

HISTOLOGICAL CHANGES OF LIGAMENTA FLAVA IN LUMBAR DISC HERNIATION AND SPINAL CANAL STENOSIS

Thamer A Hamdan^{*}, Khalida K Jbara[#], Hatem A Hatem[@]

^{*}Professor of Orthopaedic Surgery, Dept. of Surgery, College of Medicine, University of Basrah.

[#]Assistant professor, Dept. of Anatomy, Histology & Embryology. [@]MBChB, MSc in Histology, College of Medicine College of Medicine, University of Basrah

Abstract

Samples of ligamenta flava were obtained after surgical operations from 50 patients with a lumbar disc herniation, another 50 patients with a lumbar canal stenosis, and 25 patients with spinal fractures who were used as control group.

Ligamenta flava from control patients aged below 46 years consisted of large elastic fibers, thin bundles of collagen fibers, and few spindle-shaped fibroblast cells.

In close proximity to the laminal insertion, the ligamentum flavum had fibrocartilaginous features. In the control patients who were aged 46 or older, the areas that had fewer and thinner elastic fibers and a more abundant collagen component were visible occasionally. The spindle-shaped fibroblast cells were fewer compared with control patients aged below 46 years. Also remnants of necrotic cells and few, short, thin, interwoven, fragmented, non-branching elastic fibers, as well as small calcified areas, were occasionally visible.

In close proximity to the laminal insertion, the ligamentum flavum had larger fibrocartilaginous features with more collagen fibers compared with younger patients.

In patients with disc herniation, the ligamenta flava had nearly similar morphologic features to those of the control patients of similar ages. The ligamenta flava from patients with lumbar spinal stenosis aged below 46 years showed areas of fibrosis in which the cells were often represented by fibroblast cells and in stenotic patients older than 46 years, central portion of ligamentum flavum showed areas of fibrosis, in which the elastic fibers appear normal in some areas, showed little changes in others and in most of these areas showed great changes. Fibrous septa, degenerating elastic fibers as well as small calcified areas were observed often.

In conclusion, Lumbar ligamentum flavum as any tissue in human body undergo degenerative changes during aging. In lumbar canal stenosis, the degenerative changes were more obvious compared with normal spine or lumbar disc herniation. In stenotic patients, ligamenta flava show a significant decrease in the elastic component as a result of fibrosis and chondroid metaplasia of the tissue, as well as degeneration of the elastic fibers. These changes, and the presence of calcified areas within the tissue, decrease the elasticity of the ligaments. An elastic tissue can be deformed under traction and gradually return to its normal size, proportional to the decrease of the elastic tension. Ligamenta flava do not normally bulge into the spinal canal when spine is in the neutral position.

Introduction

In the last decades, several studies have analyzed the microscopic morphology of ligamenta flava. However, some studies were carried out in animals^{1,2}, whereas others have

concentrated on normal human ligaments or the calcification processes in ligamenta flava of the cervical spine^{3,4}.

Only three investigations have analyzed the microscopic morphology of ligamenta flava in patients with

Correspondence to: Thamer A Hamdan, Dean, College of medicine, University of Basrah, IRAQ.

lumbar disc herniation or spinal stenosis. One of the two⁵, studied the ultrastructural features of ligaments from patients with a herniated disc, using as controls, ligamenta flava obtained from young adults only. The second investigation⁶ analyzed, at histological and histochemical levels, ligamenta flava from patients with lumbar canal stenosis or degenerative spondylolisthesis, using as a control, the ligamentum flavum of a teenager patients. The scarcity of information on the microscopic features of human ligamenta flava in degenerative spinal diseases is surprising, because these ligaments have been implicated often in the pathogenesis of degenerative condition of the spine, particularly lumbar spinal stenosis⁷⁻⁹. In this condition, ligamenta flava compress the nerve structures, although controversy still exists on whether these ligaments increase in thickness as a result of fibrosis or degenerative changes¹⁰⁻¹².

The third investigation analyzed at histological, histochemical, and transmission electron microscopic study, the morphologic features of ligamenta flava in patients with lumbar disc herniation and spinal stenosis. Because connective tissues, including the ligamentum flavum, undergo morphologic changes with aging¹²⁻¹⁵, used as controls, ligamenta flava from patients in the second to the seventh decade of life to differentiate between age-related and pathologic changes. In the present histological microscopic study, we analyzed the morphologic features of ligamenta flava in patients with lumbar disc herniation and lumbar spinal stenosis. We used as controls, ligamenta flava from patients in the second to the eighth decade of life.

Materials and Methods

This descriptive study was conducted in Basrah province from Iraq in 2003.

Preparation and examination was conducted in the department of Anatomy, Histology and Embryology in the College of Medicine-University of Basrah. Samples of ligamenta flava were obtained after surgical operations carried at Ebn-Al-Beetar private hospital from 50 patients with a lumbar disc herniation, another 50 patients with a lumbar canal stenosis, and 25 patients with spinal fractures who were used as control group. In the group with herniated discs, there were 33 men and 17 women, ranging in age from (17-57) years old (mean age 42). Disc herniation was located at level L3-L4 in 18 patients, at level L4-L5 in 27 patients, at level L5-S1 in 5 patients. With lumbar stenosis included 26 men and 24 women, aged 35-84 years (mean age 57). The control group includes 22 men and 3 women, aged (16-65) years (mean age 49). Samples of fractures were located from lower thoracic area of 3 patients and in thoracolumbar area of 5 patients and in lumbar area of 14 patients and in lumbosacral area of 3 patients. From each patient, specimens of ligamentum flavum were taken either from the central portion of the ligament or from the area which is in close proximity to the attachment to the distal lamina. In each case, specimens were obtained from the most superficial layer, and from the middle or deep layers of the ligamentum flavum. All specimens were collected directly after surgical operations and tissue samples were placed in 10% formalin used as fixative solution. Preparation of tissue for microscopic examination¹⁶.

- 1- Fixation: The pieces of the tissue were immersed in 10% formalin for about 12 hours or over night.
- 2- Dehydration: Tissues were directly transferred from the fixative solution to successively increasing gradients of alcohol concentration

- (70% then 90%) two changes one hour for each.
- 3- Clearing: Xylene used as clearing agents to replace the alcohol in the tissue. This process completed by two changes through xylene one hour each.
 - 4- Embedding: Tissue transfer to melted paraffin at 58-60 C° to keep the wax in liquid state during the procedure. The original wax was replaced by fresh wax two times for about (1-6 hours). The clearing agent replaced by paraffin wax and paraffin penetrates all intercellular and intracellular spaces making the tissues more resistant to sectioning.
 - 5- Paraffin: Is allowed to be hardened in the form of a block contains the piece of the tissue.
 - 6- Sectioning: A paraffin block containing piece of tissue fixed on a rotary microtome used for paraffin section to cut the tissue into sections in about (3-5 μm thick) by using a sharp knife. Paraffin ribbon consisting of tissue slices adhering to one another come from sectioning process, carefully separated and floated on a surface of the hot bath at (30-40C°), then mounted over an albuminized glass slide.

Preparation for staining:

Paraffin material that infiltrates within the slices of tissue was removed before staining processes can be started by using the following procedures⁴:

- 1- We immerse a glass slide bearing a paraffin section in clearing agent for (15-30 minutes).
- 2- We wash with absolute alcohol for 2 minutes.
- 3- Then transfer to successively weaker solutions of alcohol 90%, 70% and 50%, 2 minutes for each.
- 4- We wash with running tap water for 2 minutes.

- Staining of tissue section:

By using Verhoeff's haematoxylin method which is the most commonly used, it can be prepared quickly and requires only a short staining time¹⁶.

Procedure:

- a) We bring paraffin wax sections to water. It is not necessary to treat the section with iodine if it has been in a mercuric chloride fixative before staining, as the mercuric chloride deposits are removed by the staining solution.
- b) Then we stain with haematoxylin solution for (15-30 minutes).
- c) After that we differentiate with 2% aqueous solution ferric chloride for (10-30 seconds).
- d) Then we wash off the ferric chloride solution with tap water.
- e) We examine the section under the microscope for correct differentiation. If differentiation is carried too far the section can be restained, provided that it has not been washed with alcohol.
- f) Then wash with water.
- g) We place in 95% alcohol for (5minutes) to remove the iodine.
- h) Then wash in water for at least (5minutes).
- i) After that we counter stain with van Gieson stain which has been diluted with an equal amount of distilled water for (30 seconds).
- j) Then we rinse with 95% alcohol.
- k) We dehydrate with absolute alcohol.
- l) Then clear with xylene.
- m) At last we mount in D.P.X (Diastase, plastosin, xylene).

By using verhoeffs' method, elastic fibers are selectively stained deep blue colour. Using Van Giesons as a Counterstain, acid fuchsin gives a red color to collagenous fibers. Cellular detail of fibroblasts is not revealed, but the nuclei stain deep blue. Degenerated elastic fibers are also stained but can

be distinguished from the normal by their less intense staining and presenting less distinct outlines. Then the slides were examined under light microscope and selected images were taken by using a special light microscope provided with photographic camera.

Results

1- Control ligamentum flavum:

A-Control ligamentum flavum in patients aged below 46 years

Most of the ligamentum flavum, except for the area in close proximity to the bone attachment, had a uniform

appearance and consisted of fibrous connective tissue in which more elastic fibers were observed. It was made up of large elastic fibers separated by thin bundles of collagen fibers. The elastic fibers, were oriented parallel to the major axis of the ligament. Many of the fibers had branches of varying length joining adjacent elastic fibers. The collagen fibers had small and fairly uniform diameters and were mostly oriented parallel to the elastic fibers.

The cells were few in number. They were represented by spindle-shaped fibroblast cells. Fig (1)

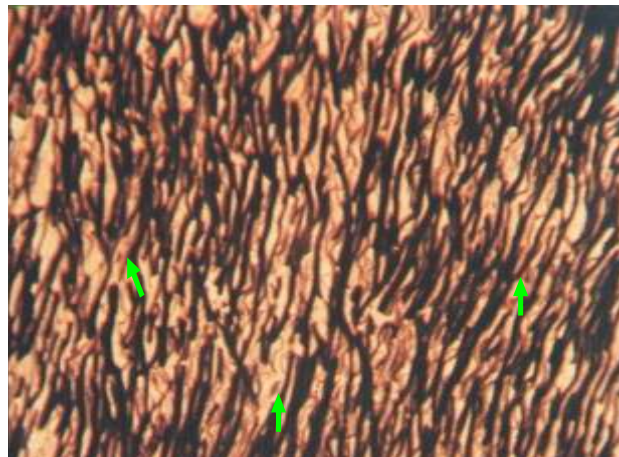


Fig (1): Control ligamentum flavum (central area) of 40 years old man showing the cells (black arrows) were few in number, represented by spindle-shaped fibroblast cells. X 495

In close proximity to the bone attachment, the ligamentous tissue showed fibrocartilagineous features. The cells had intermediate morpho-

logic characteristics between fibroblast cells and chondrocyte cells or were represented by typical chondrocyte cells. Fig (2)

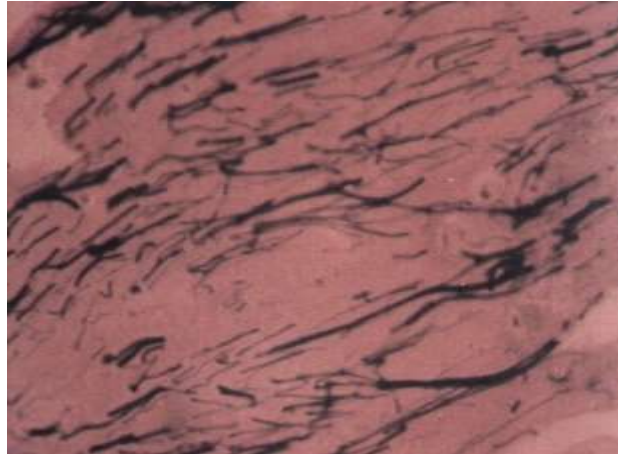
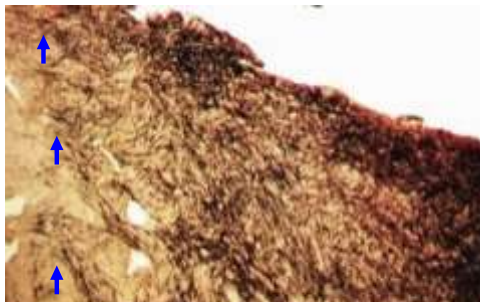


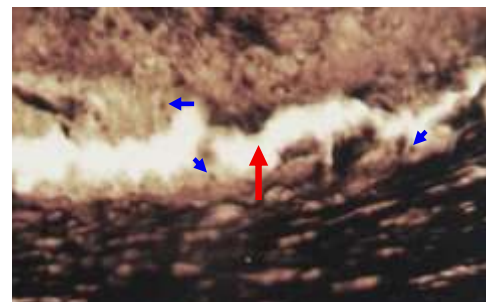
Fig (2): Control ligamentum flavum (insertional area) of 42 years old man showing cells had intermediate morphologic characteristics between fibroblast cells and chondrocyte cells (white arrows) or were represented by typical chondrocyte cells (black arrows). X 495

The elastic fibers were fewer and thinner than in the central portion of the ligament. Fig(3A). The bone

marrow cavities seen near laminal insertion filled with bone marrow cells. Fig (3B)



(A)



(B)

**Fig (3): Control ligamentum flavum (insertional area) of 44 years old man showing:-
A-The elastic fibers (black arrows) were fewer and thinner than in the central portion of the ligament. X 123.75**

B- The bone marrow cavity (white arrow) seen near laminal inseration filled with bone marrow cells (black arrows).X 495

B-Control ligamentum flavum in patients aged 46 years and above

In patients aged 46 or older, the central portion of the ligamentum flavum showed slightly different features compared with the younger patients. In some areas, the elastic fibers were less

numerous and thinner, whereas the collagen component was more abundant. Fibroblast cells were fewer and observed occasionally, particularly in the areas showing a decreased elastic component. Fig (4)

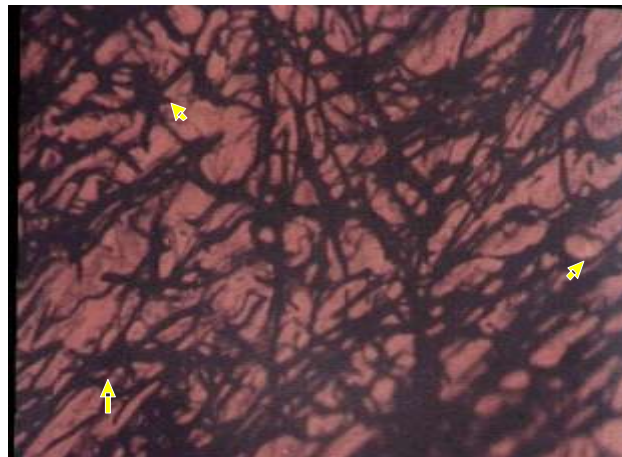


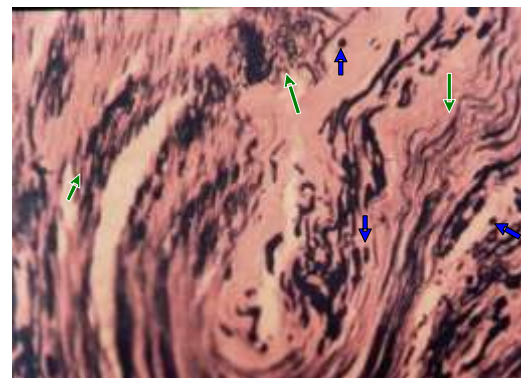
Fig (4): Control ligamentum flavum (central area) of 53 years old woman showing fibroblast cells (white arrows) particularly in the areas showing a decreased elastic component. X 495

Remnants of necrotic cells and few, short, thin, interwoven, fragmented, non-branching elastic fibers, as well as

small calcified areas, not observed in the younger patients, were occasionally visible. Fig (5 A, B).



(A)



(B)

Fig (5): Control ligamentum flavum (central area) of 59 years old man showing: - A- Large necrotic area (black arrow). X 123.75B- Remnants of necrotic cells (black arrows) and few, thin, short, interwoven, fragmented, non-branching elastic fibers (white arrows). X 495

The fibrocartilagineous zone near the bone attachment was slightly larger

and had more collagen fibers compared with the younger patients.

Summary:

Ligamenta flava from control patients aged below 46 years consisted of large elastic fibers, thin bundles of collagen fibers, and few spindle-shaped fibroblast cells. In close proximity to the laminal insertion, the ligamentum flavum had fibrocartilagineous features. And in the control patients

who were aged 46 or older; the areas that had fewer and thinner elastic fibers and a more abundant collagen component were visible occasionally. The spindle-shaped fibroblast cells were fewer compared with control patients aged below 46 years. Also remnants of necrotic cells and few, short, thin, interwoven, fragmented,

non-branching elastic fibers, as well as small calcified areas, were occasionally visible. In close proximity to the laminal insertion, the ligamentum flavum had larger fibrocartilaginous features with more collagen fibers compared with younger.

2-Disc herniation:

A-Ligamentum flavum in patients with disc herniation aged below 46 years

Ligamenta flava from patients with a herniated disc showed similar structural changes to those of the ligamenta flava from control patients of similar ages (below 46 years).

B-Ligamentum flavum in patients with disc herniation aged 46 years and above

Compared with the patients with disc herniation aged below 46 years, only two differences were noted. In the patient (above 55 years), few elastic fibers were seen more frequently in the central portion of the ligamentum flavum.

In a few patients, there were areas in which large bundles of collagen fibers were separated by small spaces which might be calcified areas. Fig (6)

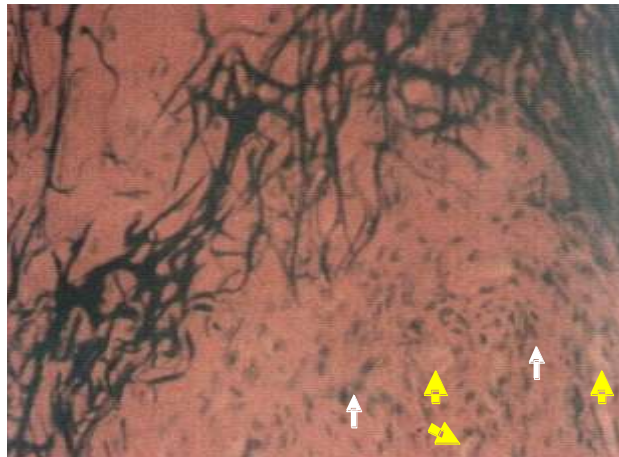


Fig (6): Ligamentum flavum (central area) of 58 years old man with disc herniation showing area in which large bundles of collagen fibers were separated by small spaces (black arrows). X 123.75

Summary:

In patients with disc herniation, the ligamenta flava had nearly similar morphologic features to those of the control patients of similar ages.

3-Spinal stenosis:

A- Ligamentum flavum in patients with spinal stenosis aged below 46 years

In stenotic patients younger than 46 years, the morphologic changes in the ligamentous tissue were less marked, though qualitatively similar to those observed in older patients (above 46 years). The morphologic changes represented by small, frequent fibrotic areas contained few fibroblast cells. Fig (7)



Fig (7): Ligamentum flavum (central area) of 44 years old woman with spinal stenosis showing fibrotic area (black arrow) contained few fibroblast cells (white arrows). X 495

B- Ligamentum flavum in patients with spinal stenosis aged 46 years and above: In the central portion of the ligamentum flavum, large fibrotic areas that had decreased elastic component and increased collagenous tissue were almost consistently observed.

In some of these fibrotic areas, the elastic fibers appear normal and in other areas show little changes. Fig (8)

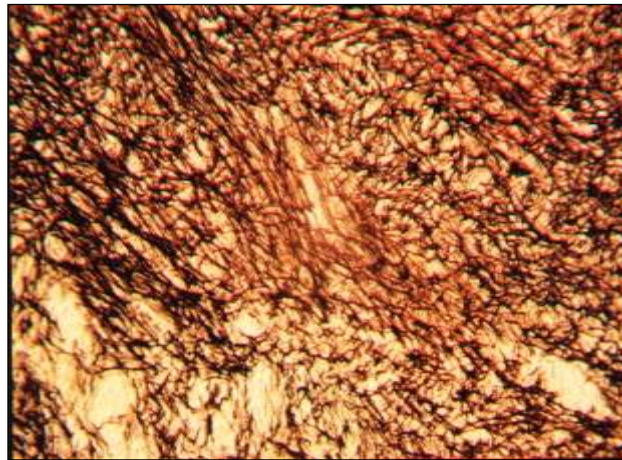


Fig (8): Ligamentum flavum (central area) of 57 years old woman with spinal stenosis showing many fibrotic areas where elastic fibers appear normal (white arrows) and in other show little changes (black arrows). X 123.75

In most of these fibrotic areas, the elastic fibers show great changes and these changes represented by small, short, thin, interwoven, non-branching,

isolated fibers separated either by small spaces or by connective tissue septa made by large bundles of collagen fibers. Fig (9)

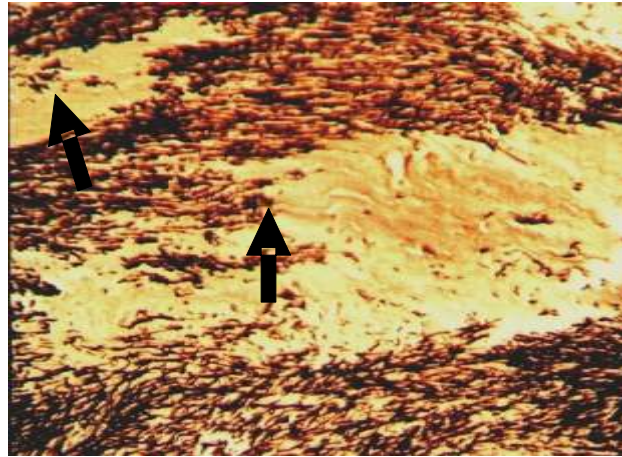


Fig (9): Ligamentum flavum (central area) of 60 years old man with spinal stenosis showing large fibrotic areas with elastic fibers (black arrows) show great changes. X 123.75

Few fibrotic areas appeared with no elastic fibers. Fig (10)

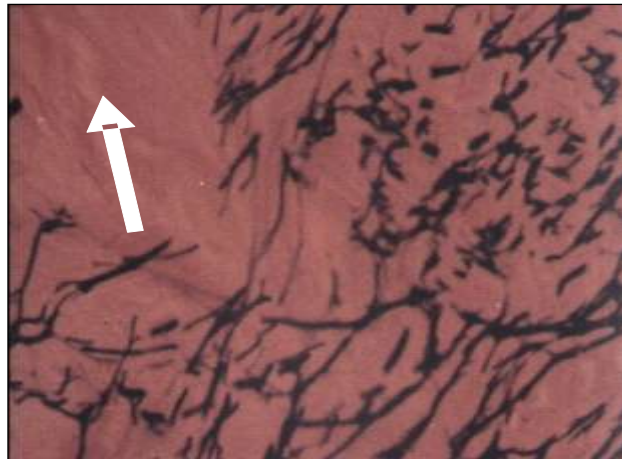


Fig (10): Ligamentum flavum (central area) of 64 years old woman with spinal stenosis showing fibrotic area appeared with no elastic fibers (white arrow). X 495

In a few patients, there were areas of collagenous tissue that contained very thin elastic fibers. Fig (11)

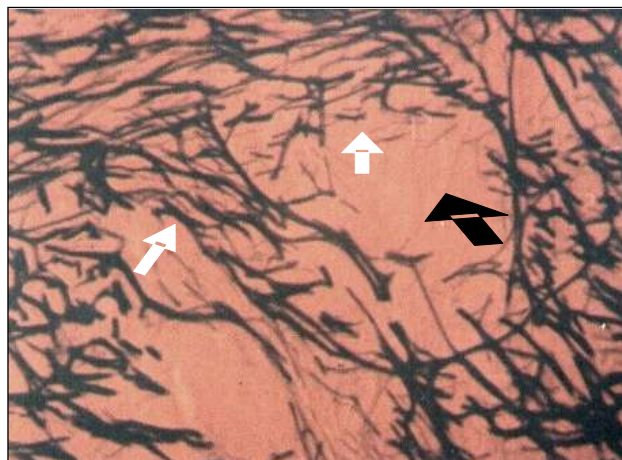


Fig (11): Ligamentum flavum (central area) of 55 years old woman with spinal stenosis showing areas of collagenous tissue (black arrow) that contained very thin elastic fibers (white arrows). X 495

The intercellular matrix in some of these fibrotic areas had no or little elastic fibers with large cellular debris and some necrotic cells.

The collagen fibers in stenotic patients had similar features to those in control ligamentum flavum of similar ages, except in the areas containing large

elastic fibers, in which very thin collagen fibers were interspersed among the large collagen fibers.

In a few specimens, the tissue was crossed by long septa made up of collagen fibers, few elastic fibers and fibroblast cells often arranged in small groups. Fig (12)

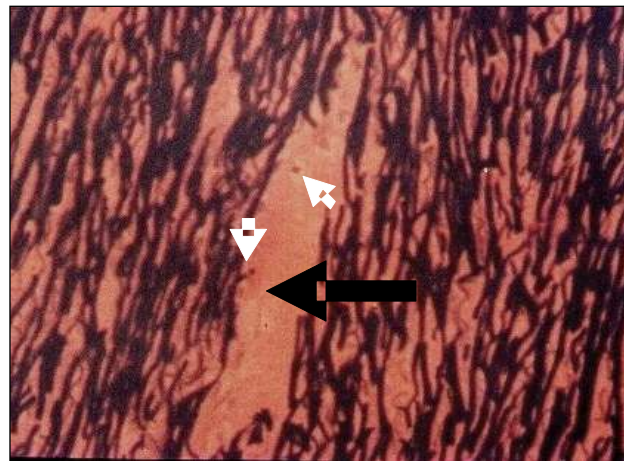


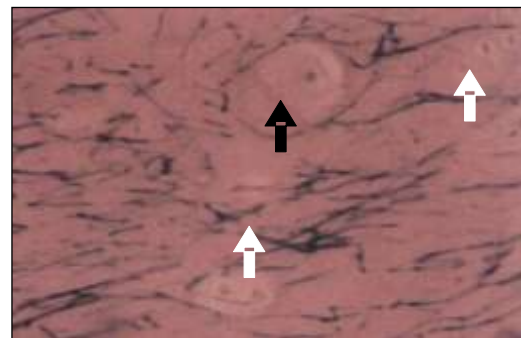
Fig (12) Ligamentum flavum (central area) of 55 years old woman with spinal stenosis showing the tissue was crossed by long septa (black arrow) made up of collagen fibers, few elastic fibers, fibroblast cells (white arrows) often arranged in small groups. X 495

Degenerating elastic fibers were seen occasionally. Some of these degenerating fibers had indistinct margins. In close proximity to the

laminal insertion, areas of chondroid metaplasia showing chondrocyte cells either isolated or arranged in pairs or clones. Fig (13 A, B)



(A)



(B)

Fig (13): Ligamentum flavum (insertional area) of 63 years old man with spinal stenosis showing: A- Area of chondroid metaplasia (black arrows). X 123.75 B-Chondrocyte cells either isolated (black arrow) or arranged in pairs or clones (white arrows). X 495

The ligamenta flava from patients with lumbar spinal stenosis aged below 46 years showed areas of fibrosis in which the cells were often represented by fibroblast cells and in stenotic patients older than 46 years, central

portion of ligamentum flavum showed areas of fibrosis, in which the elastic fibers appear normal in some areas, showed little changes in others and in most of these areas showed great changes. Fibrous septa, degenerating

elastic fibers as well as small calcified areas were observed often. In close proximity to laminal insertion; areas of chondroid metaplasia were seen occasionally.

4-Spinal stenosis associated with disc herniation:

Ligamentum flavum in stenotic patients with disc herniation aged below and above 46 years

No significant differences in the morphology of the ligamenta flava

were found between stenotic patients and stenotic patients associated with disc herniation of similar ages. In contrast, age-related differences were noted. In stenotic patients, the morphologic changes in the ligamentous tissue were less marked, though qualitatively similar to those observed in stenotic patients associated with disc herniation in both the central portion of the ligament and near the bone insertion. Fig (14)

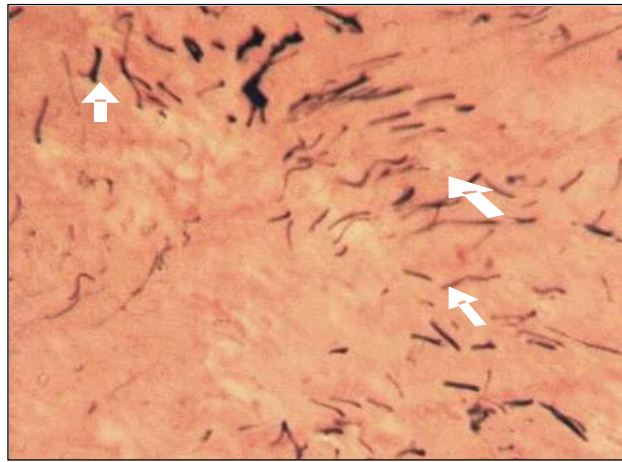


Fig (14): Ligamentum flavum (insertional area) of 63 years old woman with spinal stenosis associated with disc herniation showing large collagenous tissue and degenerated elastic fibers (white arrows). X 123.75

Discussion

The normal ligamentum flavum shows uniform morphologic characteristics throughout its length, except in close proximity to the bone attachment. In this portion, the ligamentous tissue consists of elastic fibrocartilagineous tissue i.e., an abundant collagenous component in which chondrocyte-like cells and few, thin elastic fibers are scattered.

The rest of ligament is an almost pure elastic tissue consisting of extremely large elastic fibers, thin bundles of collagen fibers, and fibrocyte-like cells. Postacchini¹ et al, in his histological, histochemical and transmission electron microscopic study found that these cells show long cytoplasmic projections, which probably make possible the

metabolic exchange that occurs between the cells and the matrix in areas far removed from cell body. In adult patients (46 years and more), the cells were extremely few, this suggests that the ligamentous tissue needs scarce metabolic exchange, probably because of low metabolic turnover of the elastic component, which represents 61% to 70% of the dry weight of the tissue⁵. Ligamenta flava undergo morphologic changes with aging. The fibrocartilagineous zone near the bone insertion undergoes slight enlargement. In the rest of the tissue, small areas of chondroid metaplasia, or fibrotic areas that have a decreased number and diameter of elastic fibers and increased collagenous component, are increasingly visible with advancing age. These

age-related changes, as well as the different structure of the ligament near the bone attachment, should be considered when analyzing the morphology of the tissue in pathologic conditions. These changes, on the other hand, may account for the decrease, with age, of some mechanical parameters of the ligaments, such as the values of stress, and the modulus of elasticity, at rupture¹⁷. Yahia et al,⁵ in an ultrastructural study of ligamenta flava from patients with disc herniation found that the fibroblasts of the normal ligament were replaced by chondrocyte-like cells. In our study, ligamenta flava from patients with a herniated disc differed only slightly from the ligaments from control patients of similar ages.

The most striking difference was the presence, in patients with disc herniation aged 46 years or older, of large bundles of collagen fibers separated by small spaces which thought to be calcified areas¹⁸ and in patients with disc herniation aged below 46 years the lesions mimic the lesions of yahia study.

The marked discrepancy between the two studies might be related to the fact that yahia et al study was ultrastructural which depend on electron microscope while our study is histological which depend on light microscope.

Furthermore, in our study, we subdivide the patients with disc herniation in two groups (group less than 46 years and 46 years and older), while yahia took only one group which were young.

In patients with lumbar stenosis, we observed three main morphologic changes: presence of areas of fibrosis, chondroid metaplasia of the ligamentous tissue, and regressive changes of the elastic fibers. Two types of regressive changes of the elastic fibers were identified. In some areas, the elastic fibers were markedly decreased in number or completely

absent, whereas in other areas the intercellular matrix contained very thin elastic fibers, presumably newly formed. Both types of fibrosis, particularly the latter where the patients younger than 46 years, were often characterized by a decrease in the cell population, represented by fibroblasts. While, Postacchini¹ et al, found that this type of fibrosis were often characterized by an increase in the cell population, represented by fibroblasts engaged in full synthetic activity, which appears in patients less than 50 years. The difference between the two studies related to the fact that we used in our study only light microscope, while postacchini used electron microscope in addition to light microscope which gave his study more details about the cells. The first type of fibrosis represents a regressive change of the ligamentous tissue where the patients older than 46 years. The second type, which appears to be peculiar of stenotic patients (less than 46 years), might represent an attempt to substitute degenerated tissue with newly formed tissue¹⁸. The areas of chondroid metaplasia showed similar characteristics to those in the control subjects and the patients with disc herniation. In stenotic patients, however, they were more numerous and larger than in the controls of similar ages. Degenerative changes of the elastic fibers were fairly rare and were observed in the most elderly patients (more than 60 years). These findings indicate that most changes of ligamentous tissue observed in patients with lumbar stenosis are qualitatively similar to those occurring during aging. In stenotic patients, however, these changes are more marked and diffuse than those related to aging, and thus, are pathologic and probably responsible for an abnormal function of the ligamenta flava. Areas of calcification were often seen in the ligamentous

tissue of stenotic patients, particularly the most elderly ones (more than 60 years). In both the controls and stenotic patients, the calcified areas were seen either in proximity of chondroid areas and in areas showing a normal morphology of the ligamentous tissue and this agree with postacchini et al¹, in his histochemical study which found mineralized deposits of calcium salts were often observed in both the controls and stenotic patients. The mechanism of calcification in ligamentous tissues is not known.

The greater frequency of calcified areas in stenotic patients could be related to the greater tendency of the tissue to undergo chondroid metaplasia or the greater amount of necrotic cells in these patients. Postacchini et al¹⁸, in his study found that the matrix vesicles originating from necrotic cells, in fact, were the initial calcification site in calcifying connective tissues, including the ligamentum flavum^{3,4,19,20}. In our study, Cellular debris originating from necrotic cells, in fact, was the initial calcification site in calcifying areas. We agree with postacchini et al¹ in this point, if we know that matrix vesicles and cellular debris originated from same source which are the necrotic cells. In lumbar spinal stenosis, ligamenta flava play a major role in the compression of the nerve structures in the standing position and extension of the lumbar spine^{7,19-21}. However, it is clear by MRI and operative findings that the ligaments bulge into the spinal canal because they are thickened or they are simply pushed into the bulging position by hypertrophied articular processes. This study clarifies this issue and may explain the role of ligamenta flava in the compression of the neural structures. In stenotic patients, ligamenta flava show a significant decrease in the elastic component as a result of fibrosis and chondroid metaplasia of the tissue, as well as degeneration of the elastic

fibers. These changes, and the presence of calcified areas within the tissue, decrease the elasticity of the ligaments. An elastic tissue can be deformed under traction and gradually return to its normal size, proportional to the decrease of the elastic tension. Ligamenta flava do not normally bulge into the spinal canal when spine is in the neutral position. When the spine is extended, they remain undeformed until such extension occurs that their elastic tension is completely satisfied, after which the ligaments bulge into the spinal canal¹².

The degree of extension necessary to satisfy the elastic tension of ligamenta flava is lessened proportionately to the reduction in the elasticity of the tissue. These observations suggest that in stenotic patients, the ligaments, due to reduced elasticity, may bulge into the spinal canal in the standing position even if they are normal in thickness and that bulging increase progressively, parallel to the increase of degenerative changes¹⁸. Nakamura et al⁶, in a histochemical study of ligamenta flava from patients with lumbar stenosis and patients with degenerative spondylolisthesis, found that the ligaments in the latter group have a greater amount of proteoglycans in the matrix and a higher degree of chondroid metaplasia of the tissue. In our study, no significant differences in the morphology of the ligamentum flavum could be found between patients with lumbar stenosis and stenotic patients with disc herniation. This related to the difference in the nature of the two studies. This contrasts the hypothesis that spinal instability (which represented her in our study in stenotic patients associated with disc herniation) accelerates the degenerative changes in the ligamentum flavum⁶. In contrast, age-related differences were noted.

At last, we can say that in our study, ligamenta flava from patients with disc

herniation slightly differed from those of normal patients of similar age.

Histological examination of the ligamentum flavum revealed scanty changes in disc herniation and obvious degenerative changes in lumbar canal stenosis.

Consequently it might play a major role in the pathogenesis and symptomatology of neurogenic claudication caused by lumbar canal stenosis.

Conclusions:

Lumbar ligamentum flavum as any tissue in human body undergo degenerative changes during aging.

In lumbar canal stenosis, the degenerative changes were more

obvious compared with normal spine or lumbar disc herniation.

In stenotic patients, ligamenta flava show a significant decrease in the elastic component as a result of fibrosis and chondroid metaplasia of the tissue, as well as degeneration of the elastic fibers. These changes, and the presence of calcified areas within the tissue, decrease the elasticity of the ligaments. An elastic tissue can be deformed under traction and gradually return to its normal size, proportional to the decrease of the elastic tension. Ligamenta flava do not normally bulge into the spinal canal when spine is in the neutral position.

References

1. Fasana F. Microscopic organization of the ligamenta flava in cercopithecinae. *Acta Anat* 1976; 94: 127-142.
2. Serafini-Fracassini A, Field JM, Smith JW, Stephens WGS. The ultrastructure and mechanics of elastic ligaments. *Journal of Ultrastructural Research* 1977; 58: 244-251.
3. Kubota T, Sato K, Kawano H, et al. Ultrastructure of early calcification in cervical ossification of the posterior longitudinal ligament. *Journal of Neurosurgery* 1984; 61: 131-135.
4. Kubota T, Kawano H, Yamashima T, Ikeda K, Hayashi M, Yamamoto S. Ultrastructural study of calcification process in the ligamentum flavum of the cervical spine. *Journal of Spine* 1987; 12:317-323.
5. Yahia LH, Garzon S, Strykowski H, Rivard CH. Ultrastructure of the human interspinous ligament and ligamentum flavum. A preliminary study. *Journal of Spine* 1990; 15: 262-268.
6. Nakamura T, Hashimoto N, Maeda Y, Ikeda T, Nakagawa H, Takagi K. Degeneration and ossification of the yellow ligament in unstable spine. *Journal of Spinal Disorder* 1990; 3: 288-292.
7. Postacchini F. Lumbar spinal stenosis. Springer-Verlag, Wien. Aulo Gaggi, Bologna, 1989
8. Ramani PS, Perry RH, Tomlinson BE. Role of ligamentum flavum in the symptomatology of prolapsed lumbar intervertebral disc. *Journal of Neurol Neurosurgery Psychiatr* 1975; 38: 550-557.
9. Yong-Hing K, Reilly J, Kirkaldy-Willis WH. The ligamentum flavum. *Journal of Spine* 1976; 1: 226-234.
10. Beamer YB, Garner JT, Shelden CH. Hypertrophied ligamentum flavum. *Arch Surg* 1973; 106: 289-292.
11. Kirkaldy-Willis WH, Paine KWE, Cauchoix J, Mclover G. lumbar spinal stenosis. *Journal of Clinical Orthopedic* 1974; 99: 30-50.
12. Ramsy RH. The anatomy of the ligamenta flava. *Journal of Clinical Orthopedic* 1966; 44:129-140.
13. Ippolito E, Natali PG, Postacchini F, Accinni L, De Martino C. Morphological, immunochemical and biochemical study of rabbit Achilles tendon at various ages. *Journal of Bone and Joint Surgery* 1980; 62 A: 583-598.
14. Postacchini F, Bellocci M, Massobrio M. Morphological changes in annulus fibrosus during aging. An ultrastructural study in rats. *Journal of Spine* 1984; 9: 597-603.
15. Dearden LC, Bonucci E, Cuicchio M. An investigation of aging in human lumbar spine. *Journal of Cell Tissue Research* 1974; 152: 305-337.
16. Clayden E.C. Paraffin wax sections. In: Clyden E.C. Practical section, cutting and staining 1962, 4thed. J. and A. Churchill LTD. (London); 31-65 and 107-109.
17. Luft J.H. Improvement in epoxyresin embedding methods. *Journal of Biophys.* 1961; 29:131-138.
18. Postacchini F, Gumina S, Cinotti G, Peruhia D, Demartino C. Ligamenta flava in lumbar disc herniation and spinal stenosis: light and electron microscopic morphology. *Spine* 1994; 19(8):917-922.
19. Anderson HC. Vesicles associated with calcification in the matrix of epiphyseal cartilage. *Journal of Cell Biology* 1969; 41:59-72.
20. Bonucci E. The locus of initial calcification in cartilage and bone. *Journal of Clinical Orthopedic* 1971; 78:108-139.
20. Tajima N, Kawano K. Cryomicrotomy of the lumbar spine. *Journal of Spine* 1986; 11:376-379.
21. Verbiest H. Stenosis of the bony lumbar vertebral canal. In: Wackenheimer A, Babin E. The narrow lumbar canal: Radiological signs and surgery. Berlin: springer-verlag, 1980.