QUANTITATIVE ANALYSIS OF COLLAGEN AND ELASTIC FIBERS IN THE TRANSVERSALIS FASCIA IN DIRECT AND INDIRECT INGUINAL HERNIA

Aldo Junqueira Rodrigues Junior, Consuelo Junqueira Rodrigues, Ana Claudia Pereira da Cunha and Jin Yoo

RHCFAP/3104

RODRIGUES Jr. AJ et al. - Quantitative analysis of collagen and elastic fibers in the transversalis fascia in direct and indirect inguinal hernia. **Rev. Hosp. Clín. Fac. Med. S. Paulo** 57(6):265-270, 2002.

PURPOSE: Our previous studies demonstrated structural and quantitative age-related changes of the elastic fibers in transversalis fascia, which may play a role in inguinal hernia formation. To verify whether there were differences in the extracellular matrix between direct and indirect inguinal hernia, we studied the amount of collagen and elastic fibers in the transversalis fascia of 36 male patients with indirect inguinal hernia and 21 with direct inguinal hernia.

MATERIAL AND METHODS: Transversalis fascia fragments were obtained during surgical intervention and underwent histological quantitative analysis of collagen by colorimetry and analysis of elastic fibers by histomorphometry.

RESULTS: We demonstrated significantly lower amounts of collagen and higher amounts of elastic fibers in transversalis fascia from patients with direct inguinal hernia compared to indirect inguinal hernia patients. The transversalis fascia from direct inguinal hernia patients showed structural changes of the mature and elaunin elastic fibers, which are responsible for elasticity, and lower density of oxytalan elastic fibers, which are responsible for resistance. These changes promoted loss of resiliency of the transversalis fascia.

CONCLUSION: These results improve our understanding of the participation of the extracellular matrix in the genesis of direct inguinal hernia, suggesting a relationship with genetic defects of the elastic fiber and collagen synthesis.

DESCRIPTORS: **Inguinal hernia. Transversalis fascia. Elastin. Collagen. Extracellular matrix.**

INTRODUCTION

The abdominal wall is the site of opposing physical forces that may eventually result in the appearance of hernias, which have social and economic consequences.

Inguinal hernia repair is the most frequent surgical intervention among all abdominal hernias. Since the mid 1990s, 2 different surgical approaches have been used for inguinal herniorrhaphies: tension and tension free (with prosthetic mesh). Tension-free repair reduces the recurrence rate, improves postoperative recovery, and lowers costs; it is becoming the gold standard procedure.

Although the etiology of adult inguinal hernias is multifactorial, all groin hernias emerge at the myopectineal orifice of Fruchaud, an opening in the lower abdominal wall, originated from the lack of a myoaponeurotic layer, which is closed off only by the transversalis fascia (TF). The common anatomic features among all groin hernias do not answer the question of why less than 5% of human beings develop inguinal hernia. Also, in a multifactorial etiology model, which factors predispose failure in TF continence?

In attempting to answer these questions, it is reasonable to remember that TF is structurally dependent on the elastic fiber and collagen components arranged in a framework to support tissue tension forces. So, any degree of alteration of the extracellular matrix that promotes loss in tissue resistance and elasticity of the TF at the myopectineal orifice may explain how inguinal hernia develops.

Some studies, although not conclusive, have tried to quantify collagen's structural modification in hernias[1-5]. Rodrigues Junior et al.[6] and Quintas et al.[7] have shown structural and quanti-

From the General Surgery Division and Investigation Lab of the Surgical Anatomy, Hospital das Clínicas, Faculty of Medicine, University of Sao Paulo.

tative age-related changes in the TF elastic fibers. These results indicate clearly that there is a morphological correlate to the loss of tensile resistance and elasticity of the TF with aging, and this loss can explain the high incidence of inguinal hernia in patients after the fifth decade of life.

In fact, connective tissue pathology plays an important role in the genesis of groin hernias. In the present paper, we have analyzed the structural and quantitative changes of collagen and elastic fibers of the TF from direct and indirect inguinal hernias in adult and aged patients to examine differences in the mechanisms of the genesis of these hernias.

MATERIALS AND METHODS

Patients

Fifty-seven male patients with nonrecurrent inguinal hernias underwent surgical treatment. These patients were distributed into 2 groups: 36 with indirect inguinal hernia (IIH) and 21 with direct inguinal hernia (DIH). During surgery, 1 fragment of 0.5 cm of intact TF from the posterior inguinal wall was excised, fixed in 10% formaldehyde solution, and embedded in paraffin for quantitative evaluation of collagen and elastic fibers.

Histomorphometric evaluation of the elastic fiber system

Three adjacent sections from paraffin blocks of 5 mm thickness were submitted to one of the following selective methods for staining elastic fibers: Verhoeff's iodine iron hematoxylin (Verhoeff[8]), which demonstrates only the mature elastic fibers; Weigert's resorcin-fuchsin (Weigert[9]), which stains mature and elaunin elastic fibers; and Weigert's resorcin-fuchsin stain with a previous oxidation performed using oxone 1% (Fullmer et al.[10]), which reveals oxytalan elastic fibers and mature and elaunin elastic fibers. The linear density (LV) of the elastic fiber system was determined in 25 random microscopic fields per histologic section stained by Verhoeff (LVV), Weigert (LVW), or Weigert-oxone (LVWO) stain. The sections were scanned randomly at a magnification of 1000X in a continuous line from edge to edge employing a test eyepiece reticule with 10 parallel lines and 100 points that contains a simple square lattice test system covering 10,500 mm^2. Each elastic fiber that was completely transected by one edge of the test line was counted. These intersections of elastic fibers with a test reticule are related to the length of those fibers per unit of area by the expression LV = 2 Na, where Na is the length of fibers per unit of area (Niewoehner and Kleinerman[11]). The area of the tissue examined was determined by counting points that fell over a tissue.

Colorimetric quantification of collagen

Eight serial sections of 10 mm thickness from paraffin blocks of TF specimens were placed on separate slides, and 2 slides were selected at random. The tissue sections of the first slide were deparaffined and stained with 0.1% picrosirius saturated with picric acid (sirius red, Gurr BDH, no. 34149), for 30 minutes in the dark and at ambient temperature. After staining, the sections were then rinsed several times with distilled water, and with the help of shaving blade were agglomerated and preserved in an assay tube. The stain was washed out from the sections using 1 mL of a solution of 0.1% NaOH in absolute methanol (1:1, v:v). The absorbency of the washed tissue was read in a spectrophotometer at 535 nm. This absorbency refers to the collagen content of the fascia transversals, which was obtained from the relationship between the absorbency and the color equivalency of 1 mg of collagen stained by picrosirius, i.e. 37 (Bedossa et al.[12]; Rodrigues et al.[13]). The second slide was deparaffined, and the tissue of the TF was weighed with an analytical balance. The TF's collagen content was expressed according to the ratio: mg collagen/ mg of TF.

Statistical Analysis

We calculated the mean and standard deviation of the measurements, and a one-way analysis of variance (ANOVA) was used to compare the data of the groups of patients. The level of significance was set at 5%.

RESULTS

Comparison of patient ages between the 2 groups showed no significant difference.

The TF from IIH patients had structurally well preserved and homogeneously distributed mature, elaunin, and oxytalan elastic fibers (Fig. 1 and 2). The TF from DIH patients had greater amounts of mature elastic fibers, which were characterized by thickening, curling, and shortening. The oxytalan fibers were less evident in TF from DIH patients (Fig. 3 and 4).

The histomorphometric study showed a significantly higher amount of all types of elastic fibers in TF from patients with DIH (Fig. 5). The linear density of mature elastic fibers revealed by Verhoeff staining for IIH patients was LVV = 0.0106 ± 0.0020mm^{-2}, and for DIH patients was LVV = 0.0139 ± 0.0019 mm^{-2}. There was a significant difference between them ($F_{1e\,55}$ = 36.36, P <0.001).

The amount of mature and elaunin elastic fibers, revealed by Weigert staining was LVW=0.0105 ± 0.0022 mm^{-2} for IIH patients and LVW = 0.0132 ± 0.0016 mm^{-2} for DIH patients. There was a significant differ-

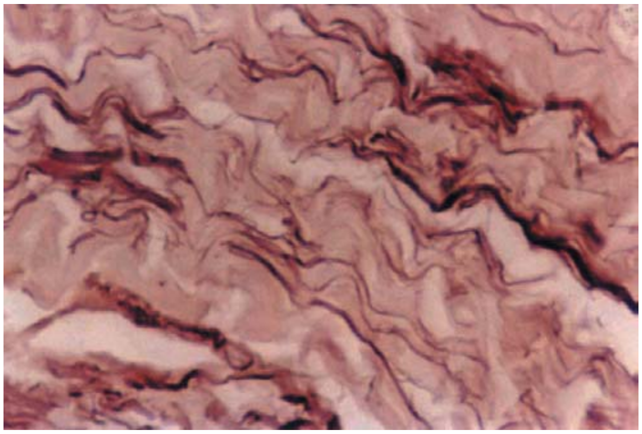

Figure 1 - TF from an adult patient with an indirect inguinal hernia. Note the long and thin elastic fibers (arrows) between collagen bundles (Weigert-oxone 400X).

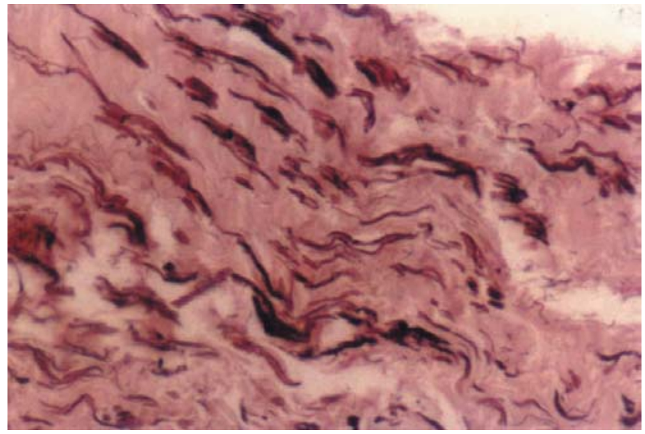

Figure 2 - TF from an aged patient with an indirect inguinal hernia. Note the thick and short elastic fibers beside some thinner fibers (arrow) (Weigert-oxone 400X).

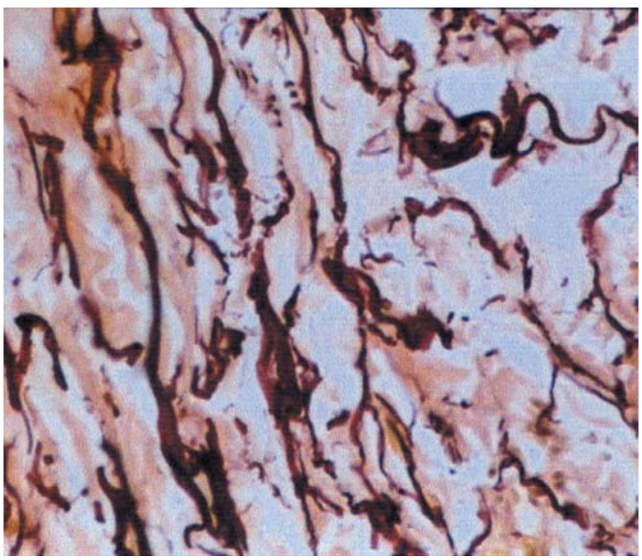

Figure 3 - TF from an adult patient with a direct inguinal hernia. Note long mature and elaunin elastic fibers (arrows). The thinner elastic fibers are not evident (Weigert-oxone 400X).

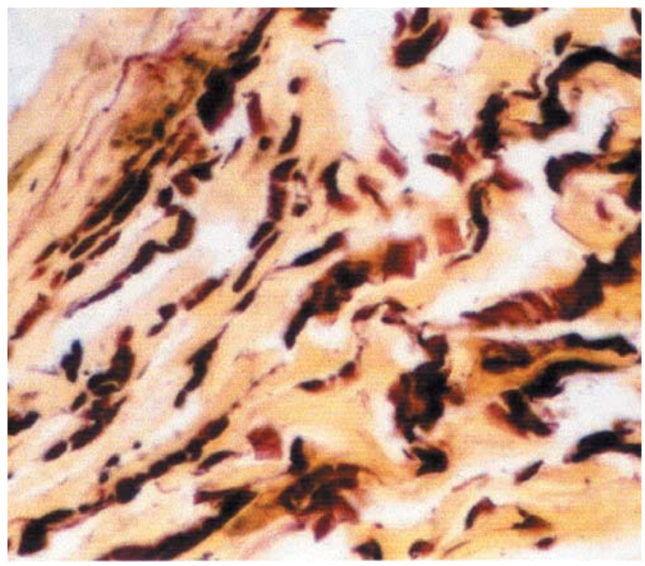

Figure 4 - TF from an aged patient with a direct inguinal hernia. The mature elastic fibers (arrows) appeared thick and curled, and the thinner fibers are not evident (Weigert-oxone 400X).

ence between them (F_{1e55} = 23.26, P <0.001).

Histomorphometric quantification of mature, elaunin and oxytalan fibers revealed by Weigert-oxone staining, showed LVWO = 0.0112 ± 0.0022 mm^{-2} for IIH patients and LVWO = 0.0135 ± 0.0016 mm^{-2} for DIH patients. Comparing these 2 data sets, we found a significant difference (F_{1e55} = 16.94, P <0.001).

The colorimetric evaluation of collagen (Fig. 6) demonstrated a significantly lower amount of collagen in the TF from DIH patients (21.28 ± 4.05 mg of collagen/mg of tissue) in comparison to the IIH patients (14.93 ± 2.58 mg of collagen/mg of tissue). Comparing these 2 groups of patients, we obtained a significant difference ($F_{1,46}$=35.35, P <0.001).

DISCUSSION

The reduction of resistance or TF rupture are considered etiologic and pathogenic factors of inguinal hernias and may be related to hereditary or acquired defects in the synthesis of extracellular matrix. Our previous reports demonstrated age-related changes of the elastic fiber system in the splenic capsule (Rodrigues et al.[14]), diaphragm muscle (Rodrigues & Rodrigues Jr.[15]), and the interspinous ligament (Barros et al.[16]). We demonstrated that these age-related changes occur in the TF and promote degeneration of mature

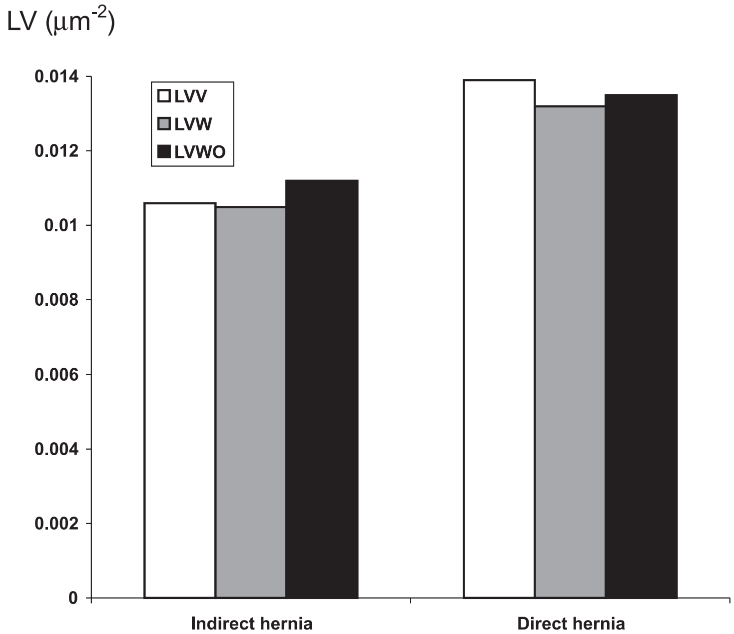

Figure 5 - Linear density of the elastic fiber system revealed by Verhoeff (LVV), Weigert (LVW), and Weigert-oxone (LVWO) staining of the transversalis fascia from patients with direct and indirect inguinal hernia.

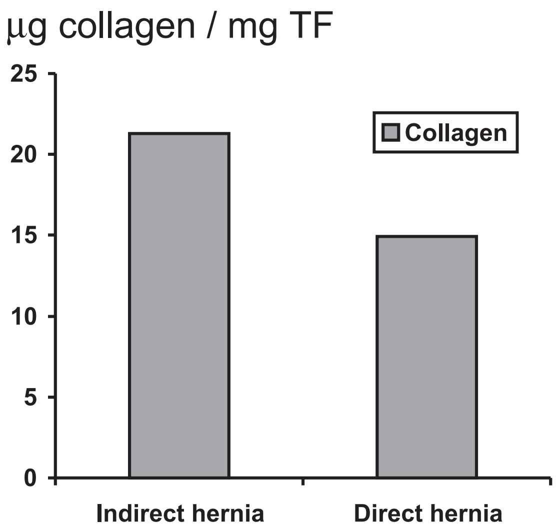

Figure 6 - Mass of collagen of transversalis fascia from patients with direct and indirect inguinal hernia.

elastic fibers and reduction in the amount of oxytalan fibers. These structural changes can explain the high incidence of groin hernias in patients after the fifth decade (Rodrigues Jr et al.[6], Quintas et al.[7]).

With aging there was a decreased collagen content in the fascias, as well as decreased extractability of collagen with pepsin (Pans et al.[17]). This is in accordance with the data of the literature reported for skin (Uitto et al.[18]) and our previous data for the diaphragm (Rodrigues et al.[13]).

To understand inguinal hernia formation, it is fundamental to verify whether there is some difference in the mechanisms leading to direct and indirect inguinal hernias. In the present study, we point out the structural and quantitative changes in the collagen and elastic fibers of the TF from DIH and IIH patients.

We found that the collagen content per milligram of TF was lower in DIH than in IIH patients. Wagh et al.[19] also demonstrated significantly decreased collagen concentration in the rectus sheaths in hernia patients. Pans et al.[17] demonstrated an increased collagen content in the TF from IIH and a significant increase in collagen extractable with pepsin (immature collagen) in TF from DIH. This alteration may be due to an enhanced collagen turnover rate or increased breakdown of collagen by metalloproteinases. Bellon et al.[20] showed an increased expression of metalloproteinase 2 in TF in direct compared to indirect hernias.

We demonstrated higher amounts of mature, elaunin, and oxytalan elastic fibers in TF from DIH patients compared to IIH patients. These higher amounts of elastic fibers did not promote increased complacency or tissue elasticity, because these elastic fibers in TF from DIH patients showed architectural distortion. They had become tortuous, thick, and fragmented, and had lost their original function. In conclusion, the TF from DIH patients had less resistance and elasticity compared to TF from IIH patients.

Based on our results, as well as the finding that there were biomechanical and structural changes in TF from both the herniated and the nonherniated sides (Pans et al. [21, 22]), we suggest that hernia disease may represent an expression of a generalized alteration of collagen and elastic metabolism that is possibly linked to genetic factors that are currently unknown.

RESUMO

RHCFAP/3104

RODRIGUES Jr. AJ - Análise quantitativa do colágeno e de fibras elásticas na fascia transversal de pacientes com hernia inguinal direta e indireta. **Rev. Hosp. Clín. Fac. Med. S. Paulo** 57(6):265-270, 2002.

OBJETIVO: Estudos prévios nossos demonstraram alterações estruturais e quantitativas de fibras elásticas na fascia transversal com o envelhecimento, tendo papel na gênese da hérnia inguinal. Com o objetivo de verificar diferenças na matriz extracellular da fascia transversal de pacientes com hernia inguinal direta e indireta, quantificamos o colágeno e as fibras elásticas na fascia transversal de 36 pacientes masculinos com hernia inguinal indireta e 21 pacientes masculinos com hernia inguinal direta.

MATERIAL E MÉTODOS: Fragmentos de fascia transversal foram retirados durante a intervenção cirúrgica e seguiram para rotina histológica para análise quantitativa colorimétrica do colágeno e histomorfométrica de fibras elásticas.

RESULTADOS: Demonstramos que a fascia transversal de pacientes com hernia inguinal direta apresenta menor quantidade de colágeno e maior de fibras elásticas em relação à fascia transversal de pacientes com hernia inguinal indireta. A fascia transversal dos pacientes com hernia inguinal direta apresentou alterações estruturais das fibras elásticas maduras e elaunínicas, responsáveis pela elasticidade e redução da densidade de fibras oxitalânicas, responsáveis pela resistência. Estas alterações promovem perda da complacência tecidual da fascia transversal.

CONCLUSÃO: Estes resultados nos dão elementos para compreender a participação efetiva da matriz extracellular na gênese da hernia inguinal direta, sugerindo uma relação com alterações genéticas do metabolismo de fibras elásticas e colágeno.

DESCRITORES: **Hérnia Inguinal. Fáscia Transversal. Elastina. Colágeno. Matriz Extracelular.**

REFERENCES

1. WAGH PV, LEVERICH AP, SUN CN et al. – Direct inguinal herniation in men: A disease of collagen. **J Surg Res** 1974; **17**: 425.

2. PEACOCK EE & MADDEN JW - Studies on the biology and treatment of recurrent inguinal hernia: II . Morphological changes. **Ann Surg** 1974; **179**: 567.

3. WHITE HJ, SUN CN & READ RC - Inguinal hernia: A true collagen disease. **Lab Invest** 1977, **36:**359.

4. CANNON DJ & READ RC - Metastatic emphysema: a mechanism for acquiring inguinal herniation. **Ann Surg** 1981; **94**: 270-277.

5. MONBOISSE JC & BOREL JP - Oxidative damage to collagen. **Experientia** 1992; **62**:323.

6. RODRIGUES Jr. AJ, TOLOSA E.MC de & FERRAZ DE CARVALHO CA - Electron microscopic study on the elastic and elastic related fibers in the human fascia transversalis at different ages. **Gegenbaurs Morphol Jahrb** 1990, **136:** 645-52.

7. QUINTAS M, RODRIGUES CJ, YOO JH et al. - Age related changes in the elastic fiber system of the interfoveolar ligament. **Rev Hosp Clin Fac Med S Paulo** 2000; **55**: 83-6.

8. VERHOEFF FH - Some new staining methods of wide applicability including a rapid differential stain for elastic tissue. **J Am Med Assoc** 1908, **50**: 876-7.

9. WEIGERT C - Uber eine Methode zur Farbung elastischer Fasern. **Zbl Allg Path Anat** 1898, **9**: 289-92.

10. FULLMER HM & LILLIE RD - The oxytalan fiber: a previously undescribed connective tissue fiber**. J Histochem Cytochem** 1958; **6**: 425-30.

11. NIEWOEHNER DE & KLEINERMAN J - Morphometric study of elastic fibers in normal and emphysemetous human lungs. **Am Rev Resp Dis** 1977; **115**: 15-21.

12. BEDOSSA P, LEMAIGRE G, BACCI J et al. - Quantitative estimation of the collagen content in normal and pathologic pancreas tissue. **Digestion** 1989; **44**:7-13.

13. RODRIGUES CJ, RODRIGUES Jr. AJ & BOHM GM - Effects of aging on the muscle fibers and collagen content of the diaphragm. **Gerontology** 1996; **42**:218-228.

14. RODRIGUES CJ, SACCHETTI JC & RODRIGUES Jr. AJ - Age-related changes in the elastic fiber network of the human splenic capsule. **Lymphology** 1999; **32**:64-9.

15. RODRIGUES CJ & RODRIGUES Jr. AJ - A comparative study of aging of the elastic fiber system of the diaphragm and the rectus abdominis muscles in rats. **Braz J Med Biol Res** 2000; **33**: 1449-54.

16. BARROS EP, RODRIGUES CJ, RODRIGUES NR et al. - Aging of the elastic and collagen fibers in the human cervical interspinous ligaments. **Spine** (in publication).

17. PANS A, ALBERT A, LAPIERE CM et al. – Biochemical study of collagen in adult groin hernias. **J Surg Res** 2001; **95**: 107-113.

18. UITTO J, RYHANEN L, ABRAHAM PA et al. - Elastin in diseases. **J Invest Dermatol** 1982; 79:160s-8s.

19. WAGH PV & READ RC - Collagen deficiency in rectus sheath of patients with inguinal herniation. **Proc Soc Exp Biol Med** 1971; **137**: 382.

20. BELLON JM, BUJAN J, HONDUVILLA NG et al. – Study of biochemical substrate and role of metalloproteinases in fascia transversalis from hernial processes. **Eur J Clin Invest** 1997; **27**: 510.

21. PANS A, PIERARD GE, ALBERT A et al. – Adult groin hernias: New insight into their biomechanical characteristics. **Eur J Clin Invest** 1997; **27**:863.

22. PANS A, PIERARD GE & ALBERT A – Immunohistochemical study of the rectus sheath and transversalis fascia in adult groin hernias. **Hernia** 1999; **3**: 45.

Received for publication on December 13, 2001.