Annals of the University of Oradea, Fascicle: Ecotoxicology, Animal Husbandry and Food Science and Technology, Vol. XVII/B 2018

Analele Universitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara, Vol.XVII/B 2018

EVIDENCE OF BIOLOGICAL AND BIOCHEMICAL ASPECTS FROM INTERVERTEBRAL DISORDERS

Moga Ioana*, MogaTitus*, Rahota Daniela*, Holt Gineta*, Beiuşan Corina*

Faculty of Medicine and Pharmacy, University of Oradea,Str. Piața 1 Decembrie, nr.10, Oradea , Romania
ioana.moga@yahoo.com, titusmoga@gmail.com
Department of morphological disciplines

Abstract

Although it is one of the most common and studied intervertebral disc lesions, lumbar disc herniation raises some questions today about its etiology and generates discussions about early diagnosis, therapeutic attitudes, and the functional impact on quality of life. Clinical manifestations are inconsistent, unpredictable; their good tolerance is generally known, often the practitioner having the opportunity to highlight a net discartosis without the patient experiencing the slightest sensation. These diseases remain clinically latent in most cases, their being highlighted by an acute clinical onset of lumbar or lumboscalia. Correct and early diagnosis is the first condition for effective treatment. That is why it is necessary to evaluate each case using a hierarchy system to classify the severity of the lesions. Thus, the staging and classification system used becomes a real "thinking tool" that helps us find the way to the right treatment faster. In this paper we wanted to achieve a biological staging by electron microscopy study of intervertebral disc lesions obtained on intraoperative material taken.

Key words: intervertebral disc, discectomy, collagen in the vertebral disc, electron microscopy

INTRODUCTION

Lumbar disc herniation is a disease primarily affecting young people in full activity.

The multitude and complexity of these cases and unsatisfactory therapeutic results have led us to deepen this pathology of the intervertebral disc.

A first objective of this paper is to find a more precise and safe way to diagnose and treat this pathology.

The complexity of the biological and biochemical, histochemical, immunological aspects of the intervertebral disc, and the complexities of its molecular biology, justify the subject of our study.

In this research we wanted to elucidate and demonstrate issues related to:

• biological changes (histological and biochemical) produced in intervertebral discs taken during surgical interventions performed in the patients of the study group.

Early diagnosis of disc hernias by the MRI method and their biological-imagistic staging allow the therapeutic intervention to be prompt and appropriate by resorting to the latest minimal-invasive and biological surgical procedures.

MATERIAL AND METHOD

47 intervertebral discs obtained postoperatively from the patients operated in the Neurosurgery Department of the Oradea County Clinical Hospital between December 2016 and January 2018 were studied. The histopathological study was performed in the Pathology Anatomy Laboratory of the mentioned hospital, as well as at the level Department of Cell Biology and Histology of the Faculty of Medicine and Pharmacy of the University of Oradea. Also, collagen determinations were performed in the intervertebral disc (DIV) by electronic microscopy at the Electronic Microscopy Center of "Babeş Bolyai" University of Cluj Napoca.

Intervertebral discs were obtained by discectomy during surgical interventions performed at the Neurosurgery Clinic of the Oradea County Clinical Hospital.

Extraction and processing of disc material

We collected intervertebral disc fragments from 47 patients who were operated for lumbar disc herniation between December 2016 and January 2018, aged between 22 and 54 years, with an average of 42.2 years.

The sex structure of the patients studied was as follows:

• 31 men (65%) with an average age of 45.3 years;

• 16 women (35%) with an average age of 39.6 years.

As a hernia localization:

- level L3 - 2 cases (4.3%)
- L4 level - 30 cases (63%)
- L5 level - 15 cases (32.6%)

Both the sex structure and the hernia localization level are very close to the proportion of the studied group and the total number of operations in recent years.

Histopathological and electronic microscopy study

The histological material was fixed in paraffin; the sections were stained with hematoxylin-eosin using the objective microscope 10.

Photographs of the blades were performed under a microscope using the XIO Polaroid device. The electron microscopy study was performed at

the Electronic Microscopy Center of the Babes Bolyai University, Cluj Napoca. The electronic microscopy study wanted to analyze the collagen types of the intervertebral disc obtained by discectomy. These determinations sought quantitative and qualitative changes of the six types of collagen (I, II, III, V, VI, IX) in the pulp nucleus, the fibrous ring and the intervertebral disc vertebral plates. Fragments of the 47 disks studied were examined.

Determination of the different types of collagen performed at the electron microscope was performed by measuring the distinct chains and the mass of the collagen fibers chains as can be seen in table 1.

Table 1

Collagen types detected by electronic microscopy

The collagen type	No. of distinct planes	Distinctive chains	Table of chains
TYPE I	**2** **1**	**Alfa 1** **Alfa2**	**95** **95**
TYPE II	**1**	**Alfa 1**	**95**
TYPE III	**1**	**Alfa 1**	**95**
TYPE IV	**2** **1**	**2** **1**	**170-185**
TYPE V	**3**	**Alfa 1** **Alfa2** **Alfa 3**	**130-200**
TYPEVI	**3**	**Alfa 1** **Alfa 2** **Alfa 3**	**140-200**
TYPEVII	**1**	**Alfa 1**	**170**
TYPE VIII	**1**	**Alfa 1**	**150**
TYPE IX	**3**	**Alfa1** **Alfa 2** **Alfa 3**	**85**
TYPE X	**1**	**Alfa 1**	**59**
TIP XI	**3**	**Alfa 1** **Alfa 2** **Alfa 3**	**100**

Objectives

Biochemical study of the intervertebral disc:

1. determination of glycosaminoglycans and collagen in the intervertebral disc components
2. determination of chondroitin sulfate and keratosulfate as degradation products of glycosaminoglycans
3. highlight the modification of the keratosulfate / chondroitinsulfate ratio

4. Detection of pulmonary nucleus metacromasis reduction.

The research was conducted within the Department of Biochemistry of the Faculty of Medicine and Pharmacy Oradea, using the adsorption chromatography method on paper and column.

The determinations were performed on intervertebral disc fragments obtained by discectomy.

RESULT AND DISCUSSION

The histopathological changes identified were as follows, shown in Table 2

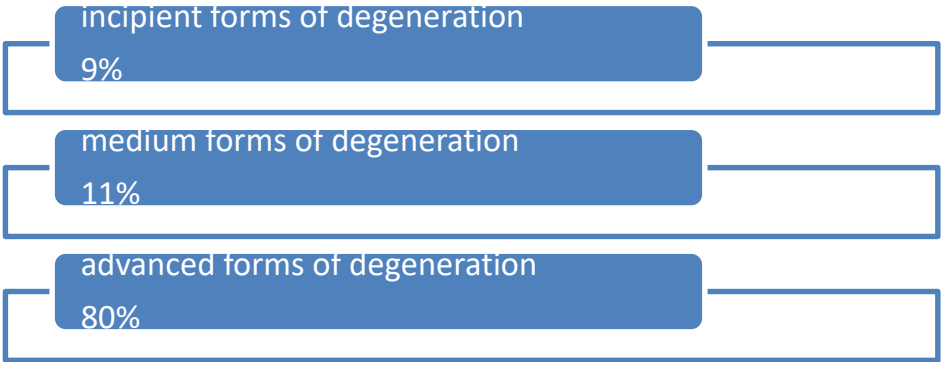
In 4 patients (8.5%) incipient forms of degeneration of the intervertebral disc, examining aspects of mixedoid degeneration, central condroplasts with chondrocytes.

Five patients (10.63%) in the middle forms of intervertebral disc degeneration were found: fibrous disc tissue with interfibrillation edema; cartilaginous disc tissue with chondrocytes in condroplasts, papillary and hemorrhagic areas; dissected cartilage tissue with hyalinization zones

In 38 patients (80.85%) in the advanced forms of degeneration of the intervertebral disc it was discovered: Hyalinated disc cartilage +/- intradiscal calcifications or ossification zones; Chronic inflammatory infiltration at the level of the disc cartilage can be seen in cartilage with intradiscal calcifications Fig. 1 and 2.

Table 2

The histopathological changes identified



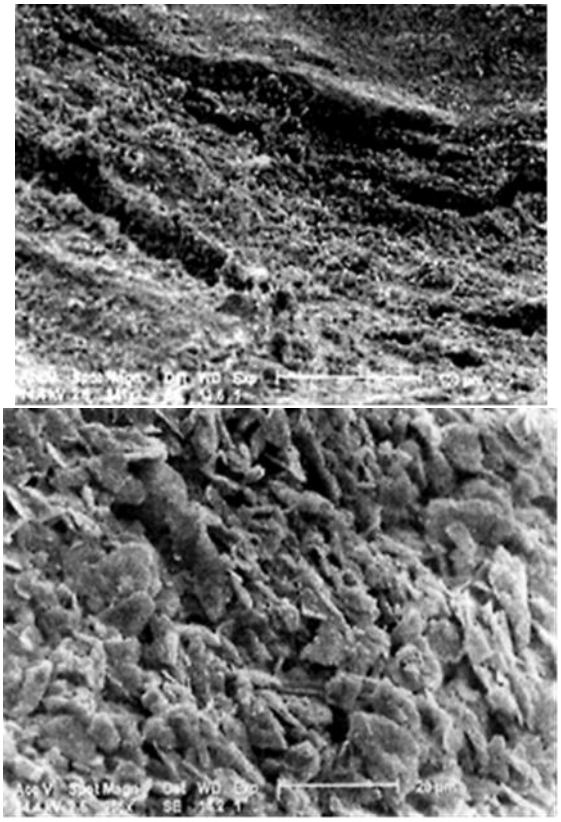


Fig. 1. Intervertebral disc fragment studied by electronic microscope (transmission method)

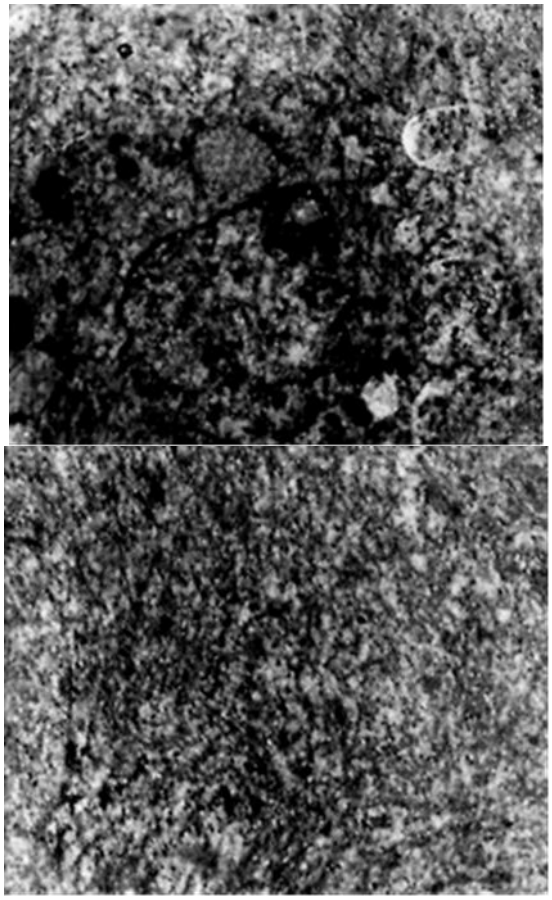


Fig. 2. Fragment of the intervertebral disc studied in the electronic microscope (scanning method)

After analyzing the 47 pieces obtained from the intervertebral discs, the following quantitative and qualitative changes were highlighted at the level of the three anatomical structures.

In type I MRI disc dislocations
(4 cases) highlighted the following aspects:

• minimal absence / increase of collagen I and II in pulpos nucleus
• Growth with a slight change of collagen I, II, III, IV in the fibrous ring
• the absence of collagen I in the vertebral plates and the presence of Type II collagen in the vertebral plates

In type II MRI disc dissections, (5 cases) the following changes were noted:

• the growth of type III collagen in the heart nucleus
• Moderate presence of type V collagen, much better represented by type III collagen in the fibrous ring
• the presence of collagen type III, V, VI on the vertebral plates

In the discarding lesions corresponding to MRI type III (38 cases) the following data were found:

• Decrease of all types of collagen in cartilage plaques with the presence of the six types of collagen in the pulp nucleus and the fibrous ring with attenuation and disappearance of proteoglycans. At this stage there are a number of changes (degradation, collagen cracks)

The analysis of biochemical composition of the intervertebral disc revealed:

- in 9 cases (19.14%) the presence of keratosulfate and condrointinsulphate in approximately equal percentages.
- In 32 cases (68.08%), a nearly 2/1 increase of keratosulfate was noted.
- In 6 cases (12.76%), an increase of up to 3/1 of keratosulfate was recorded in favor of condrointin sulphate.

Also, in cases of category 2 and 3, the reduction of intervertebral disk intervertebral disc metacromatics was noted.

CONCLUSSION

As a result of the research, the "simple" aging of the degenerate intervertebraldisc is accompanied by a slight decrease in mucopolysaccharides, from 6.5% dry weight to 20 years, to 5.5% at 60 years after they showed a maximum 9% to 40years and the increase in collagen content by one third to the third stage of degeneration. Collagen content was also increased by one-third to the third stage of degeneration.

The finding that keratosulfate / chondroitinsulphate ratio increases with the aging of the nucleus corresponds to literature data. I will present the composition of collagen in the intervertebral disc and the distribution of different types depending on the degenerative phase: 1. In 5 cases (10.63%), the following changes were noted in the early degeneration: the intervertebral disc contains predominantly collagen:

- type I outer layer of fibrous ring
- Type II in the nucleus and cartilage plates

2. In 13 cases (27.65%), the second type of degeneration, the following changes were highlighted;

- increases the concentration of normal collagen in the normal distribution areas
- increases the collagen content of types III, V and VI in the nucleus
- increases the type I collagen content in the fibrous ring

3. In 29 cases (57.44%), the third type of degeneration, the following changes were noted:

- type I collagen in the nucleus as type IV and X collagen, suggesting phenotypic changes of the chondrocytes in the pulpos nucleus

- type II collagen disappears from cartilage plaques. From a biochemical point of view, the degeneracy of the intervertebral disc is expressed by the depolymerization of proteoglycans. In this research we were able to determine MPZ degradation products (keratosulfate and chondroitinsulfate), kerato / chondroitinsulphate ratio change, pulmonary nucleus metacromasis increase and different types of collagen. Collagen changes are broadly consistent with those described by the electronic microscopy method, but not by its accuracy.

REFERENCES

1. Boos N, Rieder R, Schade V, Spratt KF, Semmer N, Aebi M., 1995, The diagnostic accuracy of MRI. Spine;20:2613–2625
2. Costello RF, Beall DP., 2007, Nomenclature and standard reporting terminology of intervertebral disk herniation. Magn Reson Imaging Clin N Am. 2007;15 (2): 167-74, v-vi. doi:10.1016/j.mric.2006.12.001
3. Cribb GL, Jaffray DC, Cassar-Pullicino VN., 2007, Observations on the natural history of massive lumbar disc herniation. J Bone Joint Surg Br.;89:782–4.
4. Erly WK, Munoz D, Beaton R, 2006, Can MRI signal characteristics of lumbar disc herniations predict disc regression? J Comput Assist Tomogr.;30:486–489.
5. Fardon DF, Williams AL, Dohring EJ et-al., 2014, Lumbar disc nomenclature: version 2.0: Recommendations of the combined task forces of the North American Spine Society, the American Society of Spine Radiology and the American Society of Neuroradiology. Spine J.;14 (11)
6. Kamei G, Kobayashi T, Ohkawa S, Kongcharoensombat W,Adachi N, Takazawa K, Shibuya H, Deie M, Hattori K, Goldberg JL, Ochi M., 2013, Articular cartilage repair with magnetic mesenchymal stem cells. Am J Sports Med;41(6):1255–64.
7. Koike Y, Uzuki M, Kokubun S, Sawai T., 2003, Angiogenesis and inflammatory cell infiltration in lumbar disc herniation. Spine;28:1928–33.
8. McGraw-Hill, 2015, Connect. connect.mheducation.com. Retrieved 2015-11-29
9. Sánchez Pérez M, Gil Sierra A, Sánchez Martín A et-al., 2012, Standardized terminology for disc disease. Radiologia. 2012;54 (6): 503-12.
10. Uchio Y, Ochi M, Matsusaki M, Kurioka H, Katsube K., 2000, Human chondrocyte proliferation and matrix synthesis cultured in atelocollagen gel. J Biomed Mater Res A.;50(2):138–43.
11. Willburger RE, Ehiosun UK, Kuhnen C, Kramer J, Schmid G., 2004, Clinical symptoms in lumbar disc herniations and their correlation to the histological composition of the extruded disc material. Spine.;29:1655–61
12. Haro H, Komori H, Kato T, Hara Y, Tagawa M, et al., 2005, Experimental studies on the effects of recombinant human matrix metalloproteinases on herniated disc tissues – how to facilitate the natural resorption process of herniated discs. J Orthop Res.;23:412–9
13. Yakovlev A, Tamimi MA, Liang H, Eristavi M., 2007, Outcomes of percutaneous disc decompression. Pain Physician. 2007;10:319–328.
14. https://www.ncbi.nlm.nih.gov/pubmed/2263967
15. https://www.spine-health.com/ebook/lumbar-herniated-disc-essential-guide Veritas Health, 2015 ISBN 0996517502, 9780996517508