AGE RELATED CHANGES IN THE ELASTIC FIBER SYSTEM OF THE INTERFOVEOLAR LIGAMENT

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SUMMARY: In order to evaluate age related changes of the elastic fiber system in the interfoveolar ligament, we studied the deep inguinal ring from 33 male cadavers aged from stillborn to 76 years. Selective and alternated staining methods for elastic fibers were performed to differentiate oxytalan, elaunin, and mature elastic fibers. We confirmed quantitative changes of the elastic fiber system with aging. There was a significant and progressive reduction of the oxytalan fibers (responsible for tissue resistance) and a significant increment in the mature elastic and elaunin fibers (responsible for tissue elasticity). Furthermore, there were structural changes in the thickness, shortness and curling of these mature elastic fibers. These changes induced loss of the elastic fiber function and loss of the interfoveolar ligament compliance. These factors predispose individuals to the development of indirect inguinal hernias that frequently emerge in adults and aged individuals, especially above the fifth decade.

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

Direct inguinal hernias seem to result from weakening of the transversalis fascia on the floor of the inguinal canal [1]. It has been demonstrated that alterations in the collagen component, and in the elastic fibers of the extracellular matrix of the transversalis fascia, occur with aging[2-4]. These factors favor the development of hernias and their recurrence after surgical repair. Consequently, after surgical repair, wall reinforcement with a layer placement on Hesselbach's triangle is indicated[5].

In contrast, the etiology of indirect inguinal hernia in children and youngsters is of congenital origin; they develop from the presence of a vaginal process or of a non-obliterated peritoneum-vaginal duct[6]. The vaginal process remains open in 15% to 37% of male individuals. However, only a small percentage develops inguinal hernia. The incidence of permeability of the vaginal process in infants, as well as in normal newborns, is 60% until 2 months of age and 40% at 2 years of age[7]. Indirect inguinal hernias may develop in 25% to 30% of newborns and in 20% of children and adults[8].

The appearance of indirect inguinal hernia in adults is correlated with the continence of the deep inguinal ring. This continence is related to the presence of a "U"-shaped thickening of the transversalis fascia, at the level of the deep inguinal ring. Lytle showed in 1945 that this sling acts as a pulley during the contraction of abdominal muscles, which places the inguinal ring more superior and lateral[9]. This mechanism occludes the deep inguinal ring during increases of intra-abdominal pressure[10,11]. Another mechanism that shuts the deep inguinal ring is that produced by the action of internal oblique and transversal muscles, which approach the inguinal ligament and clasp the deep inguinal ring. Thus the appearance of indirect inguinal hernia in the adult and elderly may derive from weakening of the transversalis fascia on the border of the deep inguinal ring[12].

This study was intended to verify the qualitative and quantitative alterations associated with aging of the elastic fiber system in the transversalis fascia (Hesselbach's interfoveolar ligament) within the deep inguinal ring.

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MATERIAL AND METHODS

We studied the deep inguinal ring from 33 male cadavers aged from stillborn to 76 years old (age = 39.99 ± 24.90). The right interfoveolar ligaments obtained by dissection were fixed in a 10% formaldehyde solution.

Staining Procedure

Three adjacent histological sections (3 micrometers thick) were submitted to one of the following selective methods for staining elastic fibers: Verhoeff's iodine-iron hematoxylin[13] method, which stains only the mature elastic fibers; Weigert's resorcin-fuchsin method[14], which stains mature and elaunin elastic fibers; and Weigert's technique with a previous oxidation performed using oxone as previously described[15], which stains the oxytalan, elaunin, and mature elastic fibers.

Morphometric Evaluation

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**), and Weigert-oxone (**LVWO**) methods. 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 an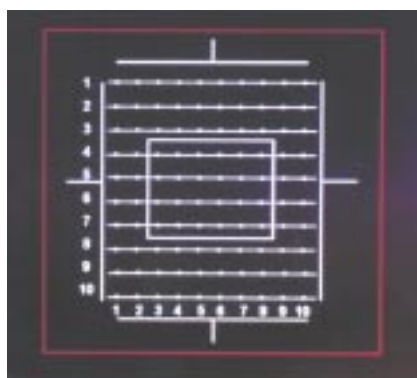d 100 points comprising a simple square lattice test area of 10 500 mm² (Fig. 1). Each elastic fiber completely intersected by any one of the lines was counted. These intersections of fibers with a test reticule are related to the length of those fibers per unit area, by the expression *LV=2Na,* where *Na* is the length of fiber per unit area[16]. The area of the tissue examined was determined by counting the number of points of intersection inside that tissue.

Statistical Analysis

The data on the linear density of elastic fiber were analyzed using a one-way analysis of variance (ANOVA). A p value 0.05 was considered statistically significant. The combined effects of age and linear density of elastic fibers were tested by linear regression analysis.

RESULTS

We demonstrated the presence of the 3 components of the elastic fiber system in the fascia transversalis of the Hesselbach ligaments. In young men, the elastic fibers showed a uniform distribution among the connective tissue and ran parallel to the collagen fibers (Fig. 2). In the aged men, the elastic fibers were shortened, fragmented, and thickened (Fig. 3).

The elastic fiber system was evaluated through the determination of linear density (LV) of these fibers in sections stained by selective methods for elastic fibers. LV values indicated the total length of mature, elaunin, and oxytalan fibers per unit area in WO-stained sections, mature and elaunin fibers in W-stained sections, and mature fibers in V-stained sections. The mean values of LVV, LVW, and LVWO from the group studied are indicated in Table 1. There were significant positive linear correlations of LVV with age and LVW with age in the interfoveolar ligament. The equation curve for LVV was 0.00439 + 0.000112 * age (r = 0.83; F = 70.68; p < 0.001); the equation curve for LVW was 0.00611 + 0.000093 * age (r=0.83; F = 69.32; p < 0.001). In contrast, there was a significant negative linear correlation between age and LVWO; the equation curve for LVWO was 0.0162 − 0.000067 * age (r = 0.69; F = 28.78; p < 0.001. The regression curves for LVV, LVW, and LVWO are shown in figure 4.

DISCUSSION

In the elastic system, the fibers with elastin deposits are the elements responsible for the reversible distensibil-
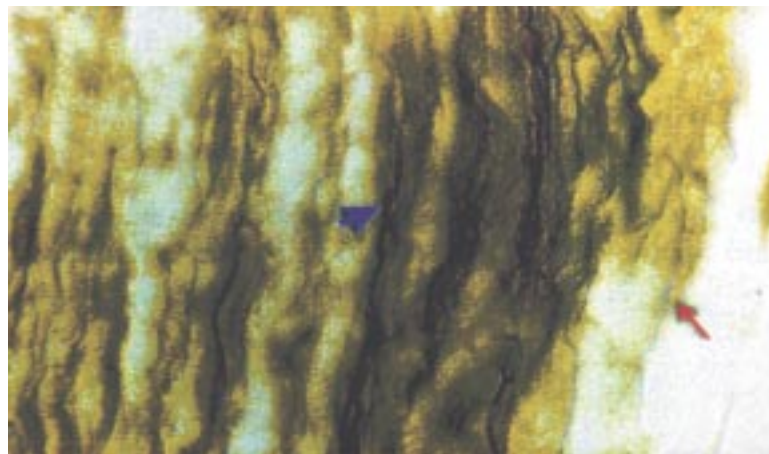

Figure 1- A test eyepiece reticule.

Figure 2 - Histological section of the interfoveolar ligament from young men showed numerous thin, long and straight elastic fibers, that ran parallel to the collagen fibers (Weigert, 400X).

ity of the connective tissue. Elastic fibers made up of microfibrils only (the oxytalanic fibers) have the mechanical resistance function[17]. It is well known that the network arrangement of elastic fibers contributes to tissue architecture, thus promoting passive withdrawal of the structure to its original size and shape when submitted to mechanical stress[18]. Hence in the aorta, cardiovascular system, lung, and tendons, the relative concentration of elastic fibers is high[19]. The integration of elastic fibers with collagen fibers is necessary for the maintenance of normal tissue resistance. If collagen and elastin composition, or its architectural arrangement is altered, tissue complacency will be jeopardized[20].

Continuous elastin synthesis would promote an increase of complacency or of tissue elasticity. With aging, however, elastic fibers undergo architectural distortion and structural alterations, becoming tortuous, thickened, and fragmented, and they lose their original functionality[21]. These alterations seem to be promoted either by an increase in collagen, which compresses the fibers through the action of tissue elastase and thus causes degeneration of these fibers, or by the arrangement of collagen fibers, which produces architectural distortion.

In 1981, Cannon and Read[2] showed evidence of increased elastolytic activity in plasma of patients around 60 years of age having direct inguinal hernias, as compared to the plasma elastolytic activity of patients without hernias within the same age range. Elastic fibers, responsible for tissue elasticity, have been demonstrated to be structurally and quantitatively altered with aging[3,23]. These structural data corroborate the observations of Pinto[24] that the low incidence of direct inguinal hernia in children, as well as its prevalence in adults — especially from fifty years of age onwards — suggest that aging humans are more susceptible to developing direct inguinal hernias.
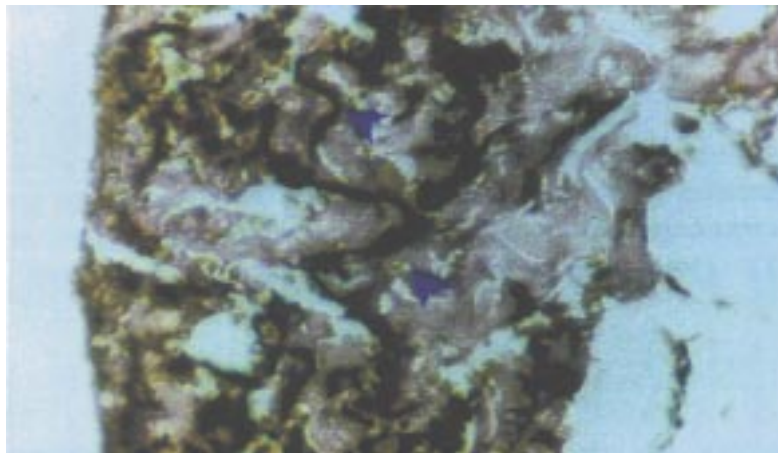

Figure 3 - Histological section of the interfoveolar ligament from aged men showed shortened fragmented, and thickened elastic fibers. There were no thin elastic fibers. (Weigert-oxone, 400X).

Table 1 – Linear density of elastic fibers in the interfoveolar ligament from adult male cadavers, according to the staining method.

Staining method	N	Mean	SD
Verhoeff	33	0.0088	0.00334
Weigert	33	0.0098	0.00281
Weigert-oxone	33	0.0134	0.00245

SD= Standard deviation.
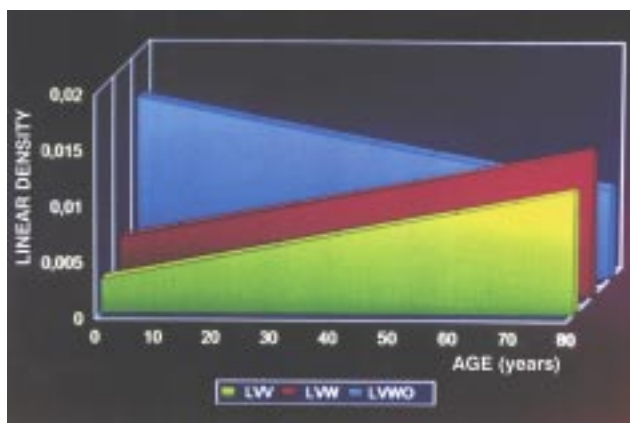
Figure 4 - Elastic fibers as a function of age in the interfoveolar ligament from male cadavers. (a) LVV; (b) LVW; and (c) LVWO. The linear regression is shown by the line. Individual data are plotted at each age.

In order to better understand the etiology of indirect inguinal hernia, we looked for an explanation from the possible structural alterations with aging—both qualitative and quantitative—suffered by the transversalis fascia within the deep inguinal ring region (Hesselbach's interfoveolar ligament). We found evidence that aging is associated with a progressive increase of elastic fibers containing elastin (mature elastic fibers and elauninic fibers). However, these fibers were found to be structurally altered — they were tortuous, thickened, and fragmented. On the other hand, we also found a progressive decrease with aging of the amount of oxytalanic elastic fibers, which are

responsible for resistance. Decrease of oxytalanic fibers, and structural and architectural alterations in the elastic fibers that occur with age would expose the collagenous fibrous system to deformation tensions higher than it normally endures. As a consequence, collagen fibers would be submitted to greater deformation, and since they represent a resistance element, they would end up by rupturing. This weakening of Hesselbach's interfoveolar ligament (caused by aging) would predispose individuals to the enlargement of the deep inguinal ring, where exteriorization of the peritoneal sac and of the contents of the abdominal cavity and indirect inguinal hernia.

RESUMO

<div style="text-align:right">RHCFAP/3008</div>

QUINTAS ML e col. - Alterações do sistema de fibras elásticas relacionadas ao envelhecimento do ligamento interfoveolar. **Rev. Hosp. Clín. Fac. Med. S. Paulo 55**(3):83-86, 2000.

Com o objetivo de avaliar as alterações do sistema de fibras elásticas relacionadas ao envelhecimento do ligamento interfoveolar estudou-se o anel inguinal profundo em 33 cadáveres do sexo masculino com idade variando de recém-nascido a 76 anos. Métodos de coloração seletiva para as fibras elásticas foram empregados para diferenciar fibras oxitalânicas, fibras elaunínicas e fibras elásticas maduras. Verificaram-se alterações quantitativas significantes no sistema de fibras elásticas do ligamento interfoveolar com o envelhecimento: uma redução progressiva de fibras oxitalânicas, responsáveis pela resistência do tecido conjuntivo, e um aumento de fibras elásticas maduras e elaunínicas, responsáveis pela elasticidade tecidual. Demonstraram-se alterações estruturais, como espessamento, encurtamento e encurvamento das fibras elásticas maduras e elaunínicas. Estas alterações induzem a perda de função das fibras elásticas, o que certamente levará a perda de complacência do ligamento interfoveolar, predispondo à hérnia inguinal indireta, que freqüentemente aparece em homens adultos ou idosos, especialmente acima de 50 anos de idade.

DESCRITORES: Hérnia inguinal. Envelhecimento. Matriz extracelular. Elastina. Fascia transversal.

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