# Morphohistometric study of the ligamentum flavum in cervical, thoracic and lumbar vertebrae: comparative approach

Gamal S. Abd El Aziz<sup>1</sup>, Sherif M. Zaki<sup>2</sup>, Magdy M.O. El-Fark<sup>3</sup>, Hesham N. Mustafa<sup>1</sup>, Rasha A. Al-Shali<sup>1</sup>, Raid M. Hamdy<sup>1</sup>, Mohammed Bangash<sup>4</sup>

<sup>1</sup>Anatomy Dept., Faculty of Medicine, King Abdulaziz University, Jeddah, KSA, <sup>2</sup>Anatomy Dept., Faculty of Medicine, Cairo University, Cairo, Egypt, <sup>3</sup>Anatomy Dept., Faculty of Medicine, Suez Canal University, Ismailia, Egypt, <sup>4</sup> Surgery Dept., Faculty of Medicine, King Abdulaziz University, Jeddah, KSA

#### **SUMMARY**

Anatomic characterization and fine structure of the human ligamentum flavum (LF), especially at different spinal levels, represent an attractive focus for the scientific and surgical application. Descriptive anatomical and structural study of LF at the cervical, thoracic and lumbar levels of the vertebral column in human cadavers is carried out here. The aim of the work is to clarify the anatomical features and fine structural differences in the human LF at different vertebral levels (cervical, thoracic and lumbar). Specimens of vertebral column were obtained from 34 human preserved cadavers. Their average age ranged between 56 and 69 years. Morphometric parameters including height, width and thickness of the ligament flavum at the midlevels of cervical, thoracic and lumbar regions were measured. Sections obtained from different levels were stained with different stains. Morphometric measurements involved the relative elastic area, relative collagen area, elastic area% and collagen area% were measured.

The results of the height, width and thickness of the LF at different spinal levels showed gradual increase in their mean values respectively. The LF midline gaps were found in the cervical, thoracic and lumbar regions. The morphometrical measure-

**Corresponding author:** Dr. Hesham N. Mustafa. Department of Anatomy, Faculty of Medicine, King Abdulaziz University, PO Box 80205, Jeddah 21589, Saudi Arabia.

E-mail: hesham977@hotmail.com

ments showed that the average elastic area was highest in the cervical region and lowest in the thoracic region. In the lumbar region, the percentages of both elastic area and the collagen area were nearly the same. The characterization of morphological and histological aspects of the LF at different spinal levels will be of great importance for applications in spinal surgery, biomechanical and physical rehabilitation of vertebral column.

**Key words:** Ligamentum Flavum – Spinal – Collagen and elastic fibers

# INTRODUCTION

Ligamentum flavum (LF) is a bifid ligament that is located in the posterior portion of the vertebral canal and runs vertically from axis vertebra to the sacrum. It is also called the yellow ligament, because of its larger content of elastic fibers than collagen fibers, and has been referred to as the most elastic tissue in the human body (Abbas et al., 2010; Newell et al., 2017).

Anatomically, it exists between vertebrae from 2nd cervical vertebra to the 1st sacral vertebra, where it runs vertically between laminae of adjacent vertebrae, forming the posterolateral wall of the vertebral canal and taking origin from the upper part of the inferior lamina and inserted onto the superior lamina's antero-inferior surface. It was described to consist of superficial and deep layers,

Submitted: 27 February, 2019. Accepted: 29 May, 2019.

with the fibers running in opposite direction (Rahmani et al., 2017; Takahashi et al., 2018).

Because its relations are very close with the structures enclosed in the vertebral canal, a considerable understanding of the ligament microanatomy at different spinal levels is required, where the LF serves as a relevant anatomical landmark to different structures in the spinal canal (Sengupta, 2017; Bashkuev et al., 2018). Several investigators have suggested that LF plays a significant role in vertebral diseases and in spinal stenosis, where degenerative changes in this ligament in the form of hypertrophy or thickening or ossification can lead to spinal stenosis and neural compression necessitating its resection (Safak et al., 2010).

Until recently, the LF was considered as an inactive structure closing the posterior gaps of the vertebral column. However, now it is well known that LF is an active ligament that plays a pivotal role in the biomechanics and integral support of the vertebral column, where it prevents the separation of the vertebral laminae, stops the sudden flexion, and opposes the angulations of the vertebral column, thus preventing hyperflexion and injury to the intervertebral discs (Lamer et al., 2018). The high elastic content preserves the normal curvature in the spine, and helps to return the vertebrae to norposition (Troyer and Puttlitz, Manchikanti et al., 2018).

As far as its biomechanical aspects are concerned, it resists kyphotic angulations with ligaments of the posterior ligamentous combination (Rawls and Fisher, 2018). In posterior lumbar approaches, LF faces some difficulties before risky neural surgeries. Therefore, the spatial relationship of the LF to the surrounding structures provides landmarks to surgeons for their protection. Moreover, degeneration in the LF can lead to nerve compression, obligating its surgical resection (Manchikanti et al., 2018).

Although the anatomical and histological structure of the LF have been much studied in many research works on both in human and animals, especially in the lumbar region (Viejo-Fuertes et al., 2000; Winkler et al., 2007; Lang et al., 2013; Newell et al., 2017; Ahmadi et al., 2019), the comparative detailed structure of this ligament in different regions of the vertebral column is still in need of further analysis. Hence, our aim in this study was to clarify the morphometric and structural differences of the LF at different spinal regions (cervical, thoracic and lumbar) using cadaveric specimens.

#### **MATERIALS AND METHODS**

# Study design

A morphological, observational, cross-sectional, descriptive, comparative, blinded study.

#### Cadaveric materials

Thirty-four formalin-preserved male human cadavers with their ages ranged between 56 and 69 years (mean 62  $\pm$  4 years) were used in this study. They were obtained from the dissection room of the Department of Anatomy, Faculty of Medicine, and stored at -20  $^{\circ}$ C until required for the detailed dissection. None of the cadavers had a history of traumatic injury or local disease, obvious abnormalities as shown in their files and from inspection, and no visible scars in the dorsal were present.

#### Ethical considerations

This study was approved by the biomedical Ethics Research Committee [Reference No 217-19]. The authors have no conflict of interest and no financial or commercial gain from the realization of this study. The material and data collection were performed in the period between January and June 2018.

#### Dissection technique

After putting the cadaver in prone position, the dissection was started by removing the cutaneous flap, with the subcutaneous tissues and superficial nerves. That was followed by removing the several muscular layers; then the different anatomical parts were identified. Upon exposing and removing the muscular layers, the dorsal vertebral arches and the ligamentum flavum were exposed. Vertebral blocks comprising four vertebrae at cervical level (C 3, 4, 5, 6), thoracic level (T 5, 6, 7, 8) and lumbar level (L 2, 3, 4, 5) were removed en bloc. To divide the vertebral blocks into two pieces, an electric saw sectioned the pedicles: the anterior, formed by the intervertebral discs and vertebral bodies, while the posterior consists of the spinous, transverse, articular processes, and the vertebral laminae with the ligamentum flavum.

# Morphological and morphometric studies

For each specimen, the origin and insertion of the LF on the corresponding inferior and superior laminae in addition to the ligaments' relationship to contiguous structures were observed and photographed using digital camera with prime lens (Nikon D300 + Nikkor 50 mm f/1.8). To give the optimal view, an external source of light was used with radio triggering device (Nikon Sb-900), a ruler was photographed with each block to identify the actual scale of pictures, then they were transferred to a photo editor program (Photoshop CS5) to digitalize the ruler. Then, each ligamentum flavum was measured as follows: using a digital vernier caliper (Mitutoyo 500-196-20 & accuracy ± 0.001") to measure the width and height, and using a millimeter ruler to measure the thickness of the ligament. Then, the following measurements were done for each LF including the height, width and thickness using a digital vernier caliper.

## Histological and histometric study

Standard paraffin blocks were prepared from different specimens of ligamentum flavum (middle part) at different spinal levels after decalcification of blocks in 2.5% nitric acid (Okuda et al., 2005). Sections of 5 µm in thickness were cut from the different blocks and were stained with Masson's trichrome and orcein stains. The Masson's trichrome stain was used for detection of the collagen fibers that were stained blue. The orcein stain was used for the detection of elastic fibers, which showed a dark purple color (Cuellar et al., 2017). From each specimen, 5 sections were prepared, which were examined and photographed using a light microscope (Olympus BX53, Tokyo, Japan) with an attached camera (Olympus E-330). Morphological analysis of the slides was performed by blinded morphologists; these specialists evaluated the tissue characteristics; the morphological organization; the height, width and thickness; analysis of collagen fibers and elastic fibers; relative elastic area and relative collagen area.

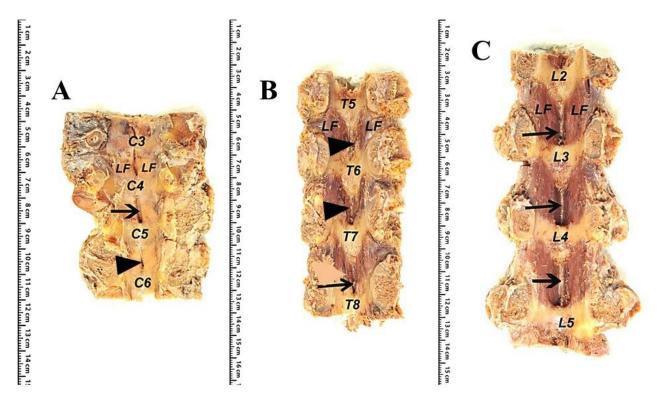
# Morphometric analysis of elastic and collagen fibers

For histometric studies, a semi-quantitative study was used for assessment of thickness and distribution of both fibrous and elastic fibers in LF at different spinal levels (Morales-Avalos et al., 2017a, b). Regarding the collagen fibers, they

were evaluated as follow: few and moderately thick fibers (+), few and thick fibers (++), abundant and moderately thick fibers (+++), abundant and thick (++++). The elastic fibers were evaluated as follow: few and thin fibers (+), few and thick fibers (++), abundant and thin fibers (+++), abundant and thick (++++). Additionally, a densitometric analysis was performed to quantify the proportion of collagen and elastic fibers using ImageJ 1.80. From the images obtained, the dark-red hue of the elastic fibers was manually selected, and the program (with the detection threshold of the control sample precalibrated) converted the interval color to grayscale and the other tissue components to white. Then, the processed images were automatically analyzed to determine the percentage of the area and intensity of the density (Int Dent) of elastic fibers in each sample. This analysis was performed in triplicate (Morales-Avalos et al., 2017a, b).

For the following parameters:

- 1) Relative collagen area was measured within a standard measuring frame of Masson's stained sections. The collagen fibers were selected and masked by red binary colors to be assessed in relation to the area of the standard frame.
- 2) Relative elastic area was evaluated in the above-mentioned way by choosing a frame of a known area in the orcein stained sections, where the elastic fibers were masked by red binary colors, which was measured in relation to the area of



**Fig 1.** Photographs showing the inner surfaces of the laminae of the vertebrae at different levels after coronal sectioning at the pedicle's most anterior aspect. **A:** Ligamentum flavum (LF) of the cervical region at C3, C4, C5 and C6 showing well defined midline gap between C4 and C5 (arrow) and ill-defined one between C5 and C6 (arrow head); **B:** LF of the thoracic region at T5, T6, T7 and T8 showing well defined midline gap between T7 and T8 (arrow) and ill-defined midline gaps between T5 and T6 and between T6 and T7 (arrow head); **C:** LF of the lumbar region at L2, L3, L4 and L5 showing midline gap in all levels (arrow).

the standard frame.

- 3) Collagen area %= relative collagen area/relative elastic area + relative collagen area X100 %.
- 4) Elastic area %= relative elastic area/relative elastic area + relative collagen area X100%.

These measurements were taken from samples of tissue sliced in an axial manner, taking into consideration the mean value of 3 measurements. This analysis was performed in the following manner: high-resolution digital images of eight consecutive sections from the orcein-stained slides were obtained with high dry objective lenses (40X). The color parameters, hue distribution, saturation, and luminance were established in the capture software and were the same for all the images obtained (Morales-Avalos et al., 2017a, b).

# Statistical study

The collected data was statistically analyzed using analysis of variance (one way ANOVA) followed by post hoc Bonferroni test to compare the relative collagen area and relative elastic area of the ligamentum flavum among the different spinal levels using SPSS (V25) statistical program. The data were studied by Kolmogrov Smirnov test for normality. The significance level was considered at P-value <0.05.

#### **RESULTS**

# Morphological and morphometrical observations of the LF at different spinal levels

The observations of the LF at cervical, thoracic and lumbar levels were shown in Fig. 1 where LF in all levels is rectangular that has four borders and two surfaces. It terminates laterally as an anteriorly and vertically oriented edge, while medially it joined with other one at an acute angle forming a posterior projecting tent with apical epidural fatty tissue. Each ligament originates from the superior part of the inferior lamina and minimally attached to the ventral surface. Furthermore, the ligament is attached broadly to the ventral surface of the superior lamina. Thus, the LF displays two parts: a superficial part, in which the fibers are more oblique in its lateral portion close to the intervertebral foramen and directed upwards and medially towards the spine; and a deep part, in which the direction of fibers is craniocaudal. Regarding the LF midline gaps, it was observed that they were existing in the cervical, thoracic and lumbar regions. Also, areas of ossification near the distal attachment of the ligaments were observed in 20% of the thoracic ligaments. In another two lumbar specimens (20%), areas of ossification mostly near the proximal attachment of the ligaments and near their middle were demonstrated.

The parameters of height, width, and thickness of LF at different spinal level are shown in Table 1. The height of the LF increased downward from the

**Table 1.** Morphometric parameters of ligamentum flavum (LF) at different spinal levels.

	Cervical	Thoracic	Lumbar
Height (mm)	$7.19 \pm 3.2^{a,c}$	17.04 ± 1.12 b	19.02 ± 2.06
Width (mm)	11.79 ± 1.25 a,c	15.5 ± 1.44 <sup>b</sup>	16.88 ± 0.46
Thickness (mr	<b>n)</b> $3.35 \pm 0.18^{a,c}$	$7.0 \pm 0.88$ b	10.39 ± 0.56

Results are expressed as Mean±SD, n=80. Significance of differences among groups was evaluated by one-way ANOVA followed by Bonferroni Post Hoc Test.

a- P<0.0001 vs. cervical region. b- P<0.01 vs. lumbar region. c-P<0.0001 vs. lumbar region.

cervical (7.19±3.2 mm), then thoracic (17.04±1.12 mm) and finally lumbar (19.02±2.06 mm) regions; in the same manner, the width of LF increased downward from the cervical (11.79±1.25 mm), then thoracic (15.5±1.44 mm) and finally lumbar (16.88±0.46 mm) regions. Regarding the thickness of LF, it was noticed that there was an increase in the thickness from cervical (3.35±0.18 mm), then thoracic (7.0±0.88 mm), and finally the lumbar (10.39±0.56 mm) regions. There was a statistically significant decrease (P<0.0001) in height, width, and thickness of the ligament in the cervical region when compared to both thoracic and lumbar regions. In addition, there was a statistically significant decrease (P<0.01) in height, width, and thickness of LF in the thoracic region when compared to that in the lumbar region.

# Histological and histometric evaluation of the LF at different spinal levels

The values obtained from the semi-quantitative analysis of the collagen fibers are shown in Table 2, where some differences are observed in the amount and thickness of these fibers, which was apparent in the cervical and lumbar regions. For the elastic fibers that are shown in Table 3, it was observed that the amount and thickness of these fibers were high in the cervical and lumbar regions than in the thoracic region.

Moreover, the examination of the Masson's trichrome stained sections (Fig. 2) showed the arrangement of collagen fibers within the LF at different spinal levels. Most of the collagen fibers were regular and organized in parallel order at different

**Table 2.** Semi-quantitative analysis of collagen fibers of ligamentum flavum (LF) at different spinal levels.

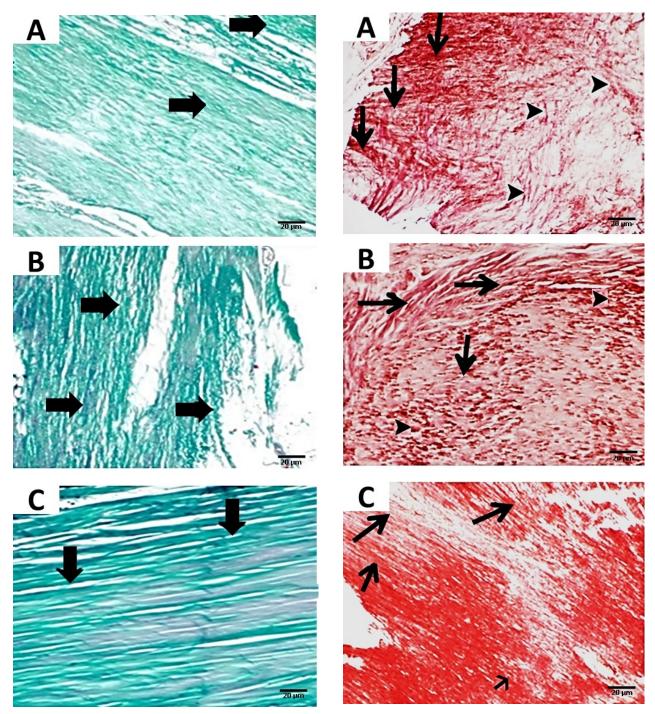
Parameters	Collagen fibers
Cervical	++, +++
Thoracic	+++, ++++
Lumbar	++++

+= few and moderately thick fibers, ++= few and thick fibers, +++= abundant and moderately thick fibers, ++++= abundant and thick.

**Table 3.** Semiquantitative analysis of elastic fibers of ligamentum flavum (LF) at different spinal levels.

Parameters	Elastic fibers	
Cervical	+++	
Thoracic	+, +++	
Lumbar	++, +++	

+ = few and thin fibers, ++ = few and thick fibers, +++ = abundant and thin fibers, ++++ = abundant and thick.



**Fig 2.** Photomicrographs of LF histological sections stained with Masson's trichrome stain. The collagen fibers (arrows) were regular and organized in parallel order at different spinal levels (x40). A: Cervical LF, B: Thoracic LF, C: Lumbar LF (Scale bar: 20μm).

spinal levels, which was apparent in the cervical and lumbar regions.

Examination of the orcein-stained sections (Fig. 3) displayed the order of arrangement of elastic fibers within the LF at different spinal levels. In both cervical and thoracic levels, most of the elastic fibers were diffuse and regular, and their orientation was craniocaudal; however, a variable direction of some fibers was seen in the thoracic re-

Fig 3. Photomicrographs of LF histological sections stained with Orcein stain showing the order of arrangement of elastic fibers, which appeared dark purple (x40). A: Most of the elastic fibers in the cervical region displayed regular, diffuse and organized in parallel order. The orientation of most elastic fibers (arrows) was craniocaudal with variation of some fibers (arrowheads). B: Many elastic fibers in the thoracic region displayed regular and diffuse with craniocaudal orientation (arrows) with variation of some fibers (arrowheads). C: Most of the elastic fibers in the lumbar region were regular and organized in parallel order (arrows) (Scale bar:  $20\mu m$ ).

gions. In the lumbar region, most of the elastic fibers appeared regular and organized in parallel order in many sections.

Table 4. Elastic and collagen fibers of ligamentum flavum (LF) at different spinal levels.

	Cervical	Thoracic	Lumbar
Relative elastic area	126.9 ± 10.7	105.3 ± 4.5 <sup>a,c</sup>	121.9 ± 13.2
Relative collagen area	109.0 ± 10.7 <sup>b</sup>	113.2 ± 2.4	121.0 ± 10.2
Elastic area %	53.9 ± 4.5	$48.2 \pm 2.9$	50.3 ± 4.6
Collagen area %	46.1 ± 4.3	51.8 ± 3.1	± 4.5

Results are expressed as Mean±SD, n=10. Significance of differences among groups was evaluated by one-way ANOVA followed by Bonferroni Post Hoc Test.

As shown in Table 4, the densitometric data showed differences in the LF at different spinal levels. The relative elastic area in the thoracic area was (105.3±4.5), which increased by 15.8% in the lumbar region to be (121.9±13.2), while it increased by 20.5% in the cervical region to be (126.9±10.7). This decrease in the relative elastic area of LF in the thoracic region was statistically significant (P<0.0001) when compared to that in both cervical and lumbar regions. The relative collagen area in the cervical region was (109.0±10.7), which increased by 3.85% in the thoracic region (113.2±2.4), and by 11.01% in the lumbar region (121.0±10.2). This decrease in the relative collagen area of LF in the cervical region was statistically significant (P<0.05) when compared to that in the lumbar region. In the cervical region, the percentage of the elastic area was higher (53.9%) than the collagen area % (46.1%) with a ratio of 1.16:1, this was reversed in the thoracic region, in which the elastic area represents 48.2%, while the collagen area represents 51.8% with a ratio of 0.93:1. In the lumbar region, the percentages of both elastic area and the collagen area were nearly the same (50.3% and 49.7% respectively, with a ratio of 1.01:1).

# **DISCUSSION**

The present study was carried out to compare both gross morphological and the microscopic structure of the ligament flavum at different spinal levels, in order to set a baseline data for different pathologies that affect this ligament. In this study, the gross examination of LF at different spinal levels revealed that they have the same intersegmental pattern of structure and attachments with differences in their dimensions.

The gross examination of the LF revealed that, at the lateral extend of the ligament, it ultimately blends into the capsule of the facet (Shemesh et al., 2018). In each level, it was reported that LF is composed of superficial and deep layers. The superficial part is inserted into the posterosuperior border of the lower lamina, while the deep part is inserted into the anterosuperior surface of the lower lamina. This observation is consistent with the studies of researchers (Abbas et al., 2010; Rahmani et al., 2017) in the thoraco-lumbar region, who found that the fibers of the superficial and

deep laminae are in opposite orders. Also, in accordance to our results, it has been reported that the LF can be divided into: pars interspinalis, near to the spines, pars interlaminar, near to the laminae and constitute the target area for flavotomy, and pars capsularis, near the capsule of the facets (Galbusera and Wilke, 2018).

In the present work, it was found that LF at each level is attached superiorly to the inferior edge and the antero-inferior surface of the lamina above; that is not remarkable in the cervical ligaments, but more notable in the lumbar ligaments. This finding was similar to the observation of others (Shi et al., 2018). However, it was mentioned that ligament upper border is inserted on the intersection between the inferior 2/3 and the superior 1/3 of the internal surface of the nearby superior lamina (Park et al., 2009), and that the upper part of the ligament is attached to the anterior surface of the lamina above (Galbusera and Wilke, 2018).

The dimensions of each LF have been stated to be of great importance. In this study, the height, width, and thickness of LF at each spinal level were measured. It was found that these measurements increased proportionally from the cervical to the thoracic to lumbar ligaments. In accordance, some previous studies have reported that some differences were found among the LF thicknesses at each level and that the thickest LF measurement was at L4-L5 and L5-S1; they suggested that LF thickness is increased by age (Sakamaki et al., 2009; Altinkaya et al., 2011). It was reported that the pathology of thickening of LF is still an object of argument as to whether this thickening is caused by a deformation or hypertrophy of the ligament (Sun et al., 2018; Takashima et al., 2018). Moreover, some reports have stated that the narrowing of the spinal canal is due to hypertrophy of the ligament, whereas other authors claim that structural abnormalities and deformities of LF inside the spinal canal compress the nerve tissues (Safak et al., 2010; Ahmadi et al., 2019).

In the present study, the width of the ligamentum flavum at the cervical, thoracic and lumbar regions was found to be 11.79±1.25, 15.5±1.44 and 16.88±0.46, respectively. In agreement, it has been reported that the average gap from the midline to the lateral extension of the LF in the lumbar region was 17 mm (Rahmani et al., 2017; Ahmadi et al., 2019). Correspondingly, it was reported that

a- P<0.0001 vs. cervical region. b- P<0.05 vs. lumbar region. c- P<0.0001 vs. lumbar region.

the width of LV varies from 12-22 mm (Takahashi et al., 2018). The lateral extension of the LF shows significant pathological changes if hypertrophied, due to the consequent stenosis of the lateral recess and compression of the nerve root (Safak et al., 2010).

In this study, the presence of the LF midline gaps was observed in the cervical, thoracic and lumbar regions. These gaps are tiny slits to permit the passage of veins communicating between the posterior internal vertebral venous plexus with the posterior external vertebral venous (Schmidt et al., 2013). In this study, it was noticed that only 10 % of the lumbar ligaments at some levels featured midline gaps, although the ligaments at lower levels were fused in the midline with absence of gaps. The exact incidence of midline gaps has been controversially discussed. Recent investigation at the cervical, high thoracic and lumbar regions has verified failure of fusion of the ligamentum flavum in the midline, which may reach up to 74% (Sung et al., 2018; Takashima et al., 2018). However, other investigators dissected six cervical columns and found that the ligaments are fused in the mid-line in all cases (Shi et al., 2018). Similarly, on dissection of to 10 lumbar vertebral columns in respectful studies, no evidence of LF midline gaps were found (Rawls and Fisher, 2018). Controversially, another study reported that 50% shows midline gaps in the cervical region (Shemesh et al., 2018). While others found that the fusion in LF midline might be absent to variable degrees (Manchikanti et al., 2018). A vital problem faced by the doctors during the administration of epidural anesthesia is the inability to recognize the epidural space. The LF midline gaps could lead to the failure of detecting the loss of resistance in some patients (Sakamaki et al.,

LF is the most elastic tissue in the human body. In comparison to other spinal ligaments, LF is rich in elastic fibers (Resnick and Sandoval-Garcia, 2017). It was reported that the elastic fibers account for 60% to 70% of the normal ligament dry weight (Qu et al., 2017), and they may reach up to 80 % of the ligament dry weight at young age (Bashkuev et al., 2018). These fibers provide elasticity and give the ligament a high compliance and elastic recoil, and prevent collapsing of this ligament into the spinal canal during extension, which might compress the canal (Park et al., 2009). In the current study, the elastic area % of the LF was found to be 53.9 % in the cervical level, 48.2 % in the thoracic level and 50.3 % in the lumbar level, which means that the average elastic area was highest in the cervical level, while lowest in the thoracic level. Contrary to our findings, researchers reported more elastic fibers in the upper thoracic level compared with that of the cervical level, with more predominant elastic fibers in the lower

spinal levels (Takahashi et al., 2018; Takashima et al., 2018). The ligamentum flavum of the thoracolumbar spine of mammals with greater range of spinal movement contains more elastin than that in animals with less spinal mobility (Okuda et al., 2005).

In this study the collagen area % increased to become 46.1 % in the cervical region, 51.8 % in the thoracic region, and 49.7 % in the lumbar region. Increased amount of collagen fibers is one of the age-related degenerative changes (Okuda et al., 2005). The collagen has very high tensile strength (Cuellar et al., 2017) and its dominance may affect the range of motion of vertebral column and reduce the elasticity of the LF. However, it was reported that the collagen fibers contents in the LF at young age are about 20% (Park et al., 2009). In the current study these ratios deceased to become 1.16:1 in the cervical region, and 1.01:1 in the lumbar region. While in the thoracic region, the ratio was inverted (0.93:1). This decrease of the ratios should be mostly attributed to the aging process and resulted in decreased elasticity and increased stiffness (Kosaka et al., 2007).

Finally, the characterization of LF at different spinal levels will be of great importance for any practical applications in spinal surgery, biomechanical and physical rehabilitation of the vertebral column. It is recognized that a limitation of this study is the use of previously embalmed cadavers, wherein tissue elements could be slightly altered due to the conservation techniques of cadavers compared to fresh or unembalmed samples (Morales-Avalos et al., 2017a, b). However, the evidence suggests that these changes are minimal, and we suggest they do not significantly affect the study results. The immunohistochemical visualization of elastic fibers can be achieved using a monoclonal antibody specific for elastin. Also, it would have been convenient to use Verhoeff's stain.

# **Conclusions**

In conclusion, the current study is a unique work to compare and observe the gross morphological and fine components of the LF at different spinal levels in human cadavers. In general, the LF has the same pattern of attachment in all spinal levels with respect of their dimensions. Its anatomic structure is peculiar, as it consists of two layers with a histological predominance of elastic fibers. Our outcomes confirmed differences in the size, orientation, quantity and distribution of collagen and elastic fibers between the cervical, thoracic and lumbar LF.

#### **REFERENCES**

ABBAS J, HAMOUD K, MASHARAWI YM, MAY H, HAY O, MEDLEJ B, PELED N, HERSHKOVITZ I (2010) Ligamentum flavum thickness in normal and stenotic lumbar spines. Spine, 35(12): 1225-1230.

- AHMADI SA, SUZUKI A, TERAI H, TAMAI K, AKHGAR J, HOSHINO M, TOYODA H, RAHMANI MS, HAYASHI K, OHYAMA S, TAKAHASHI S, NAKAMURA H (2019) Anatomical analysis of the human ligamentum flavum in the thoracic spine: Clinical implications for posterior thoracic spinal surgery. J Orthop Sci, 24(1): 62-67.
- ALTINKAYA N, YILDIRIM T, DEMIR S, ALKAN O, SARICA FB (2011) Factors associated with the thickness of the ligamentum flavum: is ligamentum flavum thickening due to hypertrophy or buckling? Spine, 36 (16): E1093-1097.
- BASHKUEV M, REITMAIER S, SCHMIDT H (2018) Effect of disc degeneration on the mechanical behavior of the human lumbar spine: a probabilistic finite element study. Spine J, 18(10): 1910-1920.
- CUELLAR CA, MENDEZ AA, ISLAM R, CALVERT JS, GRAHN PJ, KNUDSEN B, PHAM T, LEE KH, LAVROV IA (2017) The role of functional neuroanatomy of the lumbar spinal cord in effect of epidural stimulation. Front Neuroanat, 11: 82.
- GALBUSERA F, WILKE HJ (2018) Basic biomechanics of the lumbar spine. In: Biomechanics of the Spine. Elsevier, pp 51-67.
- KOSAKA H, SAIRYO K, BIYANI A, LEAMAN D, YEAST-ING R, HIGASHINO K, SAKAI T, KATOH S, SANO T, GOEL VK, YASUI N (2007) Pathomechanism of loss of elasticity and hypertrophy of lumbar ligamentum flavum in elderly patients with lumbar spinal canal stenosis. Spine, 32(25): 2805-2811.
- LAMER TJ, DICKSON RR, GAZELKA HM, NICHOL-SON WT, REID JM, MOESCHLER SM, HOOTEN WM (2018) Serum triamcinolone levels following cervical interlaminar epidural injection. Pain Res Manag, 2018: 8474127.
- LANG N, YUAN HS, WANG HL, WANG HL, LIAO J, LI M, GUO FX, SHI S, CHEN ZQ (2013) Epidemiological survey of ossification of the ligamentum flavum in thoracic spine: CT imaging observation of 993 cases. Eur Spine J, 22(4): 857-862.
- MANCHIKANTI L, SCHULTZ DM, ATLURI S, GLASER SE, FALCO FJ (2018) Lumbar epidural injections. In: Essentials of Interventional Techniques in Managing Chronic Pain. Springer International Publishing, pp 141-186.
- MORALES-AVALOS R, SOTO-DOMINGUEZ A, GAR-CIA-JUAREZ J, CARDENAS-SERNA M, ESPARZA-HERNÁNDEZ CN, CARREÑO-SALCEDO SA, MON-TES-DE-OCA-LUNA R, LOERA-ARIAS MJ, SAUCEDO-CÁRDENAS O, ELIZONDO-OMAÑA RE, GUZMÁN-LÓPEZ S (2017a) Morphological and histomorphometric evaluation of the ventral rectus sheath of the rectus abdominis muscle, fascia lata and pectoral fascia. The beginning of a morphological information bank of human fascias. Histol Histopathol, 32 (3): 271-282.
- MORALES-AVALOS R, SOTO-DOMINGUEZ A, GAR-CIA-JUAREZ J, SAUCEDO-CARDENAS O, BONILLA-GALVAN JR, CARDENAS-SERNA M, GUZMÁN-LÓPEZ S, ELIZONDO-OMAÑA RE (2017b) Characterization and morphological comparison of human dura mater, temporalis fascia, and pericranium for the cor-

- rect selection of an autograft in duraplasty procedures. Surg Radiol Anat, 39(1): 29-38.
- NEWELL N, LITTLE JP, CHRISTOU A, ADAMS MA, ADAM CJ, MASOUROS SD (2017) Biomechanics of the human intervertebral disc: A review of testing techniques and results. J Mech Behav Biomed Mater, 69: 420-434.
- OKUDA T, FUJIMOTO Y, TANAKA N, ISHIDA O, BABA I, OCHI M (2005) Morphological changes of the ligamentum flavum as a cause of nerve root compression. Eur Spine J, 14(3): 277-286.
- PARK JB, KONG CG, SUHL KH, CHANG ED, RIEW KD (2009) The increased expression of matrix metalloproteinases associated with elastin degradation and fibrosis of the ligamentum flavum in patients with lumbar spinal stenosis. Clin Orthop Surg, 1(2): 81-89.
- QU X, CHEN Z, FAN D, XIANG S, SUN C, ZENG Y, LI W, GUO Z, QI Q, ZHONG W, JIANG Y (2017) Two novel BMP-2 variants identified in patients with thoracic ossification of the ligamentum flavum. Eur J Hum Genet, 25(5): 565-571.
- RAHMANI MS, TERAI H, AKHGAR J, SUZUKI A, TOYODA H, HOSHINO M, TAMAI K, AHMADI SA, HAYASHI K, TAKAHASHI S, NAKAMURA H (2017) Anatomical analysis of human ligamentum flavum in the cervical spine: Special consideration to the attachments, coverage, and lateral extent. J Orthop Sci, 22 (6): 994-1000.
- RAWLS A, FISHER RE (2018) Developmental and functional anatomy of the spine. In: The genetics and development of scoliosis. Springer, pp 1-29.
- RESNICK DK, SANDOVAL-GARCIA C (2017) Neuroanatomy overview: Part II: Spine. In: Handbook of Neurosurgery, Neurology, and Spinal Medicine for Nurses and Advanced Practice Health Professionals. Routledge, pp 45-52.
- SAFAK AA, IS M, SEVINC O, BARUT C, ERYORUK N, ERDOGMUS B, DOSOGLU M (2010) The thickness of the ligamentum flavum in relation to age and gender. Clin Anat, 23(1): 79-83.
- SAKAMAKI T, SAIRYO K, SAKAI T, TAMURA T, OKA-DA Y, MIKAMI H (2009) Measurements of ligamentum flavum thickening at lumbar spine using MRI. Arch Orthop Trauma Surg, 129(10): 1415-1419.
- SCHMIDT RF, GOLDSTEIN IM, LIU JK (2013) Ossified ligamentum flavum causing spinal cord compression in a patient with acromegaly. J Clin Neurosci, 20(11): 1599-1603.
- SENGUPTA DK (2017) Clinical biomechanics of the spine. Spine, 42(7): S3.
- SHEMESH S, SIDON E, KAISLER E, SHEINIS D, VELKES S, OHANA N, BENAYAHU D (2018) Diabetes mellitus is associated with increased elastin fiber loss in ligamentum flavum of patients with lumbar spinal canal stenosis: results of a pilot histological study. Eur Spine J, 27(7): 1614-1622.
- SHI J-G, YUAN W, SUN J-C (2018) Surgical anatomy of the subaxial cervical spine. In: Anatomy Atlas and Interpretation of Spine Surgery. Springer, Singapore, pp 55-132.
- SUN C, WANG Z, TIAN JW, WANG YH (2018) Leptin-

- induced inflammation by activating IL-6 expression contributes to the fibrosis and hypertrophy of ligamentum flavum in lumbar spinal canal stenosis. Biosci Rep, 38(2): BSR20171214.
- TAKAHASHI T, HANAKITA J, MINAMI M (2018) Pathophysiology of calcification and ossification of the ligamentum flavum in the cervical spine. Neurosurg Clin N Am, 29(1): 47-54.
- TAKASHIMA H, TAKEBAYASHI T, YOSHIMOTO M, ONODERA M, OGON I, MORITA T, IESATO N, TERASHIMA Y, TANIMOTO K, YAMASHITA T (2018) The difference in gender affects the pathogenesis of ligamentum flavum hypertrophy. Spine Surg Relat Res, 2(4): 263-269.
- TROYER KL, PUTTLITZ CM (2011) Human cervical spine ligaments exhibit fully nonlinear viscoelastic behavior. Acta Biomater, 7(2): 700-709.
- VIEJO-FUERTES D, LIGUORO D, VITAL JM, ROM-BOUTS JJ (2000) Morphogenesis, anatomy and histology of the ligamentum flavum. Eur J Orthop Surg Trauma, 10(2): 77-83.
- WINKLER PA, ZAUSINGER S, MILZ S, BUETTNER A, WIESMANN M, TONN JC (2007) Morphometric studies of the ligamentum flavum: a correlative microanatomical and MRI study of the lumbar spine. Zentralbl Neurochir, 68(4): 200-204.