

Morphometric characteristics of fibrocartilaginous tissue in the herniated intervertebral disc

AHMED ABU-AWWAD¹⁾, ROXANA FOLESCU²⁾, DANIEL LAURENȚIU POP¹⁾, ANDREI GHEORGHE MARIUS MOTOC²⁾, DUMITRU MANUEL OPREA¹⁾, MARIANA TUDORAN³⁾, CARMEN LĂCRĂMIOARA ZAMFIR⁴⁾, COSMIN IOAN FAUR¹⁾, DINU VERMEȘAN¹⁾, BOGDAN NICOLAE DELEANU¹⁾, BOGDAN CORNELIU ANDOR¹⁾, HORIA GEORGE HĂRĂGUȘ¹⁾

¹⁾Department of Orthopedics and Traumatology, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania;

^{1st} Department of Orthopedics and Traumatology, "Pius Brînzeu" Emergency County Hospital, Timișoara, Romania

²⁾Department of Anatomy and Embryology, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania

³⁾Department of Internal Medicine II, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania

⁴⁾Department of Morpho-Functional Sciences, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Romania

Abstract

The purpose of this research was to identify a possible correlation between the morphometric characteristics of fibrocartilaginous tissue in the intervertebral herniated disc fragment and the clinical and imaging characteristics of patients with back pain. Sixty-two samples were included in this study. Intervertebral herniated disc fragments obtained during surgery (microdiscectomy) were analyzed histologically and morphologically. The analyzed fragment tissues from herniated lumbar discs were from L3–L4, L4–L5 or L5–S1 levels. The average number of chondrons encountered in a visual field was 35 (ranging from 8 to 51). The minimum chondrons surface area – 493.4 pixels² (from 188 to 925 pixels²) and the average peak area of chondrons – 5250.9 pixels² (ranging from 1171 to 11811 pixels²) and the median was 785.4 pixels² (values between 247.5 and 1621 pixels²). With age control, a correlation between the average chondron area and the Pfirrmann classification ($r=0.413$; $p=0.014$) was found but the correlation coefficient was small. The results of this study demonstrate that there is a correlation between the area of the chondrons and the clinical and imaging characteristics. The Japanese Orthopedic Association Back Pain Evaluation Questionnaire (JOABPEQ) correlated with the chondrocyte area in the presence of a lumbar disc herniation with surgical indication. It should be taken into account that the variables considered only correspond to certain patients with degenerative lumbar discopathy.

Keywords: lumbar disc disease, low back pain, fibrocartilaginous tissue, chondrons, chondrocyte.

Introduction

Lumbar disc disease (LDD) has a continuously increasing incidence possibly due to the modern lifestyle [1]. Very often, the source of low back pain is related to the intervertebral lumbar discs. Lumbar pain is also one of the most common symptoms for lumbar degenerative disc disease [2]. This pathology has many possible causes, but the most recently identified etiological factors include smoking, increased body mass index, vibrations (associated with working conditions) and genetic predisposition [1, 2].

Currently, it is believed that disc degeneration follows a predictable pattern. In the first place, the *nucleus pulposus* (NP) that is in the center of the disc begins to lose its ability to absorb the water, becoming dehydrated. In the second phase, the core becomes thick and fibrous. As a result of these changes, the NP is unable to absorb shocks and to properly transmit loads into the lower segment. Routine stress and day-by-day tensions will continue to damage the structures of the spine even more and will lead to the development of cracks in the fiber ring and thus accentuating the harmful processes [3, 4].

Microscopically, this will lead to a loss of the cell population within the intervertebral discs, alteration of the cellular phenotype, an increased activity of proteoglycans and proinflammatory cytokines and to collagen degradation [4]. If all these degenerative processes persist, all these changes will lead to an alteration of normal

biomechanics and structural instability, the intervertebral disc will lose the height, and the symptoms of the patient will increase dramatically, and finally can cause a disc herniation with root compression [5].

The incidence and prevalence of LDD according to the literature, is between 68% and 75% at men, and 74% and 78% at women, both men and women with age less than 60 years, and 88% and 94% at both men and women with age over 70 years [5].

The purpose of this research was to identify a possible correlation between the morphometric characteristics of fibrocartilaginous tissue in the intervertebral herniated disc fragment and the clinical and imaging characteristics of patients with back pain [4, 6].

Patients, Materials and Methods

This study aimed to determine the potential link between lumbar pain and nerve structures inside discs. Sixty-two people were included in this study, representing patients treated in the "Pius Brînzeu" Emergency County Hospital, Timișoara, Romania, from 2012 to 2017. Patients enrolled in the study were diagnosed with lumbar disc herniation and were consequently surgically treated according to current guidelines. All patients were initially subjected to conservative treatment for six weeks prior to surgery.

Inclusion criteria were:

- Age between 18 and 65 years old;

- Magnetic resonance imaging (MRI) presence of signs of lumbar degenerative discopathy;
- Lumbar disc herniation with neurological phenomena;
- Patients with several back pain, with no improving after conservatory treatment;
- Patients with back pain after one year of the first surgical procedure.

The exclusion criteria:

- Infectious or febrile conditions: tuberculosis, hepatopathy [hepatitis B virus (HBV), hepatitis C virus (HCV)], human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS);
- Severe mental illness, non-cooperative patients, drug or alcohol addiction;
- Pregnancy and lactation;
- Blood disorders: coagulation disorders with high bleeding risk;
- Endocrine or metabolic disorders: poorly controlled diabetes with advanced neuropathy and/or angiopathy, decompensated endocrine disorders;
- Severe respiratory and cardiovascular conditions;
- Patients with history of back pain and more than one spine surgical procedure;
- Patients with fractures, or acquired deformities of the lumbar spine.

Patients were clinically evaluated based on the criteria of the *Japanese Orthopedic Back Pain Evaluation Questionnaire* (JOABPEQ) [6].

Intervertebral herniated disc fragments obtained during surgery (microdiscectomy) were analyzed histologically and morphologically. The analyzed fragment tissues from herniated lumbar discs were from L3–L4, L4–L5 or L5–S1 levels.

For microscopic study, lumbar disc herniated fragments were fixed in 10% formalin solution for 48 hours and then embedded in paraffin, using the classical histopathology protocol. There were performed 4- μ m serial sections using Leica RM2235 semi-automated rotary microtome. The sections were stained with Hematoxylin–Eosin (HE) and Toluidine Blue.

Afterwards, the specimens were evaluated using a light microscope at 100 \times , 200 \times , 400 \times and 1000 \times magnification (Axio Imager 2, Carl Zeiss AG, Jena, Germany).

The chondrons that were identified were subsequently manually counted. With the help of specific software, an area of 20 chondrons/field of view was measured.

The study was conducted in accordance with local ethics guidelines and was approved by the Ethics Commission of the “Pius Brinzeu” Emergency County Hospital, Timișoara.

The data obtained were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) 21.0 software. The value of a $p < 0.05$ was statistically significant and very statistically significant the value of a $p < 0.001$.

Results

In the present study, 62 patients were examined clinically, the mean age being 45.34 years (19 to 61 years) and the gender distribution – 29 (42.71%) women and 38 (57.29%) men.

The mean JOABPEQ score was 6.7, with values ranging from 1 to 13. The average Visual Analogue Scale value for lumbar spine was 6.1 (with values between 2

and 10) and for sciatalgia was 7.4 (with values between 5 and 10).

Using the Pfirrmann classification, we found: 12 patients with type II modifications, which means the disk was inhomogeneous; 28 patients with type III modifications, with grey signal intensity; 20 patients with type IV modifications with no distinction *nucleus annulus*; and two patients with type V modifications, with collapsed disk.

From the histopathological point of view, on the evaluated specimens, one could differentiate the areas of tissue from the fibrous ring, areas containing collagen fibers arranged in parallel, layered but alternating as orientation, the cells being elongated, with a fibroblastic appearance.

The pulp core tissue contained circular or oval, chondrocytic arranged in a rich extracellular matrix (ECM) (Figure 1). Although chondrocytes occupy only 1–10% of cartilage volume, they play an essential role in maintaining cartilage homeostasis. They secrete all the biochemical components of the extracellular conjunctival matrix, being the essential structural elements of the cartilage. The primary role of the chondrocyte is to maintain viable cartilage by regulating macromolecular synthesis of the ECM.

In our study, some sections of hyaline cartilage could be identified, with chondrocytes of different shapes and sizes, surrounded by an intensely colored ECM (Toluidine Blue), forming functional unit that has been named a “chondron” (Figure 2).

Hyaline cartilage was not present in all evaluated sections, but the NP was a constant presence in all specimens.

The general aspect was similar to a degenerative process, because chondrocytes were in different stages of evolution and the quantities of ECM produced were insufficient. On some histological preparations, we identified areas of cartilage normally mixed with areas of degenerate cartilage and regeneration areas. We found a disorganized cartilage matrix, lax, exhibiting multiple optically empty spaces in the HE staining, suggestive of cracks (Figure 3).

In the regenerative areas the chondrons contained four or more chondrocytes, with abundant cytoplasm, basophils, with large and hypochromic nucleus. The morphology of chondrocytes and the chondrons in the regenerative regions corresponds to the three chondrocyte types: small chondrocytes, large and hypochromic nucleus (proliferative cells), adult chondrocytes and hypertrophied chondrocytes. Unfortunately, the ECM surrounding the chondrocytes in the regeneration zones was few and with an absent basophilic reaction (Figure 4). This microscopic aspect shows the inability of chondrocytes to synthesize cartilaginous matrix, as a result of the overall pathological architectural changes suffered by the intervertebral disc. We consider that for these reasons, the regeneration of the intervertebral disc is almost impossible.

The average number of chondrons encountered in a visual field was 35 (ranging from 8 to 51).

The minimum chondrons surface area – 493.4 pixels² (ranging from 188 to 925 pixels²) and the average peak area of chondrons – 5250.9 pixels² (ranging from 1171 to 11811 pixels²) and the median was 785.4 pixels² values between 247.5 and 1621 pixels².

There was no correlation between the average chondron area and the *JOABPEQ* clinical score ($r=-0.302$; $p=0.078$) with age control. With age control, a correlation between

the average chondron area and the Pfirrmann classification ($r=0.413$; $p=0.014$) was found but the correlation coefficient was small.

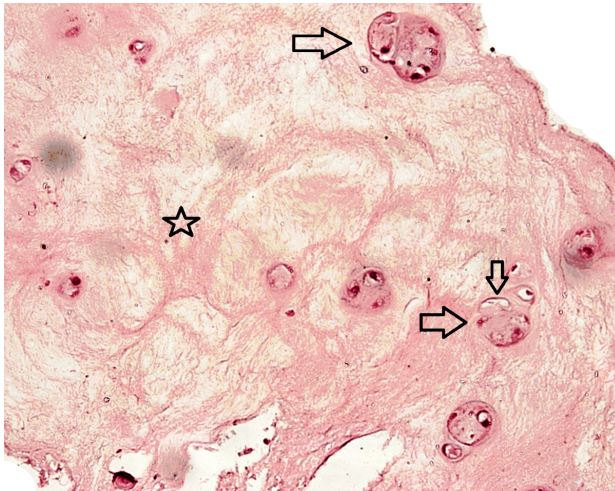


Figure 1 – Large chondrons (\Rightarrow) with pericellular matrix (\star) (HE staining, $\times 200$).

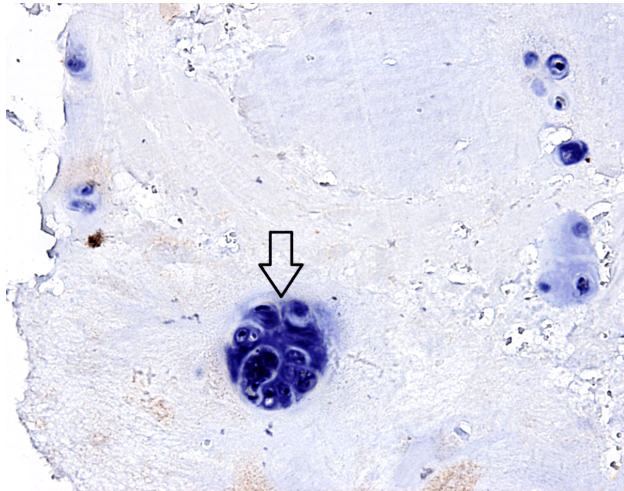


Figure 2 – Giant chondron (\Rightarrow) with multiple chondrocytes in its structure (Toluidine Blue staining, $\times 100$).

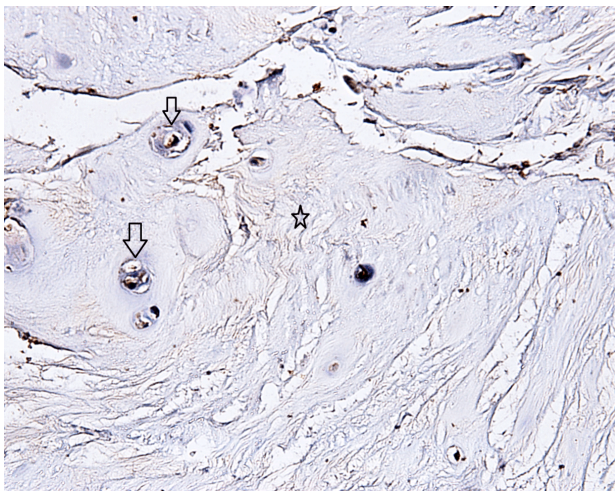


Figure 3 – Image of degenerate fibrocartilage (\star), with small chondrons (\Rightarrow) and multiple optically empty spaces, suggestive of cracks tissue (HE staining, $\times 100$).

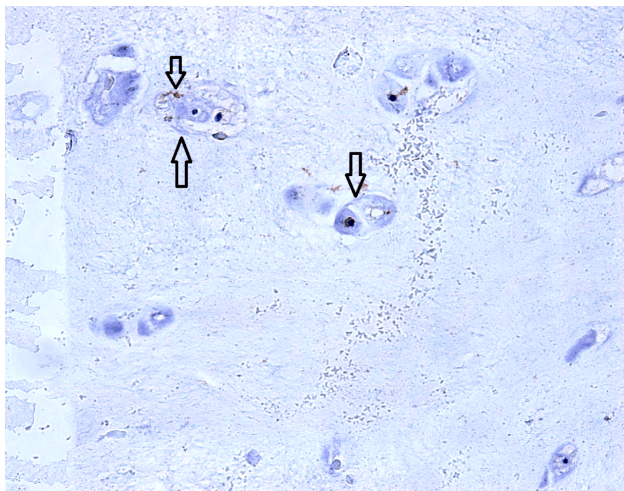


Figure 4 – Chondrons with chondrocytes (\Rightarrow) of various sizes but with absent basophilic reaction around them, which denotes a pathologically modified extracellular matrix (Toluidine Blue staining, $\times 100$).

Discussions

The purpose of this research was to identify a possible correlation between the morphometric characteristics of fibrocartilaginous tissue in the intervertebral herniated disc fragment and the clinical and imagistic characteristics of patients with back pain. Intervertebral discs are gradually changing their form and composition with age but several conditions might hasten the process even further. Such a condition is represented by an end-plate fracture that induces early degenerative modifications of the intervertebral disc. The daily lifestyle, certain types of vibration can also speed the degeneration of the lumbar disc and can induce degenerative-specific changes [7].

The fibrous and chondrocyte cells in an adult intervertebral disc represent only 1–2% of its volume. The cells we believe are responsible for the secretion and organization of the ECM that forms most of this fibroelastic cartilage tissue. Chondrocyte cells are surrounded

by a territorial matrix. Together, they form the morpho-functional and metabolic elementary unit of this fibrocartilage tissue, called chondron. Chondrons are usually composed of one or two chondrocyte cells coupled with their territorial matrix. During the degenerative process, chondrocyte cells inside the chondrons multiply, giving rise to cluster chondrons containing multiple monoclonal cells. In LDD, there is also an alteration in the function of constituent cells leading to a decreased proteoglycan secretion by the ECM [8, 9].

Normal intervertebral disc sensory innervation is limited to the first 2–3 outer bundles of the fibrous ring, containing nerve growth factor (NGF)-dependent peptide, nociceptive nerve fibers, non-peptide-dependent glial cell-derived neurotrophic factor (GDNF)-dependent and Pacini-like morphogens-like receptors, Ruffini terminations and Golgi apparatus [10, 11].

They originate in small neurons in the dorsal root of the ganglion and express the tropomyosin receptor kinase

A (TrkA), tropomyosin receptor kinase A (TrkB) and rearranged during transfection (Ret) receptors with increased affinity for NGF, brain-derived neurotrophic factor (BDNF) and GDNF [12].

Because these cells live in an avascular environment, and due to the limited regenerative capacity, the degenerative process is considered to be irreversible in by many researchers. Changes typical for this degenerative process include: increasing activity of matrix metalloproteinase, decreased total proteoglycan and collagen production, water loss with consequent change in volume, and the appearance of neoformation vessels in the periphery of the fibrous ring [13]. The cell population undergoes changes similar to those of aging cells, and the remaining cells change their morphology. The changes in intervertebral disc constituent cells also affect the concentration of large proteoglycans in ECM and collagen secretion. As the degenerative process continues, neoformation vessels and nerve fibers may appear starting from the periphery of the intervertebral disc [14].

The intervertebral disc cells tend to aggregate in clusters and the size of the chondrons from LDD correlated with *Japanese Orthopedic Association Score* (JOAS), Pfirrmann & Modic. JOAS correlates with the imagistic evaluation systems Pfirrmann & Modic [15].

Similar findings have been previously described in other areas, such as knee ligaments [16]. These new structures penetrate NP through existing cracks in the fibrous ring. Concurrently, the NP cells decrease their volume and turn into the so-called “chondrocyte-like” cells, forming larger cell aggregates called chondrons. These cell clusters often have a very large volume and can dissociate large amounts of ECM [17].

Lee *et al.* reported elevated levels of NGF in degenerate discs using the enzyme-linked immunosorbent assay (ELISA), and Aoki *et al.* reported increased levels in herniated discs, concluding that the neurotrophins could play an important role in disc degeneration [18, 19].

Although they, as well as Purmessur, by the immunohistochemical method, have identified higher levels of NGF, they still have not been able to identify the cells responsible for its secretion. It is believed that the secretion of the neurotrophins may be chondrocyte cells, endothelial cells of the blood vessels, but also inflammatory cells [20].

The results of this study confirm the presence of neurofilaments in chondrocyte cells that make up large cluster chondrocytes that are frequently identified in highly degenerate specimens. Greene *et al.* obtained the transdifferentiation of chondrocytes in neuronal cells, even if their functionality could not be assessed [21].

It is possible that part of the pulsed core chondrocyte cells are transdifferentiated into neuronal cells (capable of expressing TrkA, TrkB and Ret receptors), explaining the occurrence of pain during disc degeneration.

Kokubo *et al.* identified negative nerve endings positive for NGF, growth associated protein-43 (GAP-43) and substance P in the outer layers of herniated disc fragments after evaluation of 500 degenerate intervertebral discs but did not report information on immunocompromised chondrocyte cells [22]. In this study, both nerve fibers distributed in the ECM of herniated disc tissue, as described

by Kokubo *et al.*, as well as neurofilament-associated protein (NFAP)-positive chondrocyte cells were identified [22].

Of the genetic, biological and mechanical factors involved in the intervertebral disc degeneration, dehydration of NP and loss of gel properties due to decreased proteoglycan content is considered an important factor [23]. This phenomenon progresses towards fibrosis of the NP with alteration of the force transmission, alteration of the fibrous ring and various other aspects of the degenerative process. These changes are consistent with the histological analysis of the disc fragments in this study. In the early stages of degeneration, cellular proliferation is stimulated in an attempt to combat the loss of ECM [24–26].

Following a three-dimensional microscopic study on chondrocytes harvested from the pig, from the femoral condyle, Choi *et al.* measured the volume of the chondron, the average of its values being $2218 \pm 832 \mu\text{m}^3$ (in the region of the cartilage where the chondrons have a relatively spherical shape), which corresponds to an approximate area of $206 \pm 106 \mu\text{m}^2$. The chondrons evaluated in this study had an average area of $555.89 \mu\text{m}^2$ (values between 81.94 and $9923.81 \mu\text{m}^2$) but 71.14% of them had values less than $540 \mu\text{m}^2$. However, these differences highlight the morphometric differences between normal chondrons and those found in degenerated fibrocartilaginous tissue [27, 28].

As observed on the specimens studied, chondrocyte cells are rearranged in clusters, actively trying to produce matrices but this was poorly colored (suggesting a qualitative alteration) and in small quantities, while around the normal chondrocyte loopholes the matrix had a normal look. Ciapetti *et al.* (2012) reported an equal proportion of cell clusters in the fibrous ring and NP of degenerate intervertebral discs, herniated or not [29]. However, this aspect was difficult to assess in this study because the specimens used were only from the herniated fragments making it difficult to identify the regions of the intervertebral disc. The specimens in this study contained a large proportion of chondrons composed of chondrocyte of varying sizes and in various evolutionary stages, surrounded by a small amount of the tertiary matrix suggesting a tissue aspect in an inefficient or insufficient regeneration phase. The presence of these chondrons in increased numbers, the characteristic aspect of the ECM with cracks, necrosis zones, vascular and nerve structures are suggestive of a “degeneration by regeneration” process. This process can be a response to mechanical stress, forcing the intervertebral disc to reorganize to cope with new biomechanical conditions [29–31].

Within the fibrocartilaginous tissue of the degenerate intervertebral disc there are aggregated chondrocyte cells forming large chondron-cluster structures. They contain monoclonal cells from various evolutionary stages surrounded by a poorly colored and ECM. The overall appearance of herniated disc tissue is degenerative.

The JOABPEQ clinical score used to evaluate patients with lumbar spine correlated with the chondrocyte area in the presence of a lumbar disc herniation with surgical indication [31].

We appreciated, as other authors, as in disc herniation as in many acute or chronic conditions, inflammatory or neoplastic, immunohistochemistry studies can bring important histopathological diagnostic elements [32–34].

Quantitative degenerative imaging changes using the Pfirrmann & Modic staging systems were correlated with each other and with histological changes and were not the focus of this study [35, 36].

However, in the present study, a correlation between the Pfirrmann type and the median of the chondron area, as well as a correlation with the duration of the symptomatology, were not observed. These observations emphasize the important role played by the chondrons morphometry in the pathophysiology of disc degeneration and of his clinical expression.

Conclusions

The results of this study demonstrate that there is a correlation between the area of the chondrons and the clinical and imagistic characteristics. It should be taken into account that the variables considered only correspond to certain patients with degenerative lumbar discopathy. These patients have a lumbar disc herniation with severe symptomatology indicating surgical treatment, which may suggest the presence of more pronounced degenerative changes.

Conflict of interests

The authors declare that they have no conflict of interests.

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Corresponding authors

Roxana Folescu, Senior Lecturer, MD, PhD, Department of Anatomy and Embryology, “Victor Babeș” University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timișoara, Romania; Phone +40729–104 103, e-mail: roxanafolescu08@gmail.com

Dinu Vermeșan, MD, PhD, Department of Orthopedics and Traumatology, “Victor Babeș” University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timișoara, Romania; Phone +40748–331 262, e-mail: vermesan@gmail.com

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