

# Histology of the Internal Inguinal Ring in Patients with Indirect Inguinal Hernia

Marcelo A. Beltrán, Carlos Barría, Francisco Rodríguez, Javier Arcos, Karina S. Cruces

## Abstract

**Aim:** The purpose was to investigate the basic histological structure of the internal inguinal ring in patients operated on for primary indirect inguinal hernia.

**Patients and Methods:** The study involved a sample of 72 consecutive male patients over 15 years of age, submitted to elective surgery for unilateral primary inguinal hernia. The primary outcome measures were the histological findings of the internal inguinal ring. Patients were divided into three groups according to age: group 1 included patients between 15 and 40 years of age, group 2 patients between 41 and 70 years of age, and group 3 patients older than 71 years of age.

**Results:** All samples from the internal inguinal ring comprised fibrous fascial tissue with elastic fibres which were thicker in groups 1 and 2, and thinner in group 3 ( $p < 0.0001$ ). Adipose tissue between elastic fibres was absent in group 1 ( $p = 0.003$ ), and was present in group 3 ( $p = 0.001$ ). Vascular sclerosis was minimum in group 1, moderate in group 2, and important in group 3 ( $p < 0.0001$ ). Acute or chronic inflammatory cells were absent in all patients. These changes were more significant with age, according to the analysis with multiple linear regression.

**Conclusions:** The basic histological characteristics of the internal inguinal ring in patients with indirect inguinal hernia consisted of reduced density and thickness of elastic fibres, and increased adipose tissue between elastic fibres, changes which became more significant with increasing age. Vascular sclerosis was also more severe in older patients compared to younger patients. These histological changes all appeared to be related to ageing.

**Key words:** *Inguinal hernia; Indirect hernia; Internal inguinal ring; Hernia histology*

## Introduction

The basic histology of the internal inguinal ring in patients with indirect inguinal hernia has not been a traditional subject of research among surgeons dedicated to hernia surgery. Initial studies indicated that hernia formation is the result of a collagen maturation defect [1]; hence, most

research into the inguinal region in patients with hernia has since focused on molecular alterations of collagen metabolism and arrangement leading to alterations in the ultrastructure of fascial tissues in the inguinal region and abdominal wall [2-14]. As a consequence, current evidence supports the notion that primary hernia formation derives from a biological deficiency of the extra-cellular matrix, rendering it a soft-tissue disease [14]. Proper basic histological studies of the internal inguinal ring in patients with indirect inguinal hernia had not been performed until recently [15]. Moreover, only a small number of older and a few more recent articles made mention of certain histological characteristics of the internal inguinal ring [16-21]. A recent article described fibrohyaline degeneration of muscle fibres together with acute and chronic inflammation in samples taken from the internal inguinal ring of patients with indirect inguinal hernia. The authors intimated that these findings constitute the aetiology of inguinal hernia formation [15]. As already established, the aetiology of hernias in humans is related to defects in the extra-cellular matrix metabolism [2-14,22]. Accordingly, we suggest that the basic histological characteristics of the internal inguinal ring in patients with indirect inguinal hernia and any degenerative changes

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Marcelo A. Beltrán, M.D.  
Department of Surgery, Hospital De La Serena,  
Department of Clinics, Facultad de Medicina,  
Universidad Católica Del Norte

Carlos Barría, M.D.  
Department of Pathology, Hospital De La Serena

Francisco Rodríguez, M.D., Javier Arcos, M.D.  
Department of Clinics, Facultad de Medicina,  
Universidad Católica del Norte

Karina S. Cruces, R.N.  
Department of Surgery, Hospital de la Serena

Corresponding author: Marcelo A. Beltrán, M.D.  
P.O. Box 912, Manuel Antonio Caro 2629  
La Serena – IV Región, CHILE  
Tel.-Fax: +56 51 485923  
e-mail: beltran\_01@yahoo.com

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would be related to normal ageing. In consequence, such changes do not constitute the aetiology of hernia. The purpose of this study was to investigate the basic histological structure of the internal inguinal ring in patients submitted to surgery for primary indirect inguinal hernia, to describe the findings, and to investigate their correlation with time in younger, middle age and older patients.

## Patients and Methods

### Study design and inclusion criteria

A prospective observational case-only study was designed. The protocol was entered into the Clinical Trials-Protocol Registration System with the number NCT01211067 on September 28, 2010, and was approved by our institutional ethics committee on October 10, 2010. Informed consent was obtained from all included patients. A convenience sample of 72 consecutive male patients older than 15 years of age, submitted to elective surgery for unilateral primary inguinal hernia, was enrolled in the protocol. Inclusion criteria were male gender >15 years of age, unilateral indirect primary inguinal hernia, non-smokers, non-diabetic, and patients without any genetic connective tissue diseases such as osteogenesis imperfecta, Marfan syndrome, Ehlers-Danlos syndrome, hip dislocation of childhood, autosomal dominant polycystic kidney, and joint hypermobility. Exclusion criteria included female gender, patients < 15 years of age, bilateral inguinal hernia, recurrent inguinal hernia, direct or combined direct-indirect inguinal hernia and femoral hernia.

### Outcome measure

The primary outcome measure was determined by the histological findings of the internal inguinal ring of patients submitted to elective reparation of a primary unilateral indirect inguinal hernia. The time of hernia evolution was counted from the onset of symptoms to surgery. Obesity was classified according to body mass index (BMI): patients with BMI less than 29 kg/m<sup>2</sup> were considered to be of normal weight, BMI 30 to 34 kg/m<sup>2</sup> indicated overweight, BMI 35 to 40 kg/m<sup>2</sup> signified obesity, and patients whose BMI was higher than 41 kg/m<sup>2</sup> were classified as morbidly obese. The type of work that patients performed was divided into light work if it was related to office jobs or minimum physical effort, heavy work if it involved great physical effort as required of farmers or miners worker, and retired if the patient had given up any kind of work and whose activities were restricted to those of daily living. Histological findings were defined and classified as follows: Elastic fibres were divided in thicker and thinner fibres; adipose tissue was

deemed as absent or present; vascular sclerosis was classed as minimum, moderate and severe; vascular hyperaemia included pooled erythrocytes and other blood cells in venous or arterial capillaries; acute inflammatory cells comprised polymorphonuclear cells; and chronic inflammatory cells were defined as lymphocytes and monocytes whose presence or absence was recorded.

### Sampling protocol and sample processing

An informed consent to participate in the study allowing tissue sample collection was signed by all patients. The operative procedure entailed the Lichtenstein technique as described elsewhere [23,24]. Once the inguinal hernia was identified as indirect, and after proper dissection and identification of the internal inguinal ring, a sample of the internal inguinal ring was taken: a strip of tissue measuring at least 1 cm x 0.5 cm x 0.5 cm from the upper border or anterior crus of the internal inguinal ring was cut with scissors or a surgical knife between two ligatures at a distance of 1.5 to 2 cm apart. The samples were immediately stored in 10% buffered neutral formalin and sent out for routine processing by an experienced surgical pathologist. The fixed tissue sample was embedded in a paraffin block and sliced with the microtome at a thickness of 0.4 microns. The samples were analyzed with histochemical techniques: haematoxylin and eosin, van Gieson, and orcein.

### Group analysis

The cohort of patients was divided into three groups according to age. Group 1 included 20 patients (28%) between 15 and 40 years of age. Group 2 comprised 20 patients (28%) aged between 41 and 70 years. Group 3 consisted of 32 patients (44%) older than 71 years, the oldest being 85 years-old.

### Statistics

Categorical variables were expressed as proportions and continuous variables as mean, standard deviation, and range. Comparisons between groups were performed with Chi-square and T tests. Multiple linear regression was used to identify variables likely to influence the histological findings. Partial correlations coefficients were made to determine correlations between variables according to histological findings. A probability value <0.05 was considered significant. The analysis was performed with the statistical software SSPS version 11.0 (Chicago, IL).

## Results

The three groups of patients were statistically comparable in terms of the number of patients, mean BMI, and

absence of associated diseases. The older the patient, the longer the time of hernia evolution. All patients in group 1 and most patients in groups 2 and 3 had a BMI of less than 29 kg/m<sup>2</sup>. A higher proportion of patients in group 1 performed light work. Most patients in groups 1 and 2 performed heavy work. A higher proportion of patients in group 3 were retired. Arterial hypertension was more frequent among patients in group 3 while other coexisting diseases were less frequent in groups 1 and 2 (Table 1).

Internal inguinal ring histological findings are detailed in Table 2. All samples from the internal inguinal ring were constituted by fibrous fascial tissue with elastic fibres that were thicker in younger patients and thinner in older patients (Figure 1). Adipose tissue between elastic fibres was absent in group 1 and present in group 3; no muscular bundles or muscle cells were found in any of the samples (Figure 2). Vascular sclerosis, though present in groups 1, 2 and 3, was minimal in group 1, moderate in group 2, and important in group 3 ( $p < 0.0001$ ). Hyperaemia was present in groups 1, 2, and 3. All patients had thick hypercellular vascular walls which were more cellular in groups 1 and 2 (Figure 3). Acute or chronic inflammatory cells were absent in all patients.

Multiple linear regression analysis identified the variable age ( $p < 0.0001$ ) as the only factor influencing histological findings (Table 3). Partial correlation coefficients were calculated according to histology to identify positive correlations between variables: Age was associated with time of hernia evolution ( $p = 0.002$ ) and the nature of the patient's work ( $p = 0.017$ ). BMI had a positive correlation with coexisting diseases ( $p = 0.002$ ) and obesity ( $P < 0.0001$ ).

## Discussion

The results of this study showed tissue samples to bear normal histological architecture and degenerative changes related to ageing, evidenced by a marked decrease in elastic fibres in terms of thickness and density, and the appearance and increase of adipose tissue between elastic fibres in older patients; these changes led to reduced fascial elasticity. General characteristics of the patients included in this study were as expected and did not differ from published series [23–26]. Biopsy samples were taken from the anterior crus of the internal inguinal ring, a fundamental structure in hernia prevention and development; consequently, the histological findings of this study are representative of the basic histology of this structure.

**Table 1.** General characteristics

Groups	1	2	3	Total
Number of patients	20 (28%)	20 (28%)	32 (44%)	72 (100%)
Age (years)	35 ± 8 (16 – 39)	50.5 ± 5.4 (42 – 70)	72 ± 0.9 (71 – 85)	54.3 ± 18.1 (16 – 85)
Time of hernia evolution (years)	3 ± 2 (1 – 5)	4.8 ± 3.5 (1 – 7)	9.1 ± 6.4 (2 – 18)	6.3 ± 5.4 (1 – 18)
BMI (kg/m <sup>2</sup> )	24.3 ± 3.6	26.4 ± 2.4	26.2 ± 3.7	25.3 ± 3.7
Obesity				
BMI < 29	20 (100%)	15 (75%)	26 (82%)	61 (85%)
BMI 30 – 34	-	5 (25%)	4 (12%)	9 (12%)
BMI 35 – 40	-	-	2 (6%)	2 (3%)
BMI > 41	-	-	-	-
Type of work				
Light	9 (45%)	7 (35%)	7 (22%)	23 (32%)
Heavy	11 (55%)	10 (50%)	11 (34%)	32 (44%)
Retired	-	3 (15%)	14 (44%)	17 (24%)
Alcoholism				
Yes	9 (45%)	6 (30%)	19 (59%)	32 (44%)
No	11 (55%)	14 (70%)	13 (41%)	40 (56%)
Coexisting diseases				
No	11 (55%)	12 (60%)	14 (44%)	37 (51%)
Hypertension	6 (30%)	2 (10%)	18 (56%)	26 (36%)
Other cardiac conditions	-	3 (15%)	-	3 (4%)
Chronic constipation	3 (15%)	-	-	3 (4%)
Asthma	-	3 (15%)	-	3 (4%)

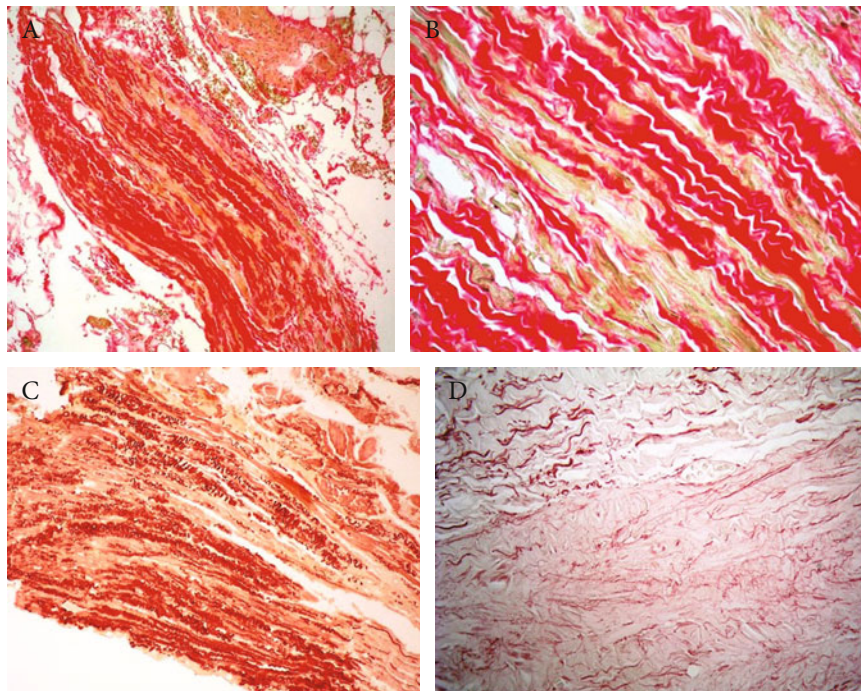
**Table 2.** Histological findings: Internal inguinal ring

Groups	1 n: 20 (28%)	2 n: 20 (28%)	3 n: 32 (44%)	Total n: 72 (100%)	p
Elastic fibers					
- Thicker	20 (100)	4 (20)	-	24 (33)	<0.0001
- Thinner	-	16 (80)	32 (100)	48 (77)	<0.0001
Adipose tissue					
- Absent	20 (100)	7 (35)	-	27 (37)	0.003
- Present	-	13 (65)	32(100)	47 (63)	0.001
Vascular sclerosis					
- Minimum	20 (100)	2 (10)	-	22 (30)	<0.0001
- Moderate	-	18 (90)	-	18 (25)	<0.0001
- Important	-	-	32 (100)	32 (45)	<0.0001
Thick vascular wall	20 (100)	20 (100)	32 (100)	72 (100)	NA
Hyperemia	20 (100)	20 (100)	32 (100)	72 (100)	NA
Acute inflammatory cells	-	-	-	-	NA
Chronic inflammatory cells	-	-	-	-	NA

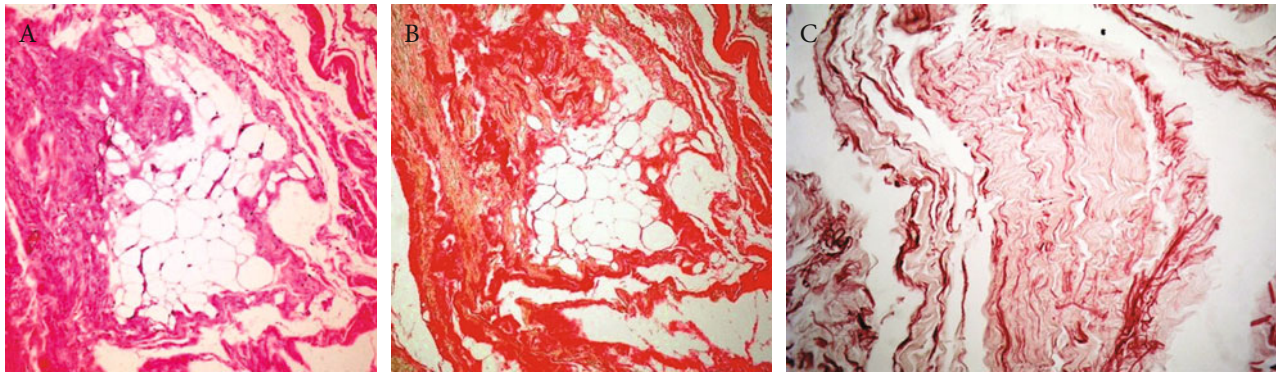
NA: Not Applicable

Increased age could predispose to inguinal hernias by a mechanism involving the reduction of oxytalan fibres responsible for tissue resistance and an increment in mature elastin and elaunin fibres responsible for tissue elasticity.

These changes are secondary to increased age together with structural changes in thickness, shortness and curling of mature elastic fibres, and induced loss of elastic fibre function [5]. Hence, it was postulated that histological ageing



**Figure 1.** A. Tissue sample from a 17-year-old patient. Elastic fibres (brownish) intercalated with connective tissue constituted by collagen fibres (red). Notice the absence of adipose tissue between fibres. (Van Gieson 10x). B. Detailed microphotograph taken from the same sample depicting elastic fibres (brownish) and collagen fibres (red). (Van Gieson 40x). C. Highly dense thick elastic fibres in a 23-year-old patient. (Orcein 10x). D. Elastic fibres in a 50-year-old patient. The elastic fibres were less dense, thinner, and fragmented. (Orcein 40x)



**Figure 2.** A. Tissue sample from an 80-year-old patient. Adipose tissue between elastic fibres was found in great quantities. (Haematoxylin-Eosin (HE) 10x). B. The same tissue sample stained with van Gieson shows the elastic fibres, collagen fibres and abundant adipose tissue. (Van Gieson 10x). C. The elastic fibres were thinner and of low density. (Orcein 10x)

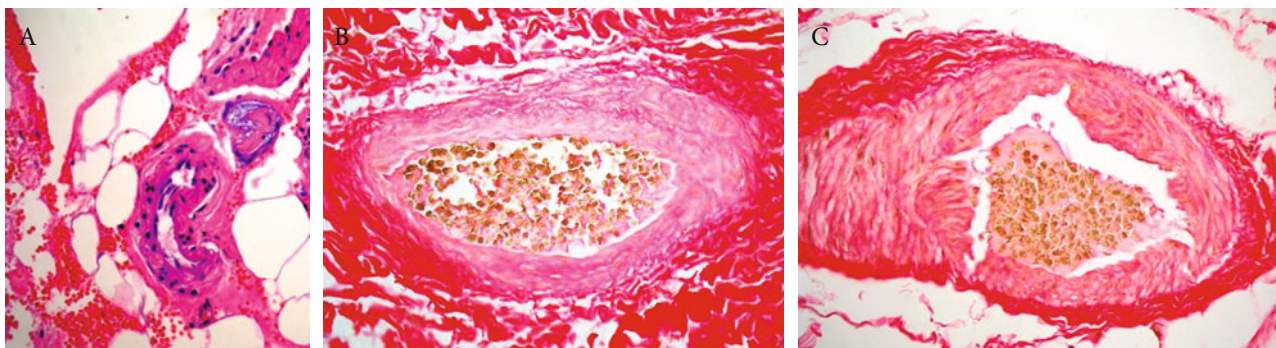
of the internal inguinal ring and internal oblique muscle could predispose to inguinal hernia. However, others have argued that all indirect inguinal hernias in adults and infants are congenital [27]; this argument remains the subject of controversy. The degenerative changes described herein were expected in fascial tissues constituted by collagen and elastic fibres with ageing, and have been previously suggested [5,21,25]. Another consistent histological characteristic was vascular sclerosis that correlated well with increased age, supporting the notion that the only histological changes in the tissues constituting the internal inguinal ring were those related to ageing. The finding of progressively thick vascular walls in patients of all ages was of particular interest; it suggested that these tissues were placed constantly under high pressures. High intra-abdominal pressure has been regarded as one of the factors leading to hernia formation [21,25,26]. This histological finding could be related to high intra-abdominal pressure placed on inguinal tissues. Previously, a report suggested that an inflammatory response

over the internal inguinal ring area was responsible for the development of indirect inguinal hernia [15]; we found no such inflammatory response. Consequently, our findings do not support that notion.

The results of the present study demonstrate that the internal inguinal ring undergoes histological degenerative changes with advancing age, though this does not exclude

**Table 3.** Multiple linear regression

Variable	p
Age	p<0.0001
Time of hernia evolution	p=0.348
Type of work	p=0.567
Alcoholism	p=0.602
Obesity	p=0.858
BMI	p=0.425



**Figure 3.** A. Vascular sclerosis was absent, although the vascular wall on this tissue sample from a 21-year-old patient, was thick and hypercellular with abundant muscle cells. (HE 10x). B. Tissue sample depicting a blood vessel from a 50-year-old patient. The vascular wall was thick although less cellular. Moderate sclerosis was present. (Van Gieson 40x). C. A hyperaemic blood vessel from a 70-year-old patient depicting severe sclerosis and a thick vascular wall. The vascular lumen was stenotic. (Van Gieson 40x)

the risk of indirect inguinal hernia at all ages. Risk factors thought to predispose to inguinal hernia and other types of hernias are related to the number and thickness of collagen fibres in fascial tissues [28]; very attenuated or even absence of fascial tissues, such as the aponeurotic fibres of the transversus abdominis muscle, can be consistently found in patients with inguinal hernia [18,28,29]. Defects in the metabolism and quality of collagen types I and III also predispose to inguinal hernia [2-7,14,29]. Hydroxyproline, a product of procollagen containing proline and lysine essential for intermolecular and intramolecular cross-linking and glycosylation of collagen, has been found to be decreased in patients with indirect inguinal hernia and smokers [29]. Other factors known to influence the development of inguinal hernias include connective tissue disorders, activation of collagenases, and activation and increased levels of neutrophil elastase and metalloproteinase in smokers [29]. In order to have a selected sample without known significant risk factors for primary inguinal hernia, smokers and patients with connective tissue disease were excluded from this study. Consequently, the histological findings of this study showing degenerative changes related to age in tissues involved in primary indirect inguinal hernia establish these characteristics as those to be expected in this condition.

## Conclusions

The basic histological characteristics of the internal inguinal ring in patients with primary indirect inguinal hernia consisted of reduced density and thickness of elastic fibres, increased adipose tissue between elastic fibres, and vascular sclerosis, all of which were more prevalent with age. These histological changes over the internal inguinal ring were related to normal ageing.

## Ethical Approval

The Authors declare that Ethical approval was obtained from their institutional board.

## Conflict of interest

There is no conflict of interest.

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