國立交通大學

生化工程研究所

碩士論文

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中文摘要

鼠蹊部疝氣是種常見的疾病。在美國，每年大約施行六十萬例以上的疝氣修補手術。傳統上，大多認爲直接型鼠蹊部疝氣是由於腹橫筋膜變得薄弱所造成。但是根據 Pans 等人的研究，腹橫筋膜的膠原蛋白發生功能性變化可能和腹股溝疝氣生成有關

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本篇研究的重點在探討直接型疝氣病患、間接型疝氣病患的腹橫筋膜所含的膠原蛋白含量，並做比較；同時研究直接型疝氣病患、間接型疝氣病患的腹橫筋膜切片的病理變化。結果顯示，直接型疝氣病患與間接型疝氣病患的腹橫筋膜所含的膠原蛋白含量並無統計學上有意義的差別，不過間接型疝氣病患的腹橫筋膜膠所含的膠原蛋白含量，比直接型疝氣病患的腹橫筋膜所含的膠原蛋白含量高。這種結果令人意外，而且至今確實的原因並不清楚。可能是由於直接型疝氣患者、間接型疝氣患者的腹橫筋膜所承受的腹內壓力不同，而導致間接型疝氣患者的腹橫筋膜產生較高的膠原蛋白替換率，及較高的膠原蛋白含量。

間接型疝氣患者的腹橫筋膜病理切片，顯示密實的膠原蛋白纖維及完整的彈性纖維。相反地，直接型疝氣患者的腹橫筋膜病理切片卻表現出凝集態的膠原蛋白纖維，斷裂狀的彈性纖維。本研究的結論顯示疝氣病患的腹橫筋膜產生病理變化、及功能性變化可能和鼠蹊部疝氣的形成有關。

A Possible Role for Transversalis Fascia in Adult Groin Hernias？

ABSTRACT

Hernia is a common disease. Hernia repair is the most frequent operation after to appendectomy in the United States. Classically direct hernia is considered the result of weakness of transervalis fascia. In light of the studies of Pans and Albert, it appeared that collagen pathology of transversalis fascia could be involved in the genesis of adult groin hernia.

In this work, we focused on investigations including collagen content and pathologic changes of transversalis fascias from patients with direct or indirect hernias. The collagen content of transversalis fascias collected from patients did not show significant difference between direct and indirect hernia patients. The slight increase in collagen content of transversalis fascias from indirect hernia patients was impressed. However, its explanation remains unclear.

Patients with indirect hernia had compact collagen fibers and intact elastic fibers. In contrast, patients with direct hernia had aggregated collagen fibers and disrupted elastic fibers. This work suggests that the functional and pathologic changes of transversalis fascia may probably play a role in the genesis of adult groin hernias.

DEDICATION

To our patients who grant us the privilege of practicing our crafts;

To my colleagues Dr. Tze-Yi Lin and Miss Mei-Hsin Chung;

To my parents, my wife Michelle and my daughter Jennifer----without your supports, this would not have been possible.

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INTRODUCTION

Hernia is a common disease (10). The importance of hernias is suggested by the following: one man in 5 and one woman in 50 eventually has a hernia (18). Each year in the United States, there are over a half million groin hernia repairs (3, 19). Groin herniorrhapy constitutes 5 per cent of primary operative procedures, and adult inguinal herniorrhapy accounts for 15 per cent of operations in general surgery. As a result, the socioeconomic impact of hernia disability is enormous. There can be little question why surgeons avidly continue to publish papers on this subject.

The exact reason for the development of inguinal hernia has not been completely determined. Stability of inguinal posterior wall depends on the integrity of muscular and fascia framework. The mechanical properties of each fascia rely on the connective tissue and its collagen. Many studies suggesting that increased collagen degradation or decreased collagen synthesis of the transversalis fascia that predisposes the genesis of inguinal hernia (1, 2). The transversalis fascia is the thinnest and most weakness layer of the posterior inguinal wall (26, 27). It is known that the transversalis fascia is one of the structures preventing development of hernias (14, 15, 20). The transversalis facia is an investing sheath of connective tissue deep to and inseparable from the transversus abdominins aponeurosis. It has a little intrinsic strength, and as transversalis fibers deteriorates, a direct hernia results. Congenital hernias result from a persisting peritoneo-vaginal canal. Acquired hernias result from a progressive weakening of the transversalis fascia depending on connective tissue insufficiency

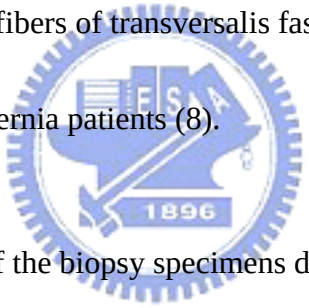
and increase of intra-abdominal pressure (28, 30). Proper wound healing requires a fibroblastic response and adequate oxygenation (34). What occurs is a complex sequence of post-translational modifications with collagen fibril polymerization and cross-liking as the final stage. An ongoing balance of collagen synthesis and enzymatic lysis continues for about a year until the remodeling process results in a stable aponeurotic structure.

Many aspects of the researcher's works have identified the etiology of hernia to be a kind disorder of collagen (21). Collagens belong to the most abundant proteins in the human body (23). Within the past two decades, the development of hernias has been shown to result from deterioration of the inguinal floor as "collagenolysis overpowers net collagen synthesis and deposition in the floor of the inguinal floor"(1, 12). The problem of hernias is an attenuation of transversalis fascia of the inguinal floor (14).

The altered ratio of collagen subtype can be either by a modified synthesis or by an imbalanced breakdown. The cleavage is regulated by the activity of the matrix metallo-proteinases (MMPs), proteins of a family of zinc-dependent endopeptidase. Among them MMP-I and MMP-III are the principal matrix enzymes cleaving fibrillar type I, II and III collagen. It has been showed that the MMPs may play a pathologic role in excessive breakdown of some connective components, e.g. in rheumatoid arthritis and osteoarthritis (16). In particular, the alternations in MMP-I and MMP-III protein expressions could been responsible for the change ratio of the type I to type III collagen on

the protein level. Nevertheless, as firstly shown in investigation by Bellon et al. in 1997, cultured fibroblasts in transversalis fascia from patient with inguinal hernia demonstrated no differences in the expression of matrix metallo-proteinase-1, whereas the same author later detected a MMP-2 overexpression in these patients (29). It is pathological changes in collagen that set the stage for the genesis of hernia (21).

In light of Borquez's work, he demonstrated that patients with inguinal hernias have alternation in skin collagen fiber quality and density (9). Rodrigues showed that significantly lower amounts of collagen and higher amounts of elastic fibers of transversalis fascias in patients with direct inguinal hernias compared to indirect inguinal hernia patients (8).



According Nyhus's studies, some of the biopsy specimens demonstrated degenerative changes in the musculoaponeurotic fibers of the transversus abdominis muscles (34). Most specimens revealed a paucity and fragmentation of elastic tissue fibers at the dilated internal ring and at the direct hernia site. Similar findings have been reported in patients with Marfan and Ehlers-Danlos syndromes. The elastic tissue structure at the site of grossly normal transversalis abdominis aponeurosis varies (34). In the older patient, the changes range minimal to marked. In the young adult male with a direct hernia and a strong history of hernia, the abnormalities are striking. This suggests that the collagen metabolic dysfunction may play a role in the younger group.

Up to now, we could not encounter any study where the transversalis fascias were evaluated histopathologically in inguinal hernia patients. In this work, we focused on the analyses including collagen content and pathologic changes of transversalis fascias from hernia patients to try to determine the possible role of trnasversalis fascia in the development of inguinal hernia.

MATERIALS AND METHODS

There are 30 male adult patients including 15 indirect and 15 direct unilateral primary hernias. The mean age of the indirect hernia group is 67±4 years. The mean age of the direct hernia group is 71±3 years. Once the anterior approach was accomplished, biopsies of a constant surface area (15× 15 mm^2) were taken from the transversalis fascias.

Each fresh sample was cleaned of remaining blood with normal saline. A constant size (10×10 mm^2) from sample was trimmed, weight was measured (wet weight) and stored in liquid nitrogen. The thawed tissue specimens were lyophilized and weighed again (dry weight).The collagen content decreased with age. It was necessary to take age into account to make a valid comparison between direct hernia patients and indirect hernia patients. In order to avoid age induced collagen content difference, we select similar age patients for study. The collagen content was measured using the technique of Bergman and Loxley (4). Masson- Trichrome method is used for the detection of collagen fibers. The collagen fibers will be stained blue. Paraffin section is at 5 μm thickness.

This procedure includes:

1. Deparaffinize and rehydrate through 100% alcohol, 95% alcohol 70% alcohol.
2. Wash in distilled water.
3. Stain in Weigert's iron hematoxylin working solution for 10 minutes.

4. Rinse in running warm tap water for 10 minutes.

5. Wash in distilled water.

6. Stain in Biebrich scarlet-acid fuchsin solution for 15 minutes. Solution can be saved for future use.

7. Wash in distilled water.

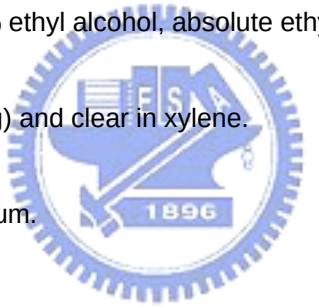
8. Differentiate in phosphomolybdic-phosphotungstic acid solution for 15 minutes or until collagen is not red.

9. Transfer sections directly (without rinse) to aniline blue solution and stain for 5-10 minutes. Rinse briefly in distilled water and differentiate in 1% acetic acid solution for 2-5 minutes.

10. Wash in distilled water.

11. Dehydrate very quickly through 95% ethyl alcohol, absolute ethyl alcohol (these step will wipe off Biebrich scarlet-acid fuchsin staining) and clear in xylene.

12. Mount with resinous mounting medium.



Weigert stain is applied for the investigation of elastic fibers. Paraffin section with 5 μm thickness is suitable. The elastic fibers will be stained blue black.

This procedure includes:

1. Bring sections to water via xylene and ethanol.

2. Place into Weigert's solution for 20 minutes to 1 hour.

3. Wash with 95% alcohol to remove excess solution.

4. Differentiate with 1% acid alcohol.

5. Wash in water.

6. Counterstain with iron hematoxylin and van Gieson’s solution.

7. Dehydrate with ethanol, clear with xylene and mount with a resinous medium.

Pathologic checks were performed using Masson-Trichrome stain for collagen fibers, Weigert stain for elastic fibers.

Results are expressed as means±SD. Two-sample Student *t* tests were performed for comparison of different groups. Significance levels were conservatively two-tailed ($P \leq 0.05$).

RESULTS

The wet weight and the collagen content of samples from direct hernia patient and indirect hernia patients were (24.0±8.3, 131±23), (22.2±9.8, 119±18) respectively. Patients with indirect hernia had compact collagen fibers and intact elastic fibers(Fig. 1,3). In contrast, patients with direct hernia had aggregated collagen fibers and disrupted elastic fibers(Fig. 2,4).

Comparison of the indirect and direct hernia patient Transversalis Fascias

	Indirect hernia (mean ± SD)	Direct hernia (mean ± SD)
Age®	67±4 (n= 15)	71±3 (n= 15)
Wet weight*®	24.0±8.3 (n= 15)	22.2±9.8(n= 15)
Dry weight*®	11.8±3.2 (n= 15)	10.5± 2.5(n= 15)
Collagen content[a]®	131±23(n= 15)	119±18 (n= 15)

* Expressed as mg/100mm².

[a] Expressed as μg/mg dry eight.

® P>0.05

DISCUSSION

Up to now, there are a few studies focusing on transversalis fascias of inguinal floor. However, this study is aimed to explore the possible mechanisms leading to inguinal hernia, because the transversalis fascia is one of the structures preventing development of hernias (14, 15, 20).

We observed that the collagen content of transversalis fascias in indirect hernias is slightly increased as compared to direct hernias. This phenomenon is similar to the work of Pans (5). The increase in collagen content of transversalis fascias in indirect hernias is unexpected and its explanation remains unclear. It is well-known that one mechanism of collagen metabolism is regulated by mechanical forces (6). The distribution of mechanical forces, induced by the intra-abdominal pressure, onto the posterior wall of inguinal canal, could be different in the indirect and direct hernias. Therefore, the increase in collagen content of transversalis fascia in the indirect hernia patients could reflect an increased collagen synthesis and a remodeling process of the connective tissue (5).

In the etiology of the inguinal hernias, disorders in collagen metabolism have been proposed and the role of matrix metallo-proteinase in remodeling the collagen has recently been of great importance. Collagen fibers are imbedded in extracellullar matrix, which is on continuous process of synthesis and degradation under the action of matrix metalloproteinase (24). The altered ratio of collagen subtype can be either by a modified synthesis or by an imbalanced breakdown. The cleavage is regulated by the activity of the matrix metallo-proteinases (MMPs), proteins of a family

of zinc-dependent endopeptidase. Among them MMP-I and MMP-III are the principal matrix enzymes cleaving fibrillar type I, II and III collagen. Based on immunomorphological data, an increased expression of MMP-2 (matrix metallo-proteinase-2) was observed in transversalis fascias from direct hernia patients, when compared to the indirect hernia patients (7, 17). According Bellon's studies, significant active MMP-2 expression was shown by transversalis fascia fibroblasts from young patients with direct hernias. These results indicate that MMP-2 may be involved in the transversalis fascia matrix degradative process in patients with direct hernia (29). The persistence of changes in MMP-2 levels in cell cultures may suggest a genetic defect or irreversible change as the pathologic factor of hernia formation (29). Type I collagen is mature and mechanically stable, whereas type III collagen is immature and instable. Friedman et al. showed increases in type III collagen synthesis by skin fibroblasts in patients with inguinal hernias (25). Rosch et al revealed that the ratio of type I to type III procollagen mRNA by skin fibroblasts was decreased in patients with primary hernias, showing significant differences as compared to controls (16). A defective collagen metabolism contributes to a decreased tensile strength and mechanical stability of both the connective tissue and the induced scar tissue (31, 32, 33).

We demonstrated chaotic arrangement of collagen fibers and fragmented elastic fibers in the tansversalis fascias from direct hernia patients. In light of the works of Rodrigues's (8, 13), lower amounts of collagen fibers in transversalis fascias from direct hernia patients were seen as compared

to indirect hernia patients. Under transmission electron microscope, collagen microfibrils of transversalis fascia in direct hernias were unevenly arranged and rarely formed compact fibers (22). Dysplastic collagen microfibrils with variable diameters and non uniform profiles were also encountered (22). According to mechanical study of transversalis fascias (9), extensibility and biological elasticity were significantly increased in transversalis fascias from the direct hernia patients as compared to indirect hernia patients.

According Nyhus's studies, some of the biopsy specimens showed degenerative changes in the musculoaponeurotic fibers of the transversus abdominis muscles (34). Most specimens revealed a paucity and fragmentation of elastic tissue fibers at the dialated internal ring and at the direct hernia site. Similar findings have been reported in patients with Marfan and Ehlers-Danlos syndromes. The elastic tissue structure at the site of grossly normal transversus abdominis aponeurosis varies (34).In the older patient, the changes range minimal to marked. In the young adult male with a direct hernia and a strong history of hernia, the abnormalities are striking.

Functional and structural changes in the transversalis fascias from hernia patients suggest that a kind of connective tissue pathology may be implicated in the genesis of groin hernias.

Hernias are considered a result of multifactor including anatomical and dynamic processes. The slight increase of collagen content of transversalis fascias in indirect hernias may suggest that the different distribution of the intra-abdominal pressure onto the transversalis fascias. There would be

enhanced collagen turnover rate that producing an increased portion of collagen. The aggregated and chaotic arrangement of collagen fibers, disrupted elastic fibers of transversalis fascias from the direct hernia patients may be responsible for alteration in the resistance of transversalis fascias and result in the development of hernia formation. Further studies are needed to understand this complex problem.

FIGURES

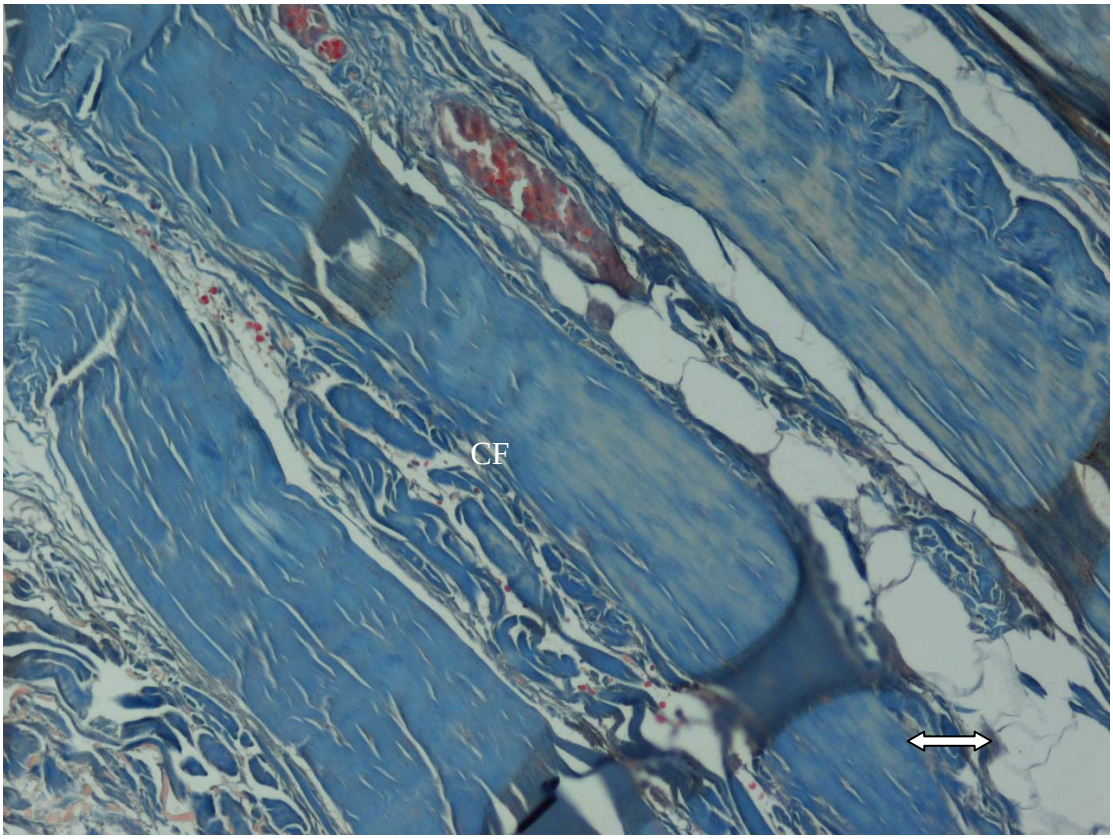


Fig. 1

Masson-Trichome stain 200 x

Transversalis fascia in indirect hernia

Uniform and even arrangement of CF (collagen fibers)

⟷ = 10μm

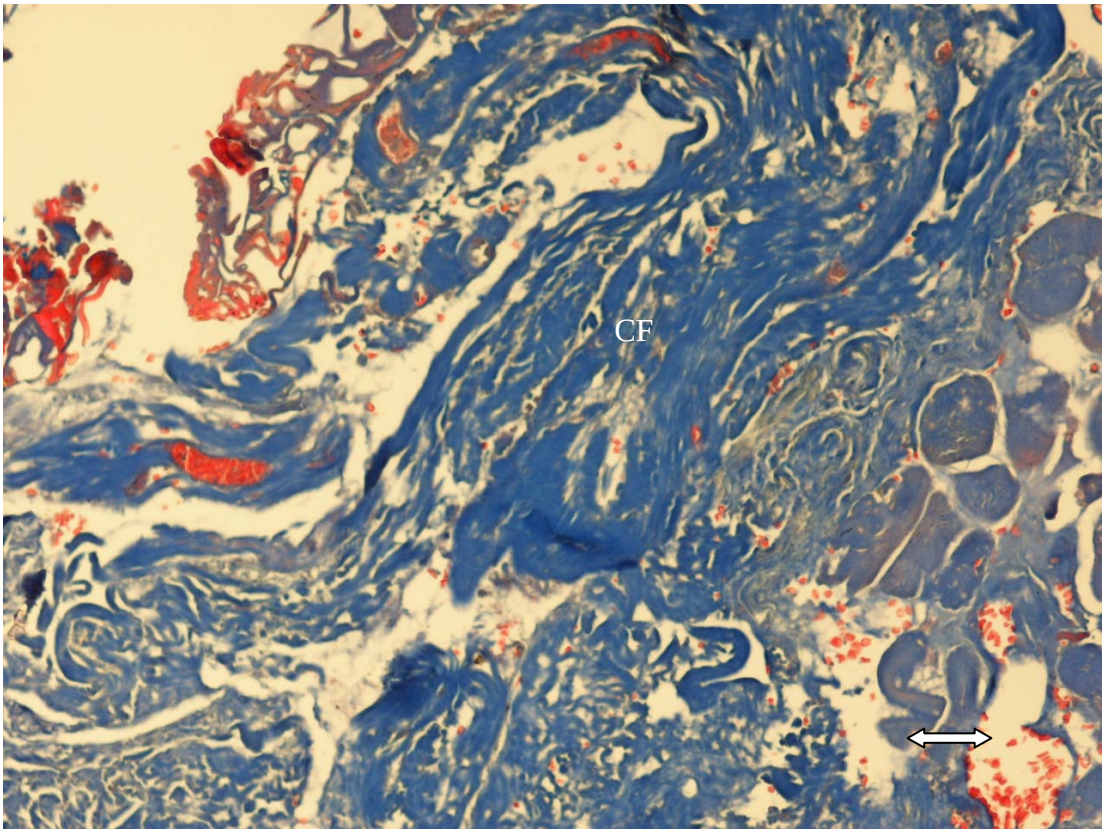


Fig. 2

Masson-Trichome stain 200 x

Transversalis fascia in direct hernia

Aggregated and chaotic arrangement of CF (collagen fibers)

⟷ ＝10μm

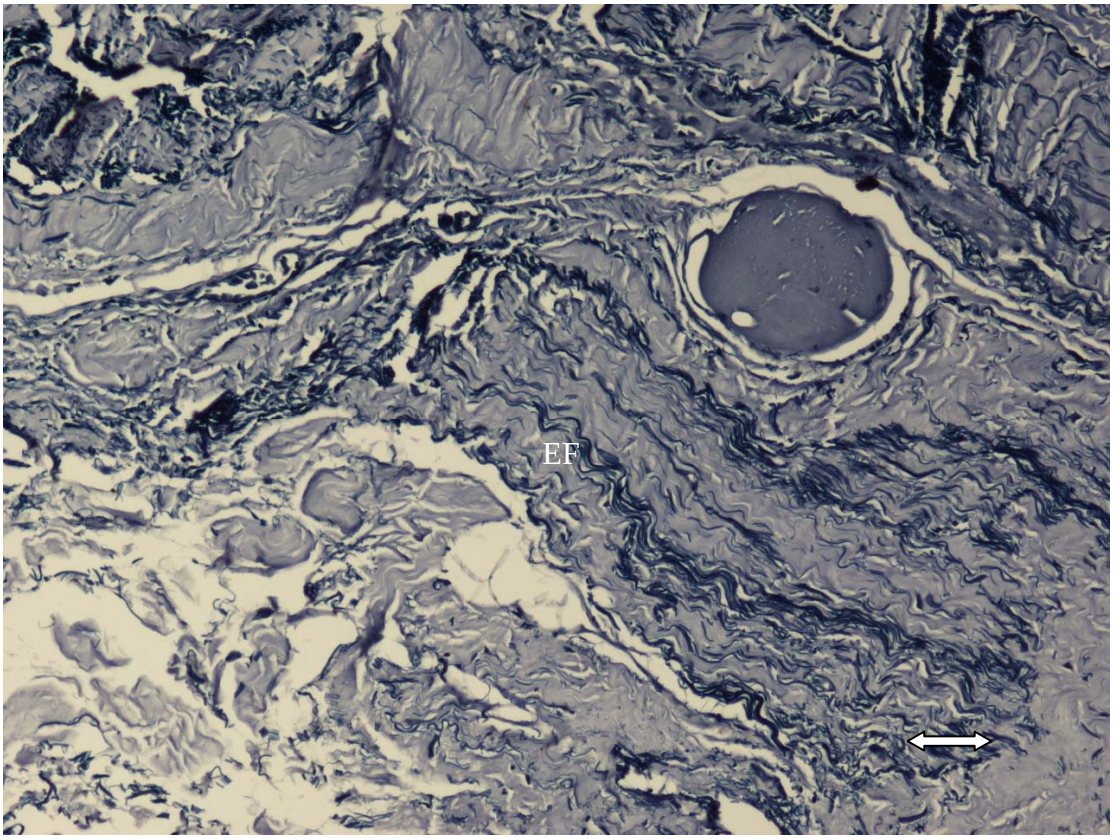


Fig. 3

Weigert stain 200 x

Transversalis fascia in indirect hernia

Intact and smooth EF (elastic fibers)

⟷ = 10μm

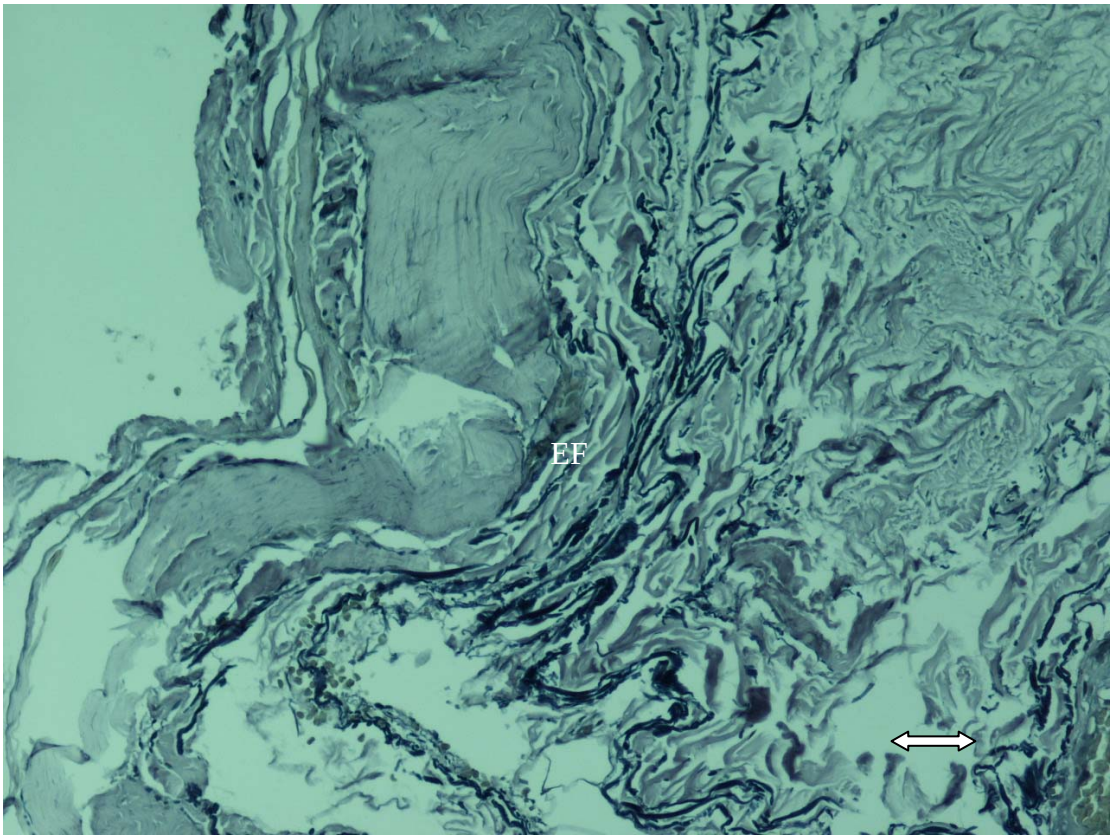


Fig. 4

Weigert stain 200 x

Tranversalis fascia in direct hernia

Disrupted and tangled EF (elastic fibers)

＝10μm

REFERENCES

1. Lichtenstein IL,. Shulman AG,. Amid PK., et al. The tension-free hernioplasty. Am J Surg. 1989; 157: 188.

2. Peacock EE. Internal reconstruction of pelvic floor in recurrent groin hernia. Ann Surg. 1984; 200: 321.

3. Social-economic factbook for surgery, 1984-1985. Chicago, IL. American College of Surgeons. 1985; 35: 32.

4. Bergman I., Loxley R. Two improved and simplified methods for the spectrophotometric. determination of hydroxyproline. Anal Chem. 1963; 35: 1961.

5. Pans A., Albert A., Nusgens B., et al. Biochemical study of collagen in adult groin hernias. J Surg Res. 1974; 17: 425.

6. Lambert CA., Soudant EP., Nusgens BV., et al. Pretranslational regulation of extracellular matrix macromolecules and collagenase expression in fibroblast by mechanical forces. Lab Invest. 1992; 66: 444.

7. Bellon JM., Bujan J., Honduvilla NG., et al. Study of biochemical substrate and role metalloproteinase in fascia transversalis from hernial processes. Eur J Clin Invest. 1997; 27: 510.

8. Rodrigues junior AJ., Rodrigues Cj. Quantative analysis of collagen and elastic fiber in the transversalis fascia in direct end indirect inguinal hernia. Rev Hosp Clin Fac Med. Sao Paulo 2002; 57: 265.

9. Borquez P., Garrido L., Manterofa C., et al. Study of collagen and elastic fibers of connective tissue in patients with and without primary inguinal hernia. Rev Med Chil. 2003; 13(11): 1273.

9. Pans A., Albert A. Adult groin hernias: new inslight into their biochemical characteristics. Eur J Clin Invest. 1997; 27: 510.

11. Hay JM., Bondet MJ., Fingerhunt A., et al. Shouldice inguinal hernia repair in the male adult: the gold Standand? Ann Surg. 1995; 222: 179.

12. Pans A. New prospects in the etiology of groin hernias. Chirurgie. 1999 Jun; 124 (3): 328

13. Rodrigus Junior AJ., de Tolosa EM., De Carvallio CA. Electon microscopic study on the elastic and elastic related fibers in the human fascia tranversalis at different ages. Gegenbaurs Morphol Jahrb. 1990; 136 (6): 645.

14. Stoppa R., Verhaeghe P., Marrasse E. Mechanism of hernia of the groin. J Chir (Pairs). 1987 Feb; 124 (2): 125.

15. Mattioli F. Surgical physiopathology of the inguinal region. Chir Ital. 2002 May-Jun; 54 (3): 311.

16. Rosch R., Klinge U., Shongyi SI., et al.. A role for the collagen I/III and MMP-1/-13 genes in primary inguinal hernia? BMC Med Genet. 2002; 3: 2.

.

17. Rosch R., Junge K., Knops M. et al. Analysis of collagen-interacting Protein in patients with incisional hernias. Langenbeks Arch Surg. 2003 Feb; 387 (11-12): 427.

18. George D. Zuidema. Shackelford's surgery of the alimentary tract. 3rd ed. Saunders. 1991.

19. Hoizheimer GR. Inguinal hernia: classification, diagnosis and treatment- classic, traumatic

and sportsman's hernia. Eur J Med Res. 2005 Mar 29; 10(3): 121.

20. Abci I., Bilgis S., Aitan A. Role of TIMP-2 in fascia transversalis on development of inguinal hernias. J Invest Surg. 2005 May-Jun; 18(3): 123.

21. Bendavid R. The unified theory of henia formation. Hernia. 2004 Aug; 8(3): 171.

22. Nikoiov S., Beitschev B. Several ultrastructural peculiarities of fascia transversalis in direct hernias of senile men. Anat Anz. 1990; 170(3-4): 265.

23. Lynen Jansen P., Klinge U., Merien PR. Hernia diasease and collagen regulation : are they clues for intervention? Hernia. 2006 Sep 21(4): 169.

24. El Sherif A., Yano F., Mittal S., et al. Collagen metabolism and recurrent hiatal hernia: cause and effect? Hernia. 2006 Oct 5(5): 187.

25. Friedman DW., Boyd CD., Norton P., et al. Increases in type III collagen gene expression and protein synthesis in patients with inguinal hernias. Ann Surg. 1993; 218: 754.

26.Wolloscheck T., Gaumann A., Terzic A., et al. Inguinal hernia: measurement of the biomechanics of the lower abdominal wall and the inguinal canal. Hernia. 2004 Aug: 8(3): 233.

27. Bendavid R., Howarth D. Transversalis fascia rediscovered. Surg Clin North Am. 2000 Feb; 80(1): 25.

28. Fiamen JB., Avisse C., Delattre JF. Anatomy and mechanism of inguinal hernias. Rev Prat. 1997 Feb 1; 47(3): 252.

29. Bellon JM., Bajo a., Ga-Honduvilla N., et al. Fibroblasts from the transversalis fascia of young patients with direct inguinal hernias show constitutive MMP-2 overexpression. Ann

Surg. 2001 Feb; 233(2): 287.

30.Condon RE., Carilli S. The biology and anatomy of inguinofemoral hernia. Semin Laparosc Surg. 1994 Jun; 1(2): 75.

31.Henkel W., Glanville RW. Covalent crosslinking between molecules of type I and type III collagen. The involvement of the N-terminal, nonhelical regions of the alph1 (I) and alph1 (III) chains in the formation of intermolecular crosslinks. Eur J Biochem. 1982; 122: 205.

32.Fleischmaier R., Perlish JS.,Burgenson ER., et al. Type I and type III collagen interactions during fibrillogenesis. Ann NY Acad Sci. 1990; 580: 161.

33. Uitto J., Perejda A. J., Abergel RP., et al. Altered steady-state ratio of type I/III procollagen in mRNAs correlates with selectively increased type I procollagen biosynthesis in cultured keloid fibroblasts. Pro Natl Acad Sci. USA 1985; 82: 5935.

34. The surgical clinics of north America. Hernias, April, 1984. .

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