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Etiology of inguinal hernia: ultrastructure of rectus sheath revisited

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Abstract In the last decade, in the search for abdominal-wall hernia etiology, attention has been brought to alterations in the connective tissue ultrastructure as the probable etiological factor. These may cause weakening of connective tissue, which in turn may form ground for hernia formation. To investigate this hypothesis in depth, we compared the ultrastructure of the connective tissue in hernia patients and the control group. The study group consisted of five patients with primary inguinal hernia (Nyhus II = 4, Nyhus IIIa = 1). Another five patients posted for emergency appendectomy created the control group. Tissue specimens, harvested intraoperatively from the rectus muscle sheath (RAMS) and fixed in 4% glutaraldehyde, underwent staining by the Masson, H-E and methylene blue techniques and were assessed by microscopy (light and scanning electron). The examinations showed significant differences in the rectus sheath ultrastructure. They included altered architecture, placement and quantity of collagen and elastic fibers, differences in the caliber of individual fibers and disrupted ground matter–to–fiber ratio. In patients with hernias, chaotic arrangement of collagen fibers was seen, as well as their thinning and a decrease in the general amount of elastic fibers, replaced by ground matter. Our research has shown significant differences in the structure of the RAMS between patients with hernias and healthy individuals. This supports the theory linking connective tissue alterations with the etiology of hernia, and stating that these alterations include connective tissue at locations distant from the hernia site as well, as the rectus sheath itself does not form a hernial defect.

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Introduction

Inguinal hernias have accompanied mankind from the earliest days, resulting partially from the development of vertical posture [1]. The cause of hernia formation remains unknown, although the definition of hernia etiopathogenesis seems close [2, 3]. The most current theory states that hernia is a polyethiological disease [3–5]. Quantitative and qualitative abnormalities of the elastic and collagen fibers, which—along with cells and extracellular matrix—form the connective tissue of the fasciae, seem to be the fundamental cause of hernia formation. These fibers are responsible for the integrity of the abdominal wall and laparotomy scar. Investigations of the cause of these alterations point to genetic factors and increased activity of metalloproteinase-type proteases, which break down elastic and collagen fibers [6–8]. A hernia can be regarded as a local manifestation of general abnormalities, occurring in anatomically weaker locations (*loci minoris resistentiae*), e.g., within a laparotomy scar [2].

The first assumptions that hernias may be a result of pathological connective tissue alterations were formulated in the 1920s. In 1923, Harrison stated that tendons and fasciae should not be regarded as dead and passive structures, but rather as living tissue, undergoing alterations with age and pathological processes. He also noticed that, aside from congenital cases, most hernias develop in old age, which might prove the influence of aging [9].

The molecular changes in connective tissue fibers lead to structural alterations. The discovery of lathyrism and the coincidence of hernias with other pathologies in Ehlers–Danlos syndrome seemed to confirm this thesis [10–12]. Wagh and Read, almost 40 years ago, found the fascia of hernia patients to be significantly thinner and

its dry mass to be lower than in healthy controls, and proved that the proliferation of fibroblasts harvested from hernia patients (particularly from those with direct hernias) is impaired [13–15].

In order to assess ultrastructural alterations, human fascia specimens from live donors were investigated. This was conducted in a few investigations, since morphological studies of the fascia are hardly a novelty. The results of current investigations indicate connective tissue pathology as a cause for hernia formation; however, the conclusions are ambiguous [16–18]. The alterations at the cellular/molecular level, if they are responsible for hernia development, should be present as well as abnormalities in the morphology of the connective tissue in each place in the human body.

Investigations of tissue samples from the immediate vicinity of the hernia defect may carry an error derived from purely mechanical damage. It appears that the assessment of locations not directly involved in the hernial defect would be relevant and yields a more complete view of the problem [19]. New investigative methods such as scanning microscopy allow for more accurate investigation of the problem of connective-tissue ultrastructure [20]. The goal of this paper was to verify hypothesis of generalized changes in the connective tissue in hernia patients by means of light and electron microscopy, with the use of proper staining techniques.

Materials and methods

The connective tissue of ten males undergoing treatment at the Department of General and Endocrine Surgery at the Nicolaus Copernicus University Collegium Medicum in Bydgoszcz was evaluated. The tissue material in the form of rectus muscle sheath (RAMS) specimens was harvested during elective primary unilateral inguinal hernia repair procedures (study group, $N = 5$, mean age 39.4, range 18–69) and emergency appendectomy (control group, $N = 5$, mean 35.4, range 17–64). The patients in the study group had Nyhus type II ($n = 4$) and IIIa ($n = 1$) hernias. In the control group, symptoms of the disease had been present for 1–2 days; all patients had appendicitis (two acute catarrhal, three suppurative). None of the controls had history or evidence of hernia upon the operation.

A fragment of approximately 0.5 cm^2 was cut out by scalpel from the macroscopically unchanged anterior wall of the RAMS, at the location marked in Fig. 1.

In order to avoid alterations of the harvesting technique, all of the samples were taken by one surgeon (W.Sz). After harvesting, the samples were fixed in 4% glutaraldehyde and stored for 2 days at 4°C. The microscopic sections were prepared as paraffin slides stained by H-E and Masson techniques, and prepared for scanning microscopy. The research protocol has been accepted by the Local Ethics Committee of the UNC Collegium Medicum in Bydgoszcz, Poland.

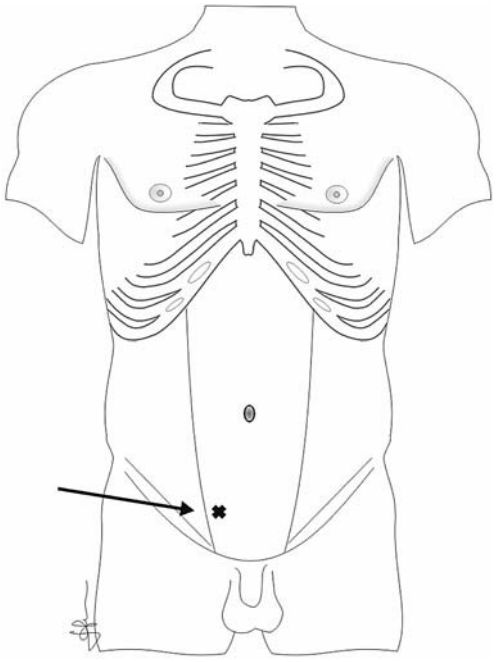

Fig. 1 Anatomical structure of the rectus abdominis muscle sheath. The *arrow* points to the site of origin of the tissue specimens

Preparation of paraffin slides

After removal from the glutaraldehyde solution, the specimens were rinsed in distilled water three times and immersed in 50% ethanol for 1 h, followed by immersion in 75% ethanol for further 2 h. The tissue was then transferred to 100% alcohol for 1 h and placed in xylene. The dehydrated specimens were placed in soft paraffin and left for 12 h in 45°C for infiltration. The next stage was placing the sample in hard paraffin. After 3 h, the material was embedded in molten paraffin and left to cool. The paraffin blocks were sectioned with a microtome into slices 4–7 μm in thickness and placed on microscopy slides.

Masson stain

The first stage was deparaffination of the slides in xylene. After 15 min they were transferred to 75% ethanol for 5 min, rinsed twice in distilled water and left in hematoxylin for 2 min. After the excess dye had been washed off with distilled water, the preparations were placed in Masson's fluid for 7 min and then moved to 1% acetic acid. The next stage was the placement of the slides for 30 s in light green solution and then transferring them to xylene. The preparations were then dried and mounted in Canada balsam. H-E and methylene blue staining was also performed.

Slide preparation for scanning electron microscopy

The specimens were transferred from 4% glutaraldehyde to a critical point drier (Baltec CPD 030) for total dehydration and left for 2 h. The dehydrated fragments were placed on so-called tables which were later sputter-coated with gold using a JEOL JFC-1200 fine coater. Thus, prepared specimens were placed in a JEOL JSM-5310 Low Vacuum scanning electron microscope (SEM). The microscope enabled viewing in 4 nm resolution at 750×, 2,000×, 3,500×, and 75,000× magnification. Vertical rotation of the samples by 90° and 360° horizontal rotation was possible. A view of the ultrastructure of the connective tissue was obtained. Selected locations were photographed for documentation.

Results

Our investigations showed alterations in the architecture of the connective tissue within the RAMS of patients with primary inguinal hernia (PIH). The structure of the connective tissue in the study group differed significantly from the control group. The alterations concerned both the direction and thickness of collagen fibers and the elastin content.

In the unaltered tissue of patients from the control group (Fig. 2), both collagen and elastic fibers are visible. Masson's staining enabled examination of tissue architecture. The structure of collagen is visible, as well as the positioning of elastin fibers. The collagen fibers form a regular pattern, and the elastin fibers are plentiful and clearly visible. Observations showed the elastin–to–collagen ratio of healthy tissue to be 1:2. The use of higher magnification (photo not included) enabled closer observation of the connective tissue stroma.

The collagen fibers are regular and aligned longitudinally, forming tight bundles. The arrangement of the elastic fibers is regular as well, and they are abundant. Ground substance can be seen between the fibers. Healthy tissue contains little ground substance.

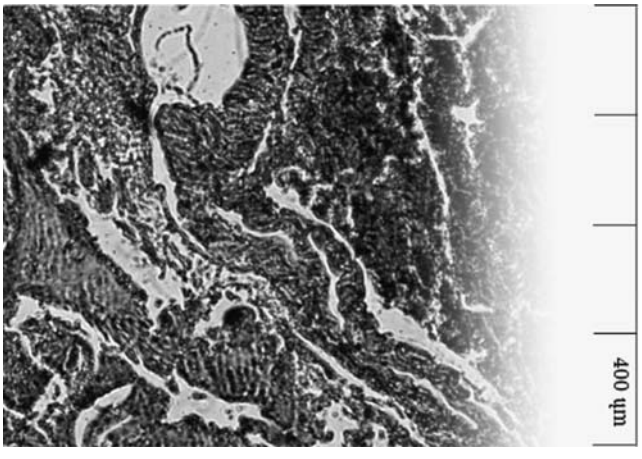

Fig. 2 Transverse cross-section of RAMS connective tissue in one of the controls (Masson's stain)

In patients with primary groin hernia (Fig. 3), the arrangement of the collagen fibers was found to be irregular, in most places chaotic. Collagen was less abundant than in healthy specimens. Little or no elastin was found. Ground matter was found to be overabundant in the connective tissue of the RAMS of hernia patients, and it infiltrates the spaces between the collagen fibers in place of the elastin fibers.

The above mentioned alterations have been confirmed by methylene blue and H-E staining.

With the help of SEM imaging, a three-dimensional view of the connective tissue structure was obtained. The analysis of healthy RAMS tissue structure (Fig. 4, upper left) confirmed that the collagen fibers are arranged parallel to each other, forming a dense structure.

Similar investigation of RAMS structure in patients with PIH has shown a disrupted structure of collagen fibers (Fig. 4, upper right). They are not densely packed and their course is not parallel to one another. The collagen is unevenly distributed and chaotically oriented. Both photographs were identified at a magnification of 3,500×.

Further analysis of the photographs has revealed differences not just in the architecture of the connective tissue, but also in the size of individual fibers. The thickness of an individual fiber in the RAMS connective tissue of a healthy individual is twice as much as in a patient with PIH (Fig. 4, lower left). It was stated that in the control group, individual collagen fibers could be identified at a magnification of 7,500×, whereas in PIH patients a magnification of 15,000× was necessary (Fig. 4, lower right).

Discussion

Our studies were aimed at comparison of the connective tissue at a location distant from the hernia site in patients with PIH in comparison to healthy controls. The

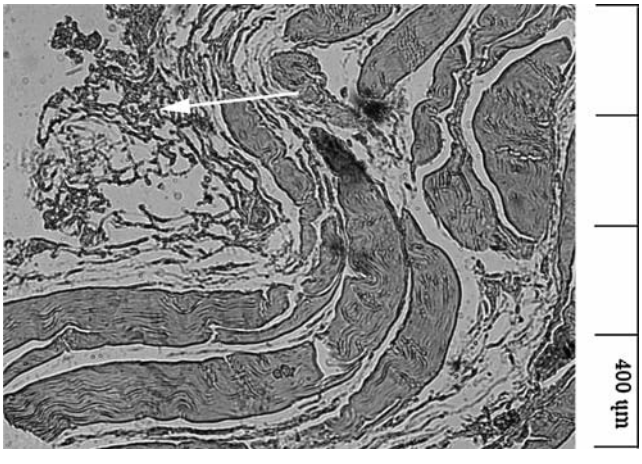

Fig. 3 Transverse cross-section of RAMS connective tissue. Sample harvested from a hernia patient. Masson's stain. *Arrow* shows the course of the collagen fibers

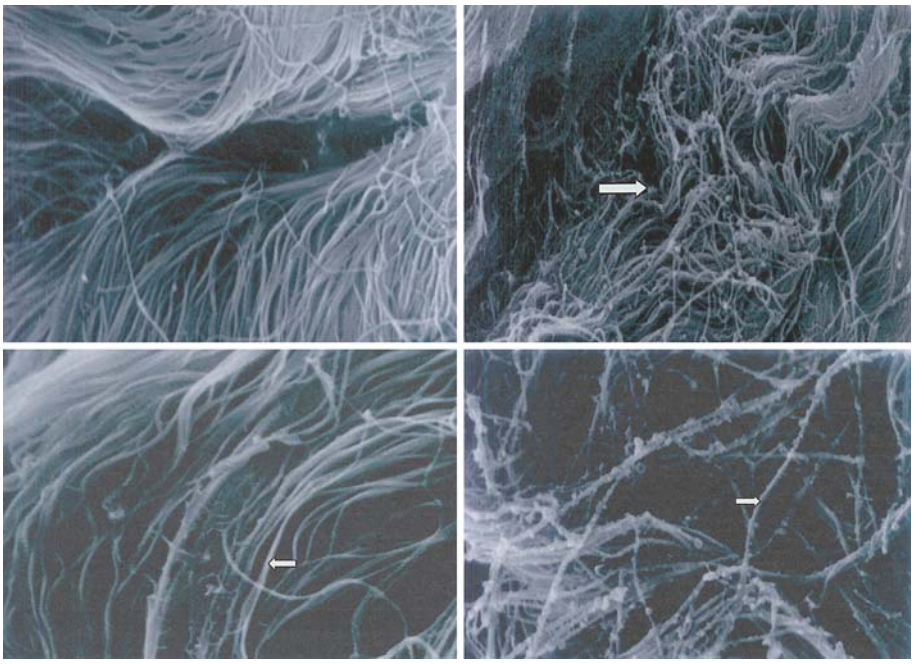

Fig. 4 Structure and arrangement of collagen fibers in the RAMS—Scanning electron microphotographs: *upper left* healthy individual; *upper right* tissue sample of a hernia patient (*arrow* points to disarranged fibers); *lower left* healthy individual, *arrow* points to single collagen fiber (×7,500); *lower right* hernia patient, *arrow* points to individual collagen fiber (×15,000)

tissue samples were harvested from the rectus abdominis muscle sheath (RAMS). This structure is considered representative of an individual's fasciae [19]. Only such a course of action can prove that hernia formation is the consequence of a generalized process. Hernias do not occur at this location, but it is one of the factors of integrity of the inguinal region. The RAMS is also an important reinforcing element in many hernia repair techniques.

As it has been mentioned before, even the first investigators of fascial abnormalities accompanying hernias, Wagh and Read in the 1970s, had already gathered samples of the rectus abdominis muscle sheath [13].

The first data on the ultrastructure of collagen fibers in samples of the RAMS was provided by transmission electron microscopy. In healthy individuals, both the distribution and thickness of the fibers were regular [15]. Similarly to our own research, significant differences in the thickness of individual fibers and an irregular fiber pattern were observed in patients with direct hernias.

Our investigations have shown alterations in the architecture of the connective tissue within the RAMS of patients with PIH. The structure of the connective tissue in the study group differed significantly from the control group. The alterations concerned both the direction and thickness of collagen fibers and the elastin content. It should be noted that the use of a scanning microscope allows for these alterations to be viewed as a three-dimensional image, yielding more information about the distribution of the fibers.

The results obtained by Wagh and Read, as well as our own observations, seem to confirm the old theory of Harrison, concerning ultrastructure alterations of connective tissue in various pathological processes. His assumption of a progressive, age-dependent degeneration of connective tissue fibers and its leading role in hernia formation is still under discussion. Publications still emerge supporting and undermining this theorem [21–23]. In our research patients, age was not a causative factor, as the connective tissue alterations were present in relatively young hernia patients as well.

Alterations of the elastic fibers are a significant part of connective tissue degeneration. In 1983, Berliner used histological techniques to evaluate the fascia surrounding the deep inguinal rings of hernia patients. His studies, similarly to our research, showed the fragmentation of elastic fibers as well as a decrease of the number of elastic fibers in favor of the ground matter [24]. This is also consistent with Weinstein's earlier investigation of the external oblique muscle aponeurosis (anterior wall of the inguinal canal) conducted in patients aged 3–80, where he was able to show no age-dependent differences in the relative collagen–to–elastic fiber ratio [25]. However, none of these authors compared their results with a group of healthy controls, which was provided in our studies, documenting the significant differences in RAMS connective tissue of healthy individuals and hernia patients.

The diminished number of collagen fibers in the tissues of patients with primary hernias, similarly to our observations, was described earlier by Rodrigues et al. [26]. His team harvested fascial samples from patients with direct and indirect hernias. Unlike our observations, their investigations showed an increased quantity of elastic fibers in the fasciae of patients with direct hernias. Mature elastic and elaunin fibers (responsible for tissue elasticity) of these fasciae showed structural abnormalities, and the density of oxytalan fibers (responsible for tissue strength) was diminished. These

data may indicate a slightly different pathogenesis of direct and indirect hernia.

The assessment of the condition of rectus abdominis muscle sheath connective tissue in patients with inguinal hernias is inconsistent in the literature. Pans et al. investigated both the RAMS and the transversalis fascia in patients with bilateral and unilateral hernias. In the latter case (using a surgical repair technique, in which the mesh is placed within the unchanged inguinal region as well), specimens were harvested from macroscopically unaltered sites. In this study, contrary to what we have observed, no differences were seen in the ultrastructure of the RAMS. In their study alterations were, however, present within the transversalis fascia. A disrupted structure of collagen fibers was seen, especially in patients with direct hernia. Moreover, these alterations were also present in the contralateral, unchanged groin, which the authors consider a pre-hernia state [19]. Further investigations performed by this team on material harvested from deceased donors have yielded similar results. Again, no changes were found in the structure and dry mass of the RAMS. It has, however, been noticed that the collagen obtained from the RAMS fascia shows greater solubility in NaCl and pepsin, which could indicate metalloproteinase activity or increased collagen type III content. The authors arrive at the conclusion that the quantity of collagen in the transversalis fascia of patients with indirect inguinal hernia might even be greater than in healthy controls. This is explained by the remodeling hypothesis based on the stimulating influence of tensions in the inguinal region on collagen synthesis [17].

Our studies confirmed that in hernia patients the structure of the connective tissue changes, the elastic fibers decrease in number, the ground matter builds up, and the mutual proportions of the fibers and their relative positioning are altered, disrupting tissue architecture. Such changes presumably give ground for the connective tissue to lose its mechanical strength. Our researches provide no answer as to the exact cause of the pathologies. It is highly improbable that all the changes can be linked to one causative factor. The beginning of the events may have been the weakening or degeneration of the fibers due to faulty collagen synthesis and increased breakdown. The cause of the latter is currently believed to be metalloproteinase hyperactivity or a deficiency of their inhibitors [27–29]. The fasciae of the human body, regardless of their location, exist to counteract mechanical forces and preserve tissue integrity. The fascial site, from where the fascia fragments have been harvested, however, distant from the dynamically changing inguinal region, remains under constant mechanical stress as well. The alterations observed may, thus, result from the action of external mechanical forces, disrupting the weakened tissue. Significant biomechanical changes seen in transversalis fascia in hernia patients have been confirmed by the Liege team [30].

The etiopathogenesis of hernia remains a subject of discussion [16]. It appears prudent to continue with detailed analyses of the available material to approach the goal of explaining hernia etiology, and thus improve treatment options. Our own morphological studies have in many aspects confirmed the general weakening of the connective tissue in patients operated on for inguinal hernias. The difference between the study and control groups was evident in every case investigated by us.

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