plates at different ages, as shown by Masson's trichrome stain, reduced from ×200. Note the structural changes that occur with age, particularly the organization and increase of collagen fibers. (A) Normal fetal urethra, at 18 weeks of gestation. Urethral plates of 2 year old (B) and 12 year old (C) boys. (D) Urethral plate of a 53 year old man.

and typical vascular sinusoids surrounded by an ECM, with smooth muscle cells, collagen and elastic fibers, and fibroblasts were found (Fig. 1). The collagen component was predominant in both urethral plates and fetal urethras. The concentration of total collagen increased significantly with aging ($r = 0.798$; $p = 0.006$) (Fig. 2). Prepubertal urethral plates presented significantly lower concentrations of total collagen than pubertal and adult urethral plates (56.0 ± 2.5, 63.4 ± 6.3 and 65.9 ± 6.7 μg hydroxyproline/mg dry tissue, respectively; $p = 0.035$). No subepithelial inflammatory changes were identified. ECM of the older urethral plates was characterized by scarce elastic fibers (Fig. 3), an abundant collagen content (types I and III) (Fig. 4), low cellular density and rare vascular sinusoids. The elastic fibers formed an irregularly oriented network onto which collagen interrelated.

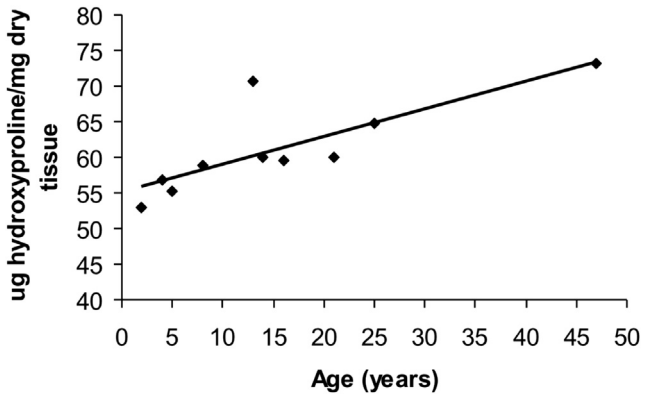

Figure 2 Concentration of total collagen in urethral plates at different ages, as estimated by hydroxyproline assay. The collagen concentration correlated significantly with aging ($r = 0.798$; $p = 0.006$).

We found no evidence of dysplasia in urethral plates, and no structural difference was observed between straight penises and those with chordee. The concept of fibrotic remnants of dysplastic corpus spongiosum thought to be implicated in the genesis of the chordee was previously studied, and the authors applied the term to the urethral plate considering any fibrotic bands or dysplastic elements, atypical for the usual epithelium overlying well vascularized connective tissue [16].

Discussion

Currently, age-related effects in different human tissues are of great scientific interest, essentially due to the opportunity for translation to clinical practice. The mammalian external genitalia are highly developed structures that permit efficient internal fertilization [17]. Although some experimental models for hypospadias have been described [18,19], the human male urethra is structurally unique with functional specialization not similar to that of other mammals and, thus, a suitable ideal experimental model does

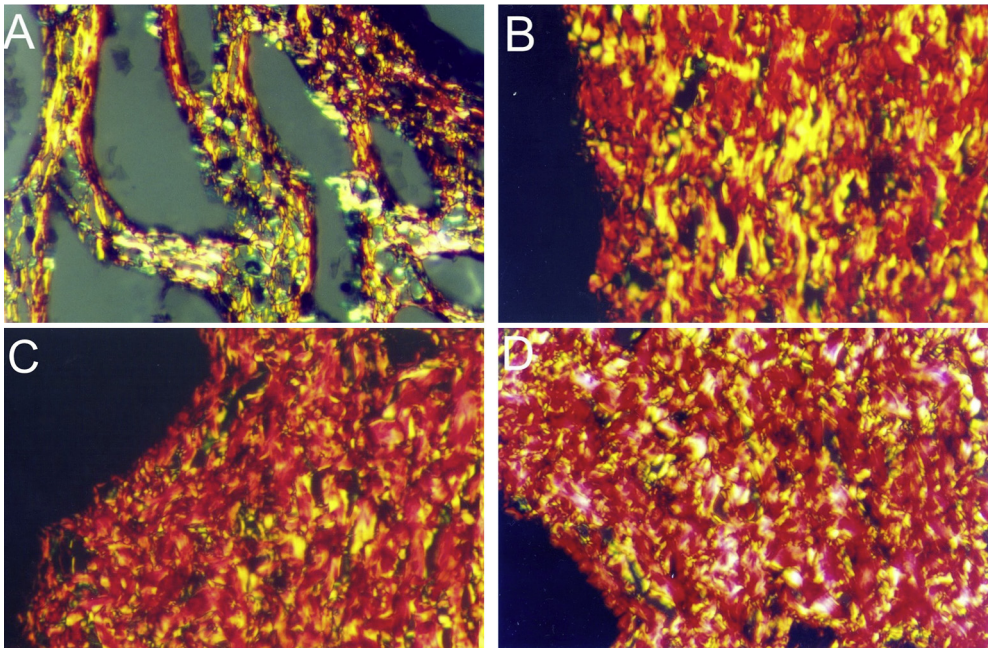

Figure 3 Distribution of the collagen fibrillar system in the spongy urethra of a human fetus and in urethral plates at different ages, as shown by Picrosirius red under polarization light, reduced from ×400. Thick type I collagen fibers appeared strongly birefringent and yellow or red-colored, whereas thin type III collagen fibers exhibited weak birefringence and greenish color. The predominant collagen is type I and its concentration is increased with age. (A) Normal fetal urethra, at 18 weeks of gestation. Urethral plates of 2 year old (B) and 12 year old (C) boys. (D) Urethral plate of a 53 year old man. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

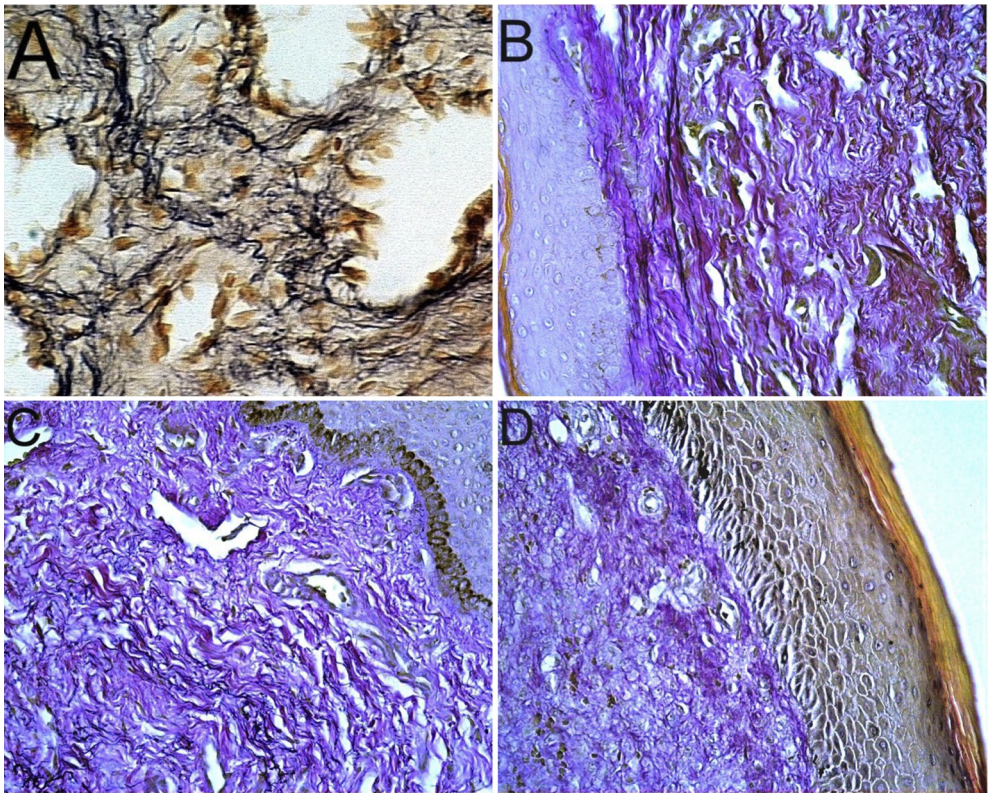

Figure 4 Distribution of the elastic component in the spongy urethra of a human fetus and in urethral plates at different ages, as shown by Weigert's resorcin—fuchsin stain after oxidation, reduced from ×400. Note the low concentration of elastic fibers in the extracellular matrix. (A) Normal fetal urethra, at 18 weeks of gestation. Urethral plates of 2 year old (B) and 12 year old (C) boys. (D) Urethral plate of a 53 year old man.

not exist [17,20]. The ECM presents significant structural differences in the phylogenetic scale and the translation of animal data to human healing may induce error.

Consequently, it is imperative that studies of human male urethra tissues should be performed to achieve better knowledge of the ECM components (i.e. collagen and elastic fibers). We evaluated human urethral plates obtained from hypospadias repair and the control group consisted of normal urethras of human fetuses. In our opinion this is the best possible control group, since urethral plate is exclusively an embryonic condition or related to hypospadias. Well-developed normal urethra cannot be regarded as an adequate control since it is not submitted to direct influence of the external environment.

Although the same techniques for urethral reconstruction are used in children, adult hypospadias repair is prone to a higher complication rate (e.g. fistula) and the outcome may be less satisfactory [6—8]. As surgical material and techniques had been similar to those used in the pediatric population, these authors hypothesize that the high complication rate is due to the impaired healing process in adults. Herein, we have described age-related structural changes in the ECM of urethral plates emphasizing the elastic fibrous elements that play a key role in wound healing.

The human urethral plate morphology was previously described in a small number of fetuses and prepubertal boys using usual histological staining, and an extensive well vascularized connective tissue was observed [16,21]. The tissue beneath the urethral plate has also been studied for the distribution of fibrillar collagen in prepubertal boys, and collagen type I was found to be distributed in the interfascicular space only and surprisingly collagen type III was not detected [22]. We found no microscopic evidence of scar-like or dysplastic tissue in the urethral plate and our findings support the theory that the urethral plate does not play a major role in chordee.

Although the histological pattern of hypospadias was previously described in boys, to our knowledge, we performed for the first time the structural characterization of the urethral plate in adults, as well as providing original data about the association of the elastic and collagen system with aging. The ECM of the urethral plate presented important age-related changes, as evidenced by the increase of the total collagen content (mainly type I fibers), scarce elastic fibers, lower cellular density and rare vascular sinusoids.

Sex hormones changes can variously affect the modulation of inflammatory reaction and ECM molecule turnover by mesenchymal cells [23,24]. Due to the marked increase of total collagen concentration that occurs at puberty and remains unchanged at adulthood, we hypothesize that androgen is a decisive contributing factor to ECM remodeling of the urethral plate.

Fibrillar type I and III collagens are major components of the ECM and have an active role in the structural integrity of tissues, providing the extracellular framework for all multicellular organisms [10]. The evaluation of the fibrillar collagen showed progressive decrease of the collagen type III:I ratio in the urethral plates with aging. The type III collagen fibers occur early in the healing process, but mainly in developmental tissues or organs exhibiting elastic properties. The presence of a higher collagen type I concentration, to the detriment of type III fibers, can be considered an age-related feature of normal tissues. However, this pattern of fibrillar collagen, in addition to other matrix changes, could implicate negatively on the healing process.

Penile and urethral extensibility decreases with aging and this is important with regard to the surgical repair of urethral defects [25,26]. The elastic system fibers have a key role in tissue compliance, mainly in organs that change shape under physiological conditions (e.g. the spongy human urethra). We found a progressive reduction in elastic fiber content of the urethral plate with aging.

Aging is associated with delayed epithelialization resulting from impaired migration and proliferation, excessive inflammation leading to increased levels of proteases (matrix metalloproteinases, elastases) and matrix degradation [27].

The true role of the age-related structural changes of the urethral plate with regard to the overall success rate of hypospadias repair is unclear. Careful follow-up of a similar but larger sample size of patients submitted for hypospadias repair is necessary to provide correlation between surgical outcomes and histological findings of the urethral plate.

Conclusions

The human urethral plate in hypospadias presents important age-related structural changes. These may play a role in urethral healing following surgical repair of hypospadias, although this subject deserves more investigation. Further understanding of the complex interaction between the aging cell and its microenvironment is essential in order to develop focused therapeutic strategies to improve urethral healing.

Conflict of interest statement

No conflicts of interest to disclose.

Ethical approval

The study has been approved by the local ethical committee on human research of Rio de Janeiro State University, Pedro Ernesto Memorial Hospital.

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