Journal of Investigative Surgery, 18:123–128, 2005

ISSN: 0894-1939 print / 1521-0553 online
DOI: 10.1080/08941930590956147

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Original Research

Role of TIMP-2 in Fascia Transversalis on Development of Inguinal Hernias

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ABSTRACT The exact reason for the development of inguinal hernia has not been completely determined. However, it is known that the fascia transversalis (FT) is one of the structures preventing development of hernias. In the etiology of the inguinal hernia, disorders in collagen metabolism have been proposed, and the role of metalloproteinases in remodeling the collagen has recently been of great importance. We could not encounter any study where the role of metalloproteinase inhibitors was evaluated in inguinal hernia. We obtained samples of FT from patients with direct and indirect hernia and used an immunohistochemical method to determine tissue inhibitor of metalloproteinase-2 (TIMP-2) expression. In the study group, samples of FT were taken during the operation from 45 patients, of which 35 were indirect and 10 were direct inguinal hernias. In the control group, samples of FT from various abdominal incisions were also taken from 45 patients with no hernia and operated upon for another pathology. TIMP-2 scores of a direct inguinal hernia were significantly less than those of the control group. However, no difference has been found between the TIMP-2 scores of an indirect inguinal hernia and those of the control group. Decreased TIMP-2 scores in patients with a direct inguinal hernia, compared with both the indirect inguinal hernia group and the control group, explain the reason for the increase in matrix metalloproteinase-2 (MMP-2) that has been proposed in some studies. Therefore, it can be expressed that a decreased activity of TIMP-2 plays a role in inguinal hernia development.

KEYWORDS etiology, fascia transversalis, inguinal hernia, TIMP-2

Received 19 July 2004;
accepted 24 January 2005.

This study was supported by the Trakya University Research Fund.

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Inguinal hernia is an ailment seen in both sexes, in all races, and in all age groups. Although it is still one of the most common targets of surgical treatment and the majority of inguinal hernias represent a defect in the fascia transversalis (FT), there continues to be debate

among physicians about many points ranging from etiology to proper management [1–3].

Extracellular matrix collagen is synthesized, and it is also destroyed at the same time. During this remodeling stage, the collagenases, which are responsible for collagen degradation, are released from endothelial cells, fibroblasts, inflammatory cells (macrophage, neutrophil), and keratinocytes. The lysis of different types of collagens is controlled by the balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [4].

MMPs are a zinc-including endopeptidase family. They represent optimal enzymatic activity at neutral pH and play an important role in the pathological and physiological destruction of connective tissue. MMPs are known to play an important role in cancer-cell invasion by mediating the degradation of extracellular matrix proteins [4–6].

TIMPs connect to the active sides of the MMPs and inhibit them via covalent composition [4]. Tissue inhibitor of metalloproteinase-2 (TIMP-2) is a 22-kD molecular mass nonglycolysis protein that is synthesized by many cells [5, 6].

In recent years, MMPs, which play a role in the remodeling phase of extracellular matrix, have been evaluated from the samples of FT in inguinal hernias [3, 7]. However, we could not encounter any study about the role of TIMPs in the development of an inguinal hernia in the literature.

In this study, we evaluated the role of TIMP-2 in the development of an inguinal hernia. For this purpose we obtained samples of FT from patients with direct and indirect inguinal hernias and used immunohistochemical methods to determine TIMP-2 expression.

MATERIALS AND METHODS

The patients with an inguinal hernia were graded according to the classification recommended by Gilbert (Table 1).

In the study group, samples of FT were taken during operation from 45 consecutive patients with an inguinal hernia, of whom 35 had an indirect and 10 had a direct inguinal hernia. Ages ranged from 20 to 75 years, with a mean age of 35.9 years, and all of them were male.

TABLE 1 Gilbert classification

Type 1: Peritoneal sac that protrudes through an intact deep orifice.
Type 2: Peritoneal sac that protrudes through a moderately dilated deep orifice measuring no more than 4 cm in diameter.
Type 3: Peritoneal sac which protrudes through a deep orifice greater than 4 cm in diameter; the sac frequently has a sliding component, and there is medial displacement of the deep epigastric vessels.
Type 4: Weak, defective inguinal floor with a normal deep ring.
Type 5: Diverticular defect no greater than 2 cm in the inguinal floor, generally in a suprapubic position.

In the control group, samples of FT were taken from various abdominal incisions in 45 consecutive patients operated on for another pathology (Figure 1a). Ages ranged from 19 to 78 years, with a mean age of 46.5 years; 30 (66.6%) were male and 15 (33.3%) were female. The mean age was 42.4 in males and 54.8 in females.

In each case of the inguinal hernia group, biopsy specimens were taken from the same central area of the posterior wall of the inguinal canal (Figure 1b). These tissue samples, measuring 5 × 10 mm, were fixed in 10% formaldehyde solution. In the control group, FT samples of the same size were taken from the edge of various abdominal incisions. In each group, patients receiving steroid therapy and those who had previous laparotomy or inguinal hernia repair were excluded.

Tissue samples without any information about the patient were sent to the Pathology Department. They were embedded in paraffin and cut to 4-μm thickness. Using polyclonal TIMP-2 (Biogenesis, Poole, UK) antibody, TIMP-2 expression of the samples was determined by a routine immunohistochemical method [8].

The preparations were examined by light microscope under ×20 magnification. The entire cross section was screened on preparation. It was scored as 0 if there was no staining, it was scored as 1 if a indistinct staining was seen, and it was scored as 2 if there was intense staining, by taking into consideration the stain concentrated areas (Figure 2).

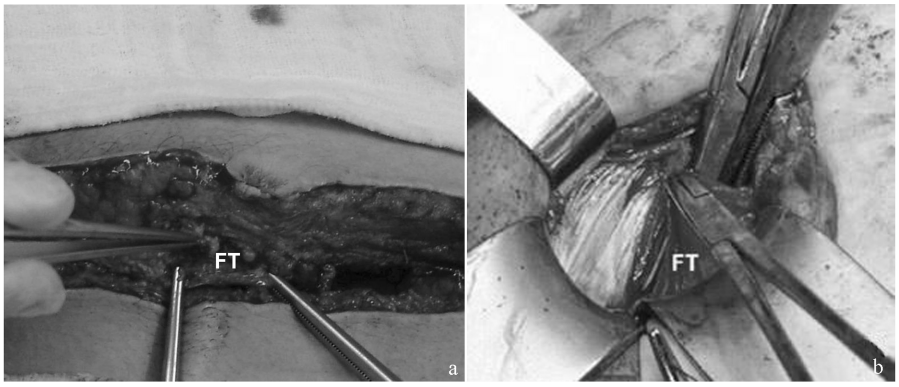

FIGURE 1 (a) Collecting the specimens of FT in the inguinal hernia cases. (b) Collecting the same specimens from abdominal incision in control group.

This study was approved by the Ethics Committee of the Medical Faculty, Trakya University, and informed consent was obtained from each patient.

Statistical analysis was performed using the chi-square test, and results were considered significant at the 5% critical level ($p < .05$).

RESULTS

Inguinal hernias were classified according to the Gilbert classification (Table 1) [9]. Four of them were Gilbert 1 (8.89%), 13 were Gilbert 2 (28.89%), 18 were Gilbert 3 (40%), 8 were Gilbert 4 (17.78%), and 2 were Gilbert 5 (4.44%). In other words, 35 (77.78%) cases had an indirect (Gilbert 1, 2, 3) and 10 (22.22%) had a direct (Gilbert 4, 5) inguinal hernia. The distribution of inguinal hernia group by age and Gilbert classification is shown in Table 2.

There was no significant difference between the mean ages of the inguinal hernia group and the mean ages of the males in the control group ($p > .05$).

Thirty cases of the control group were male (66.67%) and 15 were female (33.33%). There was no significant difference between the TIMP-2 scores of the males and the females in the control group ($p > .05$).

The control group was divided into 2 groups according to age, those under 40 years and those over 40 years. No significant difference was found between the TIMP-2 scores of these two groups ($p > .05$) (Figure 3).

In the control group, samples of FT were taken from the upper abdominal wall in 32 cases (71.11%) and from the lower abdominal wall in 13 cases (28.89%). Level 2 staining was observed in 46.88% and 61.54% of the upper and lower abdominal

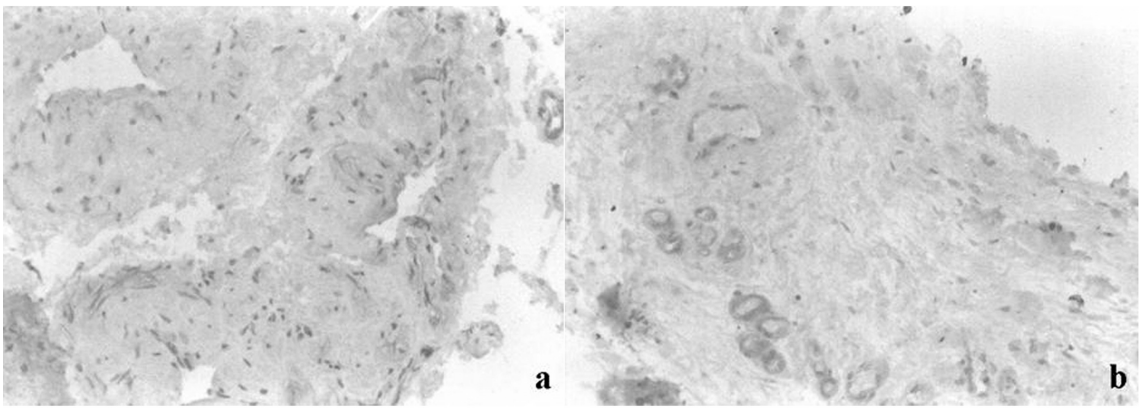

FIGURE 2 Microscopic appearances by TIMP-2 scores: (a) score 1 and (b) score 2 (immunohistochemistry ×20).

TABLE 2 Distribution of patients by hernia type (Gilbert classification) and by age

Age	Gilbert 1	Gilbert 2	Gilbert 3	Gilbert 4	Gilbert 5
0–39 years	3	10	10	5	1
≥40 years	1	3	8	3	1
Total	4	13	18	8	2

specimens, respectively. Also, significant difference was found in the TIMP-2 scores between these two groups ($p > .05$).

The control group was compared with the two hernia groups. The TIMP-2 scores of the direct hernia group were significantly lower than those of the control group ($p = .002$). No statistical difference was found between the control and the indirect hernia group ($p = .432$). The percentages of TIMP-2 scores in the three groups are shown in Figure 4.

On the other hand, the TIMP-2 scores of the direct hernia group were significantly lower than those of the indirect hernia group ($p < .05$).

DISCUSSION

Inguinal hernia repair is one of the operations most often performed by surgeons; however, the etiology of the inguinal hernia has not yet been completely suggested [1–3, 10]. Patients with a congenital indirect inguinal hernia only present an open processus vaginalis. However, numerous etiological factors have been proposed for adult inguinal hernias, such as increased intra-abdominal pressure, impaired obturator and sphincter mechanism, genetic predisposition, and iatrogenic factors [1, 2, 7, 11–13].

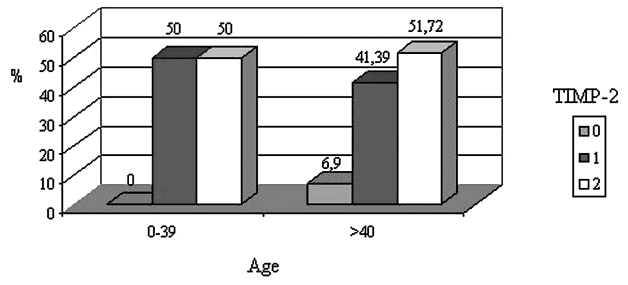

FIGURE 3 Percentage of TIMP-2 scores for those under and over 40 years in the control group.

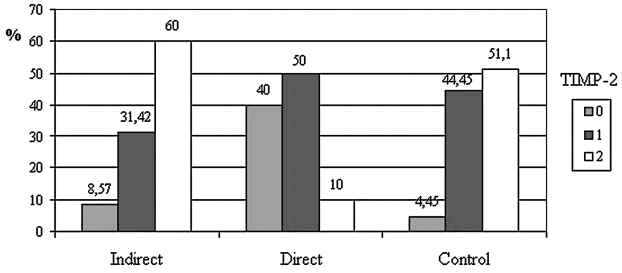

FIGURE 4 Percentage of TIMP-2 scores for direct inguinal hernia, indirect inguinal hernia, and control groups.

In the 1970s, biological factors in the etiology of the inguinal hernia became important, and studies focused on this subject. Wagh [14], Peacock [15], and Read [16, 17] are leading researchers who have suggested that inguinal hernias could be the result of disorders in collagen metabolism.

In the elderly, increased recurrences after hernia repair are related to the fibroconnective tissue weakness and impaired collagen metabolism. Physical activity plays a role only as a trigger or secondary reason in inguinal hernia development [18].

A decrease in extracellular matrix proteins and a marked increase in inguinal hernia frequency in abdominal aortic aneurysms have been reported [19]. The studies dealing with collagen have mostly been carried out on anterior rectus sheath and skin, but there are a few reports examining collagen changes in FT [19].

Patients with inguinal hernia and without connective-tissue disease have been found to have increased collagen synthesis and destruction in their FT obtained from the region of hernia [20]. Friedman et al. [21] have shown increased type III collagen synthesis and a decreased type I/type III collagen ratio in inguinal hernia patients. Type III collagen 2 is a thin fibrillary collagen with decreased mechanical strength; it is usually seen during fetal organogenesis and is found in tissues with high elasticity, such as the aorta, esophagus, and uterus. Therefore, increased type III collagen synthesis contributes to hernia development due to a weakening of the abdominal wall [21, 22].

Nicolov and Beltschev [23] have carried out an ultrastructural study of fascia transversalis in elderly patients with direct hernias. They have found dysplastic collagen fibrils of variable diameters and

nonuniform profiles, as well as a marked collagenophagia in fibroblast-containing collagen vacuoles in different stages of degradation.

The remodeling of the collagen is controlled with the balance between matrix metalloproteinases and their inhibitors [4]. Synthesis of the MMPs in connective tissue is affected by interleukin-1, tumor necrosis factor, and growth factors. MMPs cause the destruction of the extracellular matrix content [4–6].

MMP-2, especially types IV, V, VII, X, and XI, destroys collagen, gelatin, proteoglycan, fibronectin, and elastin. High levels of MMP-2 cause basal membrane destruction, tumor invasion, and metastasis [24].

Bellon et al. [25] found increased levels of MMP-2 in FT obtained from patients with direct inguinal hernia. Klinge et al. [22] have determined type I and type III collagen in skin specimens of patients with inguinal hernia by the immunohistochemical method and found increased levels of type III collagen.

TIMP-2 is strongly stained, particularly in extracellular matrix, fibroblasts, and the endothelium of vessels [26]. In our study, TIMP-2 dissolved homogeneously in extracellular matrix and strongly stained in the endothelium of vessels.

The role of TIMP-2 has been studied in chronic liver disease [27], colorectal adenocarcinoma with liver metastasis [28], and stomach cancer metastasis. Moreover, it has been proposed for use as an anticancer treatment.

Bellon et al. [3] have found that FT obtained from patients with direct hernia showed very strong staining for MMP-2 when compared to that observed in indirect hernia. TIMP-2, which is an inhibitor of MMP-2, has not previously been studied in patients with inguinal hernia.

In our study, the samples of fascia transversalis were taken from the posterior wall that was repaired during surgical approach in inguinal hernia. In the control group, they were taken from FT during abdominal incisions of patients operated on for other pathologies, because having such a sample from the posterior wall of the inguinal canal in a healthy patient was unethical because of the risk of subsequent herniation.

There was a significant difference between the ages of the inguinal hernia group and the control group ($p < .05$). All patients with an inguinal hernia were male. No significant difference was determined between the ages of inguinal hernia and the ages of the males in the control group. The control group was divided into two groups according to their age, those under 40 years and those over 40 years, in order to elucidate whether TIMP-2 could decrease with aging; however, no significant difference was found between the two groups.

The influence of incision site and sex on TIMP-2 scores of the control group were evaluated. There was no significant difference between TIMP-2 scores of the samples taken from upper and lower abdominal wall incisions, and no significant difference was found between the TIMP-2 scores of male and female subjects in the control group ($p > .05$). These findings suggested that the control group could wholly be compared with the hernia groups.

When all the inguinal hernia groups compared with the control group, TIMP-2 scores did not differ significantly. However, TIMP-2 scores in the direct inguinal hernia group were found to be significantly lower than those of the control group. This finding supports the result of the study made by Bellon et al. [3], who found increased activity of MMP-2 in direct inguinal hernias. Finally, we found that TIMP-2 scores of the direct inguinal hernia group were significantly lower than those of the indirect inguinal hernia group ($p < .05$).

In conclusion, this study has confirmed decreased TIMP-2 activity of FT in direct inguinal hernias. Therefore, it can be expressed that decreased activity of TIMP-2 plays a role in direct inguinal hernia development, and mesh repair can also be recommended as a proper technique in these patients.

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