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G. Morgia · A. Saita · M. Falsaperla
A. Spampinato · M. Motta · S. Cordaro

# Immunohistochemical and molecular analysis in recurrent urethral stricture

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**Abstract** The authors have analyzed the most recent additions to the literature of immunohistochemical and molecular assessment of acquired urethral strictures and report on their data obtained in a selected clinical series. Innovative immunohistochemical studies in patients presenting with plurirecurrent symptoms suggest that urethral mesenchymal changes caused by tissue de-epithelialization may be the underlying cause of stricture. This condition may be congenital or acquired. It may determine aberrant connective tissue formation induced by abnormal fibroblastic activation with formation of over abundant hyperdense collagen scar tissue.

**Key words** Urethral strictures · Mesenchymal disease

## Introduction

Our study aimed to show that recurrent urethral stricture is caused by irritation that, in turn, is determined by urethral mucous membrane lesions and connective tissue scar characterized by abnormal connective tissue proliferation. We postulate that inherited or acquired mesenchymal urethral alterations associated with tissue damage are predisposing factors of stricture and that stricture can extend to the corpus spongiosum and provoke spongiofibrosis.

G. Morgia (✉)
Via Fisichelli 32, 95037 S.G. La Punta (CT), Italy
e-mail: morgia@mbox.unict.it
Tel.: +39-95-7412868; Fax.: +39-95-7513576

A. Saita · M. Falsaperla · A. Spampinato · M. Motta
Department of Urology, University of Catania, Catania, Italy

S. Cordaro
Histopathology Unit, M. Ascoli Hospital, Catania, Italy

## Material and methods

We recruited ten male patients (aged between 52 and 79 years; mean age 61.5 years) affected by recurrent postoperative urethral stricture in the period March–December 1998. Diagnosis had been made at least 6 months prior to therapy and the patients had undergone repeated (mean 3.9 treatments per patient) Sachse's uretrotomy treatments. The study was in line with the Helsinki Declaration and approved by the Ethical Committee of Catania University. All patients gave informed consent to the investigation.

Biopsies of strictured tissue and normal urethral tissue were removed from all the patients. Histologic examination was carried out using an optical microscope. Mallory's and Masson's trichrome staining was used to identify elastic, reticular and collagen fibers. Immunohistochemical examination used vimentin and desmin staining (Fig. 1). The collagen fibril period was assessed by scanning electron microscopy (SEM). Ultrastructural studies were performed on tissue biopsies prepared as described hereafter. They were fixed in 2% glutaraldehyde in a pH 7.4 phosphate buffer for 5–10 min at 4 °C immediately after removal and then postfixed in 1% osmium for 1 h at 4 °C. They were washed in phosphate buffered saline and dehydrated in stepwise increasing quantities of acetones to a critical point. Then they were mounted on stubs, metalized (gold, pale gold) and observed by electron microscopy. The initial preparation stages for SEM observation followed the previously described procedure. However, dehydration was carried out by embedding the samples in Durcupan and once polymerization was complete (after 1 week), an ultramicrotome was used to cut 700 Å slices. The slices were stained with lead citrate and acetate uranyl negatively enhanced (potassium pH 7) to identify banded collagen fibrils.

## Results

Optical microscopy showed that strictured urethral mucous tissue presented normal chronic inflammation with complex pseudostratified cubic epithelium coating (between 7 and 12 cell layers). It also revealed typical plasmacytoid inflammation and granulation tissue. Trichrome staining did not show specific changes, apart from normal scarring. Nonstrictured urethral tissue was normal. Immunohistochemistry revealed marked structural changes in the strictured area where the normal components of the urethral wall, i.e., muscle and elastic fibers, were replaced by collagen repair tissue. SEM

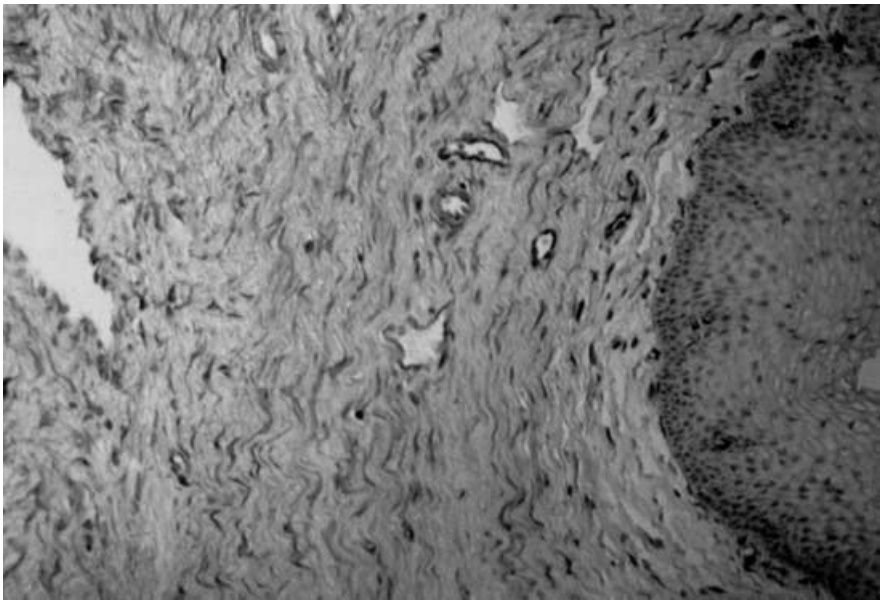

**Fig. 1** Vimentin stain: connective tissue component

(Fig. 2) revealed cell distress in the strictured areas and the various stages of cell turnover. SEM provided the following information: (1) a detailed 3-D ultrastructural picture of the collagen fibers, (2) the spatial relationship between the various polymers in the tissue examined and consequently (3) a specific variation in the collagen fibril period. This variation was shown by our results at 740 Å (74 nm) in both strictured and nonstrictured tissue in patients with recurrences as compared with those at 680 Å (68 nm) seen in classic banded fibrils in normal structures (Fig. 3).

## Discussion

### Pathogenic hypothesis

The SEM findings of different fibril periods in scar strictured tissue and in macroscopically healthy tissue may indicate primary mesenchymal changes and connective tissue disease. The latter can enhance scarring and structural changes in the urethral wall caused by fibroblast hyperactivation associated with hyperdense and anomalously ultrastructured collagen replacing the normal elastic and muscle component, all of which favor onset of stricture. The underlying pathogenic processes may be ascribed to changes in the mechanisms regulating collagen biosynthesis and fibroblastic activation.

Our study may shed light on the analysis and understanding of intrinsic biochemical mechanisms, changes in which seem to determine abnormal and anomalous recurrent scars in subjects with presumed inherited or acquired connective tissue disease. This

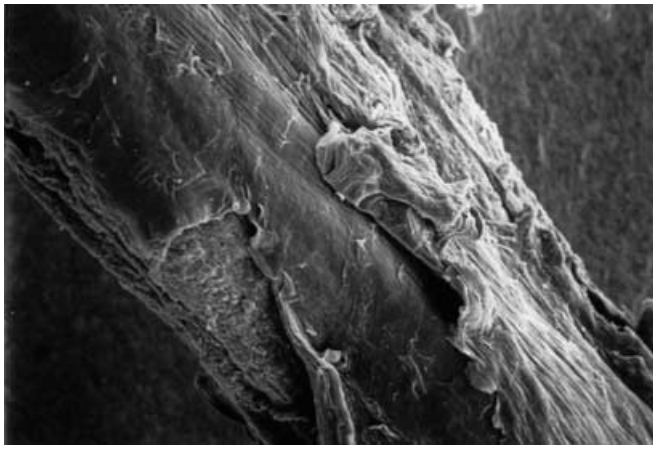

**Fig. 2** Scanning electron micrograph: strictured urethral epithelium showing cell swelling caused by tissue distress

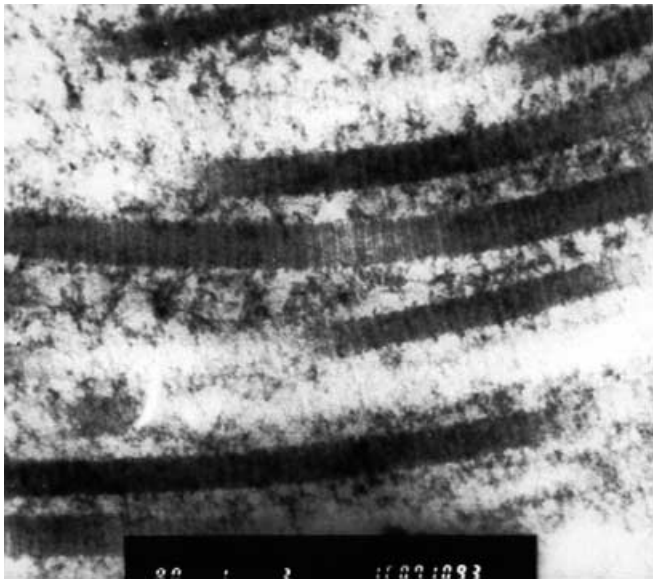

**Fig. 3** Transmission electron micrograph: collagen fibril period = 740° in normal and pathologic strictured urethra

hypothesis is supported by immunohistochemistry studies and 2-dimensional gel electrophoresis quantitation using cyanogen bromide. Important comparative studies in subjects with recurrent urethral stricture conducted by the University City Science Center, Philadelphia, achieved characterization and biochemical quantitation of the collagenous components of macroscopically healthy and scar tissue [2]. These studies revealed the characteristic morphostructural architecture caused by densely packed connective tissue fibers interspersed with fibroblasts replacing urethral spongiosum tissue. Furthermore, scanning densitometry performed in patients and controls revealed a change in the ration of Types I and I collagen in urethral spongiosum and strictured tissue. The proportion of Type I collagen fibers was increased (75.1% $\Rightarrow$ 83.9%), while that for Type II was decreased (24.9% $\Rightarrow$ 16.1%). This condition and the concomitant increase in fibrillar period observed in our study may explain the fibrotic noncompliant nature of urethral stricture scar tissue. This was also observed in macroscopically healthy urethral tissue and may be due to: (1) altered regulation of the type of collagen processed by the fibroblasts that have different functions and (2) anomalous tropocollagen fibril spatial distribution caused by fibroblasts.

Changes in biosynthetic regulation, whether associated with focal variations in amino acid polypeptide chain sequences or not, may be ascribed to changes in the tissue matrix that, in turn, induce changes in gene expression of fibroblasts and cells normally not producing fibrillar protein, and collagen production [1–9]. Exogenous stimuli triggered by specific characteristics of the interstitial matrix seem capable of influencing the expression of one or more codifying genes for the various types of fibrillar procollagen molecules and/or the enzymes involved in intra and extra posttransduction reactions regulating collagen fiber organization and arrangement [11].

Aberrant production of collagen could be a result of: (1) the release of specific hexones by physiological inhibition mechanisms with consequent increase in the formation of II messengers and/or; (2) posttransduction factors influenced by the modified fibrillar period that, in turn, modifies the geometric distribution of tropocollagen molecules. Lysyl oxidase (A) builds normal inter and intrafibrillar cross-linking to stabilize the collagen triple helix and to define the fibrillar period, thus making protein of primary importance in collagen biosynthesis [10–12].

Limited and focal gene or posttransduction changes in lysyl oxidase increase functional activity or synthesis and determine a rise, albeit negligible, in inter- and especially intrafibrillar cross-links [6, 7] This may provoke secondary changes in the collagen molecule and determine latent hyperstabilization of the collagen triple helix shown by diffuse increased fibrillar period in urethral wall collagen [4]. The histological picture of the strictured area is probably triggered focally after circumscribed tissue damage occurs.

Altered enzymatic synthesis does not always involve all collagen types and can be triggered under particular conditions (e.g., iatrogenic urethral damage) and in specific sites. The changes in collagen fibrillar period revealed by SEM ultrastructural observation and studies reported in the literature indicate that recurrent urethral stricture requires careful clinical and immunohistochemical studies to assess the mechanisms triggering connective tissue hyperproliferation and the underlying molecular causes of collagen changes. We believe that immunology studies of fibroblasts obtained from comparative biopsies from macroscopically healthy tissue and scar tissue can be used to study the enzymatic activities responsible for biosynthesis and posttransduction alterations of collagen polypeptides [5].

Chemical analysis and other examinations (e.g., X-ray diffraction) of urethral tissue could furnish additional direct or indirect information on specific molecular deficiencies of collagen [3–8]. Collagen solubility in saline and gelatin extractability in denatured solvents indirectly determine polymerization and the efficacy of inter and intra molecular cross-links between collagen fibers. Polyacrylamid gel electrophoresis of the molecular species extracted and the fission products using cyanogen bromide would furnish direct data on the cross-links. Molecular studies of the biochemical structure and biosynthetic and metabolic processes of the complex collagen biological macromolecule may furnish data on latent connective tissue diseases and define the pathogenic mechanisms underlying the changes in collagen observed in patients with plurirecurrent urethral stricture. We believe that our assessment of ultrastructural collagen changes sheds light on this issue and is useful in paving the way for newly directed therapeutic approaches.

Penicillin, cysteamine and amino nitrile seem to act on lysyl oxidase. They inhibit enzymatic functional activity and interfere with the formation of cross-links and consequently may inhibit scar tissue formation and reduce hyperstabilization of collagen molecules and deposits of hyperdense collagen.

## Conclusions

Our study confirms widespread involvement of the mucous membrane subepithelial connective tissue during stricture. Moreover, it revealed definitive histologic morphostructural disarrangement in strictured areas requiring both surgical and prosthetic treatment to prevent urethral stricture and maintain optimal urethral caliber. Investigations have shown that aspecific predisposition to anomalous repair tissue may be present in patients with plurirecurrent stricture, and that it may lead to stricture after direct urethral trauma. Studies on a large series of patients could confirm the existence of congenital or acquired collagen disorders that may be characterized by biochemical molecular changes in collagen biosynthesis, architecture and arrangement of

collagen fibers. The latter is linked to changes in gene expression of the fibroblasts and may induce hyperactivation and prime the onset of the immunohistochemical picture described. Suitable therapeutic protocols could be established to prevent or reduce recurrence in subjects predisposed to stricture formation.

Further studies are required to investigate the existence of congenital or acquired connective tissue pathologies caused by molecular alterations at the biochemical level, including changes in connective tissue biosynthesis and geometrical arrangement of the fibers. The latter is linked to altered fibroblast gene expression that may induce hyperreactivity and thus trigger off the histologic findings described herein.

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