COMPARATIVE STUDY OF NORMAL AND DEGENERATED INTERVERTEBRAL DISCS' MECHANORECEPTORS OF HUMAN LUMBAR SPINE BY X-RAY, MAGNETIC RESONANCE AND ANATOMOPATHOLOGIC STUDY

VALDECI MANOEL DE OLIVEIRA[1], EDUARDO BARROS PUERTAS[2], MARIA TERESA DE SEIXAS ALVES[3], HÉLIO KIITIRO YAMASHITA[4]

SUMMARY

The authors conducted a study on human lumbar spine, aiming to assess and determine the different kinds of nervous fibers in normal and degenerated intervertebral discs. Ten cadaver's lumbar spines with approximately 48 – 72 of death have been used. The pieces were submitted to simple X-ray and magnetic resonance tests. Subsequently to the tests, discs were divided into normal and degenerated. Then, they were dissected, divided into anterior and posterior according to the region, included in paraffin and an immunohistochemical study with S100 protein was performed. With the aid of Image-Pro Plus computer software (media cybernetics)®, nervous fibers' diameters were measured as micrometers and classified into four kinds of fibers. Four types of nervous fibers were found on different disc regions. The number and kind of fibers varied according to the region and degree of intervertebral disc degeneration. It was concluded that type-III fibers are more common at the anterior region; type-II and type-IV fibers are more common at the posterior region, and type-I fibers do not show any differences regarding anterior and posterior regions; in addition, a degenerated disc has a higher number of nervous fibers than a normal one.

***Keywords:** Mechanoreceptors; Intervertebral disk; Spine; Comparative study; Magnetic resonance spectroscopy*

***Citation:** Oliveira VM, Puertas EB, Alves MT, Yamashita HK. Comparative study of normal and degenerated intervertebral discs' mechanoreceptors of human lumbar spine by x-ray, magnetic resonance and anatomopathologic study. Acta Ortop Bras. [serial on the Internet]. 2007; 15(1):35-39. Available from URL: http://www.scielo.br/aob.*

INTRODUCTION

Vertebral spine is originated from mesoderm around the fourth week of embryonic life. It develops due to an influence of the notochord and neural tube. The disc initially develops in this environment with few blood vessels and surrounded by perichondral tissue [(1)].

The disc is structurally characterized by three integrated systems: the pulpous nucleus, the fibrous annulus, and the cartilage on vertebral plateau.

Nervous endings found on intervertebral disc have been associated to lumbar pain, were pain path starts with pain receptors' free nervous endings [(2)] (nociceptors). Where the action potential generated is continued on the axon of the small C fibers or A-delta fibers and goes into the dorsal horn of spinal cord, where the first synapses occur. The most important ascending way of pain – the second neuron transmitting the message through spinal cord – on the white substance of the spinal-thalamic tract of the thalamus, where the second synapse occurs. The third neuron continues transmission at the somatosensory cortex in the brain.

The intervertebral disc structure is well defined where we know the origin and composition of the fibrous annulus and pulpous nucleus, as well as its innervation; however, many studies still exist addressing apparently normal and degenerated discs.[(3,4)] Using the immunohistochemical technique, these reported the presence of nervous fibers on innermost layers of the fibrous annulus, as well as the presence of nervous fibers on a degenerated disc's pulpous nucleus.[(3)]

The objective of our study was to make a comparison between the number and kind of nervous endings on normal and degenerated discs, at anterior and posterior planes, identifying the kind of endings according to nervous cells' morphologic constitution and size [(5)] (Table 1).

MATERIALS AND METHODS

Ten fresh adult cadavers (approximately 24 hours after death), between forty and eight-five years old, five females and five males were used.

The whole lumbar spine was removed, from L1 to L5 during necropsy performed at the Death Examining Service, Medical College, University of São Paulo, excluding any cadaver with visible intervertebral disc injury or previous surgery on vertebral spine (Figure 1).

X-RAY METHOD

Weighted images were performed (repetition time 452 and echo time 14) at T1 and T2 (repetition time 3367 and echo time 140). 4-mm sections were made with the aid of a 27-cm F.O.V. (fielder of view). Twelve sections L1 L2 to L5S1 were made (Figures 2 A and 2B), as well as X-ray images at anteroposterior and lateral planes with KV 55/16 (Figure 3).

Discs were classified by using the Pfirmann and cols.' classification in 2001 (Table 2).

Figures 2B represent axial sections of discs L1L2 and L5S1 on weighted images of T1 and T2, respectively, of the anatomic piece.

Study conducted at the Department of Orthopaedics and Traumatology, Department of Pathology, and Department of Diagnostics by Imaging, Federal University of São Paulo – Paulista Medical School.
Correspondences to: Rua Halfeld, 1240, Centro, Juiz de Fora, MG, CEP: 36016-000- Brasil – E-mail; val.ort.@terra .com .br

1 – Associate Professor, Discipline of Anatomy, Medical and Health Sciences College, Juiz de Fora - MG and Substitute Professor of the Discipline of Orthopaedics and Traumatology, Medical College, UFJF – MG. Master, PhD in Sciences by the Department of Orthopaedics and Traumatology, UNIFESP-EPM,
2 – Associate Professor, Head of the Discipline of Traumatology, Department of Orthopaedics and Traumatology, UNIFESP–EPM.
3 – Associate Professor, Head of the Discipline of General, Systemic, Forensic, and Bioethical Pathologic Anatomy, Department of Pathology, UNIFESP–EPM.
4 – Associate Professor, Department of Diagnostics by Imaging, UNIFESP-EPM.

Received in: 04/04/06; approved in: 09/26/06

Figure 3 represents X-ray images at anteroposterior and lateral planes with KV 55/16 for one of the specimens.

HISTOLOGICAL METHOD

Discs were identified, dissected and divided at transverse plane and subdivided into two parts at sagittal plane, each part being identified as anterior and posterior region, according to anatomical references.

After fixation, pieces were dehydrated with increasing concentrations of alcohol, diaphanized in xylol and included in paraffin.

Paraffin blocks containing the matter histologically processed were cooled in a freezer and cut by rotational microtomes, were histological sections 3μm to 4μm thick were obtained by using disposable razors specially designed to this end.

Paraffin ribbons obtained from the cutting process with the microtome were submitted to histological double-boiler and the sections were deposited on slides previously prepared with 3-aminopropyltriethoxisilan (APTS). The slides with sections were taken to stove at 60^{o} C remaining there for 24 hours for a better tissue adhesion.

Technical procedure

- Paraffin removal with heated xylol (60°C) for 15 minutes at room temperature.
- Hydration with alcohol at several strengths ranging from absolute alcohol to vats containing diluted distilled water (100%, 95%, 80%, and 70%, respectively), refreshed at each 30 seconds.
- Wash with tap water.
- Endogenous peroxidase blockage with 6% hydrogen peroxide diluted at 20 volumes in three baths of 5 minutes each, followed

Kind	Morphology	Diameter (μm)	Functional characteristic	Eponyms
I	Globular or ovoid	5-8 (myelinized)	Mechanoreceptor; slow-adapting, low threshold.	Ruffini's, Golgi-Mazzoni's endings, Meissner's corpuscle.
II	Cylindrical or tapered	8-12 (myelinized)	Mechanoreceptor, low threshold, fast-adapting.	Krause's, vater, Vater-Pacine's corpuscles
III	Spindle-like	13-17 (myelinized)	Mechanoreceptor, high threshold, very slow-adapting.	Golgi's endings, Golgi-Mazzoni's corpuscle
IV	a)non-myelinized plexus	2-5 (myelinized)	Pain receptor	-
	Non-myelinized free endings	‹ 2 (non-myelinized)	Pain receptor	-

Table 1 *- Classification of joint nerves, according to Freeman and Wyke.*

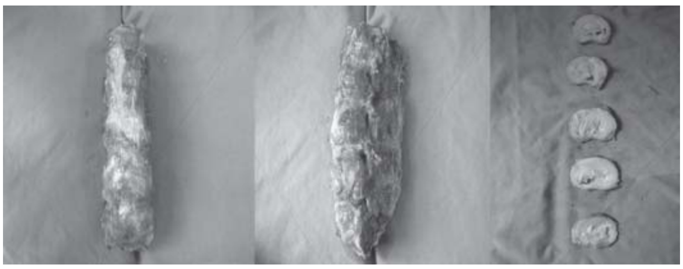

Figure 1 – *Anatomical piece AP and P and discs L1L2 to L5S1.*
Source: Death Examining Service, São Paulo city.

Figure 2A - *NMR: sagittal T1 and T2.*

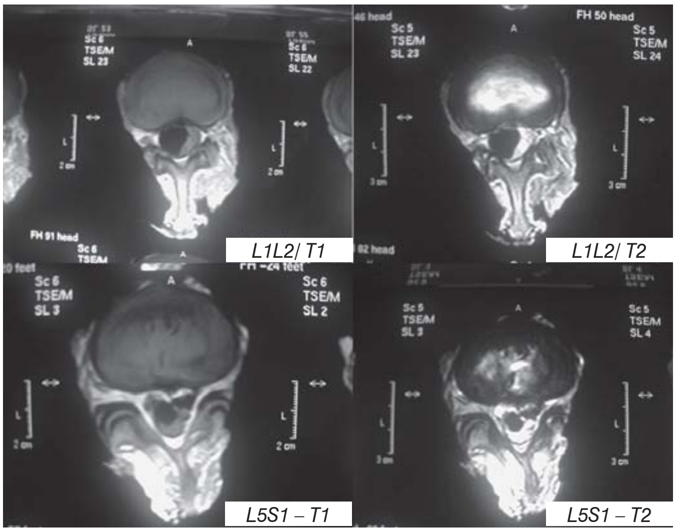

Figure 2B – *Axial sections of discs L1L2 and L5S1 on weighted images of T1 and T2 of the anatomical piece, respectively.*

Figure 3 – *X-ray image: Anteroposterior and lateral planes.*

Kind	Structure	Nucleus	Sign intensity	Disc height
I	Homogenous	Light	Hyper intense	Normal
II	Heterogeneous with horizontal line	Light	Hyper intense	Normal
III	Heterogeneous gray	Not light	Intermediate	Normal
IV-a	Heterogeneous gray	Not light	Intermediate	Reduced
IV-b	Heterogeneous black	Lost	Hypo intense	Reduced
V	Heterogeneous black	Lost	Hypo intense	collapsed

Table 2 – *Classification by Pfirmann and cols.*

by wash with tap water and PBS buffer (10mM, pH 7.4 phosphate-buffered saline solution).
- Primary antibody incubation (diluted in bovine albumin Fraction V Inlab Brasil code 1870 in wet chamber refrigerated at 4·C overnight. The antibody Protein S100 –RxS100 –Dako/Z311/batch 129 (072) titre 1/3000c) was used.
- Wash with PBS solution.
- Incubation DAKO LSAB+KIT, peroxidase code KO690, in two vials:
- 1st vial: Solution DAKO LSAB+ SYSTEM, LINK n°Ko690: ("Biotinylated antimouse, rabbit e goat") for 30 minutes at room temperature.
- Wash with PBS solution.
- 2nd vial: Solution DAKO LSAB+HRP, LINK ("Streptavidin conjugated to horseradish peroxidase") for 30 minutes at room temperature.
- Wash with PBS solution.
- Reaction delivery using substrate solution:
60mg Diaminobenzidine (3,3- Diaminobenzidine Tetrahydrochloride Sigma Chemical CO USA code D5637), 600 μl oxygenated water at 30 volumes, 100ml PBS and 1ml dimethylsulfoxide, leaving slides soaked in this solution for 5 minutes, protected from light, tissue showing a dark brown color, which is the end product of the reaction.
- Wash with tap water.
- Counter-staining using Harris' hematoxylin for 1 minute.
- Slides soaking into ammoniacal water (0.2% ammonium hydroxide solution).
- Wash with tap water.
- Dehydration of sections with absolute alcohol. Slides soaked into Xylol.
- Slide mounted with coverglass and Entellan® (Merck-Germany code 1.07961.0100).
We performed the nervous fibers counting throughout the disc, interactively stained by immunohistochemical method with 400 x magnification, with the aid of a Pro-Image digital analysis system, which consists of a JVC videocamera TK 1180V model, which shows the image captured by Olympus microscope (model BX40 with plan-achromatic objective lenses) to a computer Pentium MMX 233 MHz, 64 Megabytes RAM, mounted with a digitalizing plate and Pro-Image software working in Windows platform.
All nervous fibers were measured for length and width using the Image-Pro R plus software, with specific calibration in micro millimeters. After fibers measurements, averages were calculated and classified according to size and format by following the classification method by Freeman and Wyke[5]. Each measurement was double checked by an experienced pathologist at UNIFESP, expert in bone-muscular diseases. When there was no question among researchers, the numbers found were referred to statistical analysis.
During fibers counting, no researcher was aware of the disc degeneration degree and of the involved region.
Figure 4 shows an intervertebral disc field captured by the computer, as described above. We saw different kinds of nervous fibers stained by immunohistochemical method.
Figure 5 shows the method for measuring nervous fibers to fit the classification by Freeman and Wyke. The smaller fibers, the free nervous endings were directly counted.
Figure 6 shows a photomicrography of an intervertebral disc stained by immunohistochemical method for protein S100. Magnification: 400 x.

STATISTICAL METHOD

By using the software SPSS for Windows (v. 8.0) Statistical Package for Social Sciences, we assessed the differences between anterior and posterior regions compared to the kind of mechanoreceptor and nociceptor for different kinds of normal and degenerated discs. As this was a heterogeneous sample, we used the Chi-Square test and the significant samples were those with Z0, 01= 1,64.

RESULTS

On the ten spines studied, we found nervous fibers of different sizes and shapes. Those fibers were found throughout the disc region in study and in different degeneration degrees. We found significant differences regarding the kind of nervous fiber at different disc degeneration degrees and at normal discs.
In our sample, we found degenerated discs ranging from grade II to IVa. We had two cases where the disc was lying between two degeneration degrees, one between grade two and III, and the other between grade II and IV. We considered, then, the highest degeneration degree. We didn't find any disc with degeneration grade IVb or V in our sample. Table 3 shows the different degrees.
We studied the differences between posterior and anterior regions to the kind of nervous fiber (Table 4).
H0: kind of ending does not depend on the region
H1: kind of ending depends on the region
On Table 4, there are six significant residuals. By combining this result with the percentages found, the overall conclusion is that type-II nervous fibers are more frequently found at posterior region (51.7%) and less common at anterior region (48.3%). For type-III nervous fibers, the incidence is higher at anterior region (54,5%) and less common at posterior region (45.5%). For type-IV nervous fibers, the incidence is higher at posterior region (55.2%) and less common at anterior region (44.8%). For type-I nervous fiber, the percentage of occurrences does not differ significantly for different anterior or posterior regions from those seen for total sample.
By assessing Table 5, the null hypothesis **H0** was ruled out ($p<<<0.1$), therefore, in the 90% confidence interval and by the Pearson's chi-square test, there are evidences showing that the kind of ending is correlated to the region.
The existence of correlation between degeneration degree and kind of nervous fiber regarding anterior or posterior regions has also been determined (Table 6).
H0: degeneration degree does not depend on the kind of nervous fiber.
H1: degeneration degree depends on the kind of nervous fiber.
On Table 6, eight significant residuals are described. By combining this result with the percentages found, the overall conclusion is that type-I nervous fiber is more frequently found in IVa-type degenerated discs (20.8%) and less common in type-I degenerated discs (6.3%). For type-II nervous fiber, the incidence is higher in type-IVa degenerated discs (19.9%) and less common in type-I degenerated discs (9.3%). For type-III nervous fiber, the incidence is higher

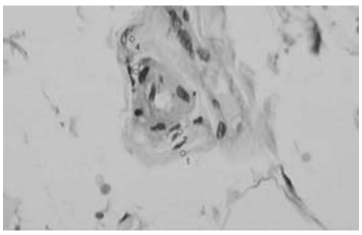

Figure 4 - *(Fibers on the anterior region of L5) type-IV fibers.*
Source: Department of Pathology, UNIFESP.

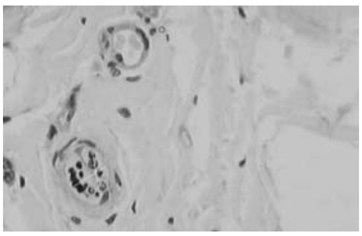

Figure 5 - *(Fibers on the anterior region of L5) type-IV and III fibers.*
Source: Department of Pathology, UNIFESP.

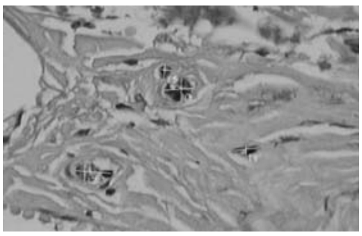

Figure 6 - *(Fibers on the posterior region of L2) type-I and II fibers.*
Source: Department of Pathology, UNIFESP.

Patient	Gender	L1/L2	L2/L3	L3/L4	L4/L5	L5/ SI
1	F	III	III	III	II	III
2	F	II	II	II	III	II
3	F	III	II	III	II	II
4	M	IVA	IVA	IVA	IVA	IVA
5	F	I	I	II	II	III/IVA
6	M	II	II	III	II	IVA
7	M		III	II	II	III
8	M	III	III	III	II	IVA
9	M	II	II	II/III	III	IVA
10	F	I	I	I	I	I

Source: Department of X-ray Imaging, UNIFESP- 2004.

Table 3 – *Represents abbreviated names, gender and degeneration degree for each intervertebral disc.*

			TN				
			One	Two	Three	Four	Total
REGION	anterior	Count	1547	921	911	5514	8893
		Expected Count	1515,1	885,5	775,9	5716,5	8893,0
		% within REGIÃO	17,4%	10,4%	10,2%	62,0%	100,0%
		% within TN	47,4%	48,3%	54,5%	44,8%	46,4%
		% of Total	8,1%	4,8%	4,8%	28,8%	46,4%
		Adjusted Residual	1,2	1,7	6,9	-6,1	
	posterior	Count	1716	986	760	6797	10259
		Expected Count	1747,9	1021,5	895,1	6594,5	10259,0
		% within REGIÃO	16,7%	9,6%	7,4%	66,3%	100,0%
		% within TN	52,6%	51,7%	45,5%	55,2%	53,6%
		% of Total	9,0%	5,1%	4,0%	35,5%	53,6%
		Adjusted Residual	-1,2	-1,7	-6,9	6,1	
Total		Count	3263	1907	1671	12311	19152
		Expected Count	3263,0	1907,0	1671,0	12311,0	19152,0
		% within REGIÃO	17,0%	10,0%	8,7%	64,3%	100,0%
		% within TN	100,0%	100,0%	100,0%	100,0%	100,0%
		% of Total	17,0%	10,0%	8,7%	64,3%	100,0%

Table 4 – *Shows the existence of difference or not between anterior and posterior region regarding the kind of nervous fiber.*

in type-III degenerated discs (33.2%) and less common in type-I degenerated disc (10.2%). For type-IV nervous fiber, the incidence is higher in type-III degenerated discs (37.4%) and less common in type-IVa degenerated disc (18.3%). For the remaining cases, the percentage of occurrences regarding the kind of nervous fiber does not significantly differ for different degrees of degeneration (one or four) from that seen for total sample.

By assessing Table 7, the null hypothesis **H0** was ruled out (p<<<0.1), therefore, in the 90% confidence interval and by the Pearson's chi-square test, there are evidences showing that the degree of degeneration is correlated to the kind of ending.

DISCUSSION

Our study shows a significant difference between the kind and the number of nervous fibers in normal and degenerated discs. We employed magnetic resonance imaging test to differentiate the different disc degeneration degrees, assessing both qualitatively and quantitatively, the kinds and numbers of existing nervous fibers. Some authors calculated those differences only by assessing macroscopic aspects [6].

The magnetic resonance imaging shows that this is a highly efficient method for differentiating disc changes, enabling to detect degeneration at its early phases. Magnetic resonance shows an evidence of disc degeneration even if the X-ray shows a normal image; however, the meaning of these early changes is still unclear [7]. Changes on signal intensity at the resonance correspond to biochemical changes [7].

Some authors have assessed cadavers and patients, and they correlated discography, macroscopic anatomy, and magnetic resonance, where this method was shown to be efficient in detecting early changes on intervertebral discs [8].

According to some authors, magnetic resonance is an efficient method for detecting disc degeneration, but, for assessing if the disc is painful or not, the use of discography is recommended [3,9].

Visibility of nervous fibers was greatly improved by immunohistochemical staining. Many kinds of stains have been used with this objective. Silver nitrate has been used by some authors [2,11-13], but

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	61,205 ª	3	,000
Odds Ratio	61,077	3	,000
Linear-by-Linear Association	13,817	1	,000
N of Valid Cases	19152		

Table 5 – *Shows the correlation between the kind of ending and region according to Pearson's test.*

			TN				
			one	two	three	four	Total
DD	one	Count	206	178	170	922	1476
		Expected Count	251,5	147,0	128,8	948,8	1476,0
		% within GD	14,0%	12,1%	11,5%	62,5%	100,0%
		% within TN	6,3%	9,3%	10,2%	7,5%	7,7%
		% of Total	1,1%	,9%	,9%	4,8%	7,7%
		Adjusted Residual	-3,3	2,8	4,0	-1,5	
	two	Count	1186	678	588	4529	6981
		Expected Count	1189,4	695,1	609,1	4487,4	6981,0
		% within GD	17,0%	9,7%	8,4%	64,9%	100,0%
		% within TN	36,3%	35,6%	35,2%	36,8%	36,5%
		% of Total	6,2%	3,5%	3,1%	23,6%	36,5%
		Adjusted Residual	-,1	-,9	-1,1	1,3	
	three	Count	1191	671	555	4608	7025
		Expected Count	1196,9	699,5	612,9	4515,7	7025,0
		% within GD	17,0%	9,6%	7,9%	65,6%	100,0%
		% within TN	36,5%	35,2%	33,2%	37,4%	36,7%
		% of Total	6,2%	3,5%	2,9%	24,1%	36,7%
		Adjusted Residual	-,2	-1,4	-3,1	2,9	
	four	Count	680	380	358	2252	3670
		Expected Count	625,3	365,4	320,2	2359,1	3670,0
		% within GD	18,5%	10,4%	9,8%	61,4%	100,0%
		% within TN	20,8%	19,9%	21,4%	18,3%	19,2%
		% of Total	3,6%	2,0%	1,9%	11,8%	19,2%
		Adjusted Residual	2,7	,9	2,5	-4,1	
Total		Count	3263	1907	1671	12311	19152
		Expected Count	3263,0	1907,0	1671,0	12311,0	19152,0
		% within GD	17,0%	10,0%	8,7%	64,3%	100,0%
		% within TN	100,0%	100,0%	100,0%	100,0%	100,0%
		% of Total	17,0%	10,0%	8,7%	64,3%	100,0%

Table 6 – *Shows the existence of correlation or not between degeneration degree and kind of nervous fiber associated to anterior and posterior regions.*

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	53,515 ª	9	,000
Odds Ratio	52,395	9	,000
Linear-by-Linear Association	6,562	1	,010
N of Valid Cases	19152		

Table 7 – *Shows that degeneration degree might be correlated to the kind of ending.*

only three of them found nervous endings at fibrous annulus. The author attributed the absence of fibers visibility to decalcification, where this destroys nervous fibers [14].

Cholinesterase has also been successfully used by several authors, where they noticed nervous fibers on the outer layer of the intervertebral disc [10-12, 15].

In our environment, we use the immunohistochemical method to stain nervous fibers with a brownish color, allowing for a good visibility [16-18]. In literature, this methodology has also been used by a number of authors [3, 4,7, 9,19-22] .

On intervertebral disc, non-myelinized fibers [15,23] and myelinized fibers[24,25] were found, leading Pedersen to determine that smaller fibers were associated to pain and the bigger fibers were associated to proprioception.

According to the classification by Freeman and Wyke, fibers with two to seventeen microns in diameter are myelinized, and those measuring less than two microns are non-myelinized. In our study, we found fibers with different diameters, a finding that is similar to other authors'.

Groen et al.[15] reported that the anterior longitudinal ligament was innervated by ventral plexus and sympathetic stem. The dorsal plexus, formed by sinuvertebral nerve fibers, innervates the posterior longitudinal ligament.

Kojima et al.[27] suggested that the fibers could be correlated to posture and motion regulation; according to Roberts et al.[28] , posture-related fibers are equal to Pacini's corpuscle and Ruffini's endings, and the Golgi's tendons are related to pain.

We found fibers similar to those mentioned by Roberts et al.[28] and concluded that those fibers are found in every kind of disc, regardless of its degeneration degree; however, we noticed that pain-related fibers are more frequent at disc's posterior region and at early phases of disc degeneration, grade III as per Pfirmann et al.[6], and balance-related fibers were most frequently found in more severely degenerated discs, regarded as Pfirmann's grade IVa.

The P substance has been studied by many authors, being found at posterior longitudinal ligament [24], around fibrous annulus surface[7,21,23,26], , and at the outer and inner layers of the pulpous annulus[3]. As staining quality and kind are improved, new discoveries about disc innervation are made. In the past, authors reported that there were no nervous endings at the fibrous annulus and pulpous nucleus, but at anterior and posterior longitudinal ligaments[13],. Other authors reported that the disc was poorly innervated[26]. However, the majority of authors found nervous endings on the outer surface of the fibrous annulus [4-12, 18,19 ,24, 25,27 ,28]

Some authors showed an increased number of fibers inside degenerated discs when compared to normal discs [4,7,9],. Freemont et al.[3] not only demonstrated the presence of these fibers on outer and inner layers but also at pulpous nucleus. William et al.[29] concluded that, in disc degeneration, an increased number of blood vessels exist, which contributes to a larger number of nervous endings inside discs.0

Oliveira et al.[18] conducted a quantitative study addressing the anterior and posterior regions of human intervertebral disc, finding a statistically significant difference between anterior and posterior regions. Fagan et al.[30] have also conducted a quantitative study, using sheep and comparing the fibrous annulus region and the cartilage of the vertebral plateau, finding no differences in these regions. Both agree that the disc is very well innervated and that those fibers show some correlation with discogenic pain.

We found that the mechanoreceptors and nociceptors play an important role in different disc degeneration phases, constituting a warning sign for pain or instability.

CONCLUSIONS

1) Fast-adjusting and low excitability threshold mechanoreceptors, as well as nociceptors, are significantly more frequent at posterior region. Very slow-adjusting and high excitability threshold mechanoreceptors are statistically more frequent at anterior region.

2) On discs L1-L2, nociceptors are more frequent when compared to other discs.

3) On degenerated discs, more mechanoreceptors and nociceptors are found when compared to normal discs.

REFERENCES

1. Humzah MD, Soames RW. Human intervertebral disc: structure and function. Anat Rec. 1988; 220:337-56.
2. Cavanaugh JM, Kallakuri S, Ozaktay AC. Innervartion of the rabbit lumbar intervertebral disc and posterior longitudinal ligament. Spine. 1995; 20:2080-5.
3. Freemont AJ, Peacock TE, Goupille P, Hoyland JA, O´Brien J, Jayson, MIV. Nerve ingrowth into diseased intervertebral disc in chronic back pain. Lancet. 1997; 350:178-81.
4. Grönblad M, Weinstein JN, Santavirta S. Immunohistochemical observations on spinal tissue innervation. A review of hypothetical mechasms of back pain. Acta Orthop Scand. 1991; 62:614-22.
5. Freeman MAR, Wyke B. The innervation of the knee joint. An anatomical and Histological study in cat. J Anat. 1967; 101:505-32.
6. Pfirmann C, Metzdorf A, Zanetti M. Magnetic resonance classification of lumbar intervertebral disc degeneration. Spine. 2001; 26:1873-8.
7. Palmgren T, Grönblad M, Virri J, Kääpä E, Karaharju E. An immunohistochemical study of nerve structures in the anulus fibrosus of human normal lumbar intervertebral discs. Spine. 1999; 24:2075-9.
8. Ehrenhaft JL. Development of the vertebral column as related to certain congenital and pathological changes. Surg Gynecol Obstet. 1943; 76:282-92.
9. Ejnisman B. Estudo imunohistoquímico dos mecanorreceptores do ligamento glenoumeral inferior em cadáveres humanos [tese]. São Paulo: Universidade Federal de São Paulo, Escola Paulista de Medicina; 2002.
10. Bogduk, N, Tynan W, Wilsom AS. The nerve supply to the human lumbar intervertebral disc. J Anat. 1981; 132:39-56.
11. Bogduk N, Windsor M, Inglis A. The innervation of the cervical intervertebral discs. Spine. 1988; 13:2-7.
12. Jackson HC, Winkelmam RK, Bickel WM. Nerve endings in the human lumbar spinal column and related structures. J Bone Joint Surg Am. 1966; 48:1272-81.
13. Jung A, Brunschwig A. Recherches histologiques sur l□innervation des articulations des corps vertebraux. Presse Med. 1932; 40:316-7.
14. Wiberg G. Back pain in relation to nerve supply of intervertebral disc. Acta Orthop Scand. 1949, 19:211-21.
15. Groen GJ, Baljet B, Drukker J. Nerves and plexuses of the human vertebral column. Am J Anat. 1990; 188:282-96.
16. Coppes MH, Marani E, Thomeer RT, Groen GT. Innervation of " painful" lumbar dics. Spine. 1997; 22:2342-9.
17. Hovelacque A. Le nerf sinuvertebral. An Anat Pathol Med Chir. 1925; 2:435-43.
18. Oliveira VM. Estudo das terminações nervosas dos discos intervertebrais da coluna lombar de humanos [tese]. São Paulo: Universidade Federal de São Paulo, Escola Paulista de Medicina; 2001.
19. Ahmed M, Bjurholm A, Kreicbergs A, Schultzberg M. Neuropeptide Y, Tyrosine Hydroxylase and Vasoactive Intestinal Polypepatide-Immunoreactive Nerve Fibers in the Vertebral Bodies, Discs, Dura Mater, and Spinal Ligamentes of the Rat Lumbar Spine. Spine. 1993; 18:268-73.
20. Kojima Y, Maeda T, Arai R, Shichikawa K. Nerve supply to the posterior longitudinal ligament and the intervertebral disc of the rat vertebral column as studied by acetylcholinesterase histochemistry. II. Regional differences in the distribuition of the nerve fibres and their origins. J Anat. 1990; 169:247-55.
21. Malinsky J. The ontogenenetic development of nerve terminations in the intervertebral disc of man. Acta anat. 1959; 38: 96-113
22. Jessel TM, Kelly DD. Pain and analgesia. In: Kandel ER, Schwartz JH, Jessel TM eds. Principles of neural science. 3rd ed. Norwalk, CT: Appleton and Lange; 1966. p.385-99.
23. McCarthy PW. Sparse substance P-like immunoreactivity in intervertebral discs. Acta Orthop Scand. 1993; 64:664-8.
24. Korkala O, Grönblad M, Liesi P, Karaharju E. Immunohistochemical demonstration of nociceptores in the ligamentous structures of the lumbar spine. Spine. 1985; 64:156-7.
25. Pedersen HS, Blunck CF, Gardner E. The anatomy of lumbosacral posterior rami and meningeal branches of spinal nerves (sinus-vertebral nerves). J Bone Joint Surg Am. 1956; 38:377- 91.
26. Ashton IK, Walsh DA, Polak JM, Eisenstein SM. Substance P in intervertebral discs. Acta Orthop Scand. 1994; 65:635-9.
27. Kojima, Y, Maeda, T, Arai, R, Shichikawa, K. Nerve supply to the posterior longitudinal ligament and the intervertebral disc of the rat vertebral column as studied by acetylcholinesterase histochemistry. I. Distribution in the lumbar region. J Anat. 1990; 169:237-46.
28. Roberts, S, Eisenstein S M, Menage J, Evans EH, Ashton IK. Mechanoreceptors in intervertebral disc. Morphology, distribution and neuropeptides. Spine. 1995; 20:2645-51.
29. Willam, EBJ, Helena E, Janis, M, Stephen, ME, Alicia, EH and Sally R (2001) Immunohistochemical Detection of Schwann Cells in Innervated and Vascularized Human Intervertebral Discs. Spine, 26(23): 2550-2557
30. Fagan A, Moore R, Roberts BV, Blumbergs P, Fraser R. The innervation of the intervertebral disc: a quantitative analysis. Spine. 2003; 28:2570-6.