Imagistic and histopathologic concordances in degenerative lesions of intervertebral disks

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Abstract
Lumbar disk lesions in 47 cases were initially diagnosed using MRI investigation, then, after surgery, biological and histopathological aspects of intervertebral disks were revealed. Pieces from intervertebral disks were used for electron microscopy studies in order to determine collagen in the components of the intervertebral disk. The aim of the present study was to highlight the correspondence between the MRI aspect in cases with clinically manifest lumbar hernia, staged according to MRI Modic classification, and the histopathological aspect in patients with surgical interventions on the intervertebral disks. 4/5 of the analyzed disks had advanced forms of degenerescence of the intervertebral disks: hyalinized disk cartilage ± intradiskal calcification or ossification zones, chronic inflammatory infiltrate at the disk cartilage level. Electron microscopy studies made on disk fragments obtained by discectomy revealed quantitative and qualitative changes of all types of collagen at the level of the three anatomical structures of the intervertebral disks, which correspond to the MRI changes.

Keywords: intervertebral disk, magnetic resonance imaging, histopathology.

Introduction
The development of molecular biology facilitated a better understanding of the processes that lead to the degenerative disease of intervertebral disks. Numerous studies aiming to identify the causes and risk factors for this degenerative disorder have been performed [1]. It is now known that degenerative disk disorder is strongly linked to genetic factors, recent research indicating that heredity has a dominant role in disk degeneration and explains about 74% of the variance in adult populations. Studies regarding genetic influences are performed since 1998 and many genes associated with disk degeneration have been confirmed, such as the genes that code for collagen I, collagen IX (COL9A2 and COL9A3), collagen XI (COL11A2), IL-1, IL-6, aggrecan, vitamin D receptor, MMP-3, VDR and CILP. For some of these, gene-gene, gene-environment or gene-age interactions may exist [2]. Recent advances have led to further understanding of the complexity of disk degenerative disease and allowed development of new treatments, targeting the biochemical pathways involved in the degenerative cascade. Gene therapy has also shown much promise as a powerful tool in the treatment of intervertebral disk degeneration. Other new targets for gene therapy have also been identified, such as TGF-beta1, TIMP-1, and LMP-1 [3]. Some studies revealed that occupational factors, such as whole-body vibration, have an additive effect to genetic risk factors increasing the likelihood of having the disorder. Of the independent genetic markers, IL1A–889T allele had the strongest association with intervertebral disk disease [4]. Additional studies, including linkage analyses and whole genome scanning in different populations and whole range of ages will improve understanding the influence of genes on disk degeneration [2].

Activity restriction in cases with lumbar disk hernia represents a solid argument that pleads for deepening studies regarding the biology of the intervertebral disk, leading to early diagnosis and effective treatment according to biological staging correlated to the stage of the disorder, which is offered by magnetic resonance imaging (MRI) investigation [5]. Classifications of lumbar spine degeneration by imaging methods were first provided by Modic MT [6]. In that study, Modic MT followed up, using magnetic resonance, the evolution of patients submitted to treatment for disk conditions with chemopapain, classifying these changes as grade I, II or III. Type I MRI changes consist of vertebral bodies parallel with the vertebral plateau of degenerated disk that shows hyposignal on ponderate images in T1 and hyperintensity in T2. There is no radiological correspondent.
Type II MRI changes consist of increased intensity of the signal on ponderate images in T1 and an iso-intense signal or slightly hyperintense in T2 images and represent the imagistic expression of disk lesions consisting of annular protrusion and relatively recent incipient disk hernia. They do not have a radiological correspondent.

MRI investigations revealing type III changes, show decreasing intensity of the signal on ponderate images both in T1 and T2, being correlated on usual planar radiographic images with extensive bone sclerosis. They are associated with disk hernia, disk extrusion, free disk fragment, lesions of the posterior vertebral ligament [7]. From the biological point of view, the intervertebral disk is characterized by the presence of collagen (its quantity increases with age), water, condroitin sulphates [8]. Disk composition changes during development, growth, ageing and degeneration and this influences the response of the disk to changes in mechanical stress [9, 10].

The aim of the present study was to highlight the correspondence between the aspect of MRI in cases with clinically manifest lumbar hernia, staged according to MRI Modic classification, and the histopathological aspect in patients with surgical interventions on the intervertebral disks.

Materials and Methods

The study was performed on a group of 47 cases with discectomy for lumbar disk hernia, selected from the patients admitted in the Medical Rehabilitation Clinical Hospital Bâile Felix, with the diagnosis of low back pain accompanied or not by radiculopathy due to lumbar hernia, between January 2008 and September 2008. All the patients were investigated by MRI before surgery. We used MRI Modic classification, which categorizes disk lesions in three classes, according to their severity. Forty-seven intervertebral disks were taken from patients operated in the Neurosurgery Department of Oradea Clinical County Hospital.

Lumbar disk lesions were initially diagnosed using MRI investigation, then, after surgery, biological and histopathological aspects of the intervertebral disks were revealed. Fragments from intervertebral disks were used for electron microscopy studies in order to determine collagen in the components of the intervertebral disk. They were studied using both transmission electron microscopy and scanning electron microscopy, but fragments from the 47 disks were also examined by column and paper adsorption chromatography methods. Electron microscopy studies aimed to analyze the collagen types from intervertebral disks obtained by discectomy. These assessments were performed in order to reveal quantitative or qualitative changes of six types of collagen (I, II, III, V, VI, IX) in nucleus pulposus, annulus fibrosus and cartilaginous end-plates from the intervertebral disk. For the histopathological examination, specimens were paraffin-embedded and stained with Hematoxylin–Eosin. Photos of the slides were made with Polaroid XIO type. Different types of collagen were determined using electron microscopy studies, by measuring distinct chains and total mass of collagen fiber chains.

Results

Patients’ age varied between 22 and 54 years, with a mean of 48.8 years. Distribution of genders was: 31 males (65%) with a mean age of 45.3 years and 16 females (35%), with a mean age of 39.6 years.

Localization of disk hernia established by MRI investigation revealed: L3 level – two cases (4.3%), L4 level – 30 cases (63%), L5 level – 15 cases (32.6%). Type I MRI Modic changes were found in four (8.5%) samples, type II changes were identified in five (10.63%) samples and type III MRI Modic changes in 38 (80.8%) samples.

Intervertebral disk degeneration is revealed by chondrocyte lesions in the fibrous cartilage: various stages of altered nuclear structure, cytoplasmic vacuolation. Cells disappear from lacunae. Intercellular cartilagenous matrix, depending upon cell alterations, suffers degradation processes: loss of tinctoriaty, fiber lysis, calcification. The cartilaginous channel contains connective tissue with many neoformation vessels, hemorrhagic suffusions and rich PMN infiltration, lympho-monocytes and macrophages. Chondrocytes show alterations of the nucleus (pyknosis) and of the cytoplasm (vacuolization). The matrix is eosinophilic, poor in fibers. Some chondrocytes are deeply altered and disappeared from lacunae.

Histopathological changes noticed in the early forms of degenerescence of the intervertebral disk have been identified in five samples (10.63%) (Figure 1):

- myxoid degenerescence of the disk cartilage, with chondroblasts and chondrocytes;
- hyalinisation and areas of interfibrillar edema on the fibrous disk tissue.

Histopathological changes – second phase of degeneration – moderate forms of degenerescence of the intervertebral disk (Figure 2), identified in 13 (27.65%) cases were:

- cartilaginous tissue with chondrocytes in lacunae, papillary aspect and hemorrhagic areas;
- chondroblasts and chondrocytes associated with areas of myxoid degenerescence.

Figure 1 – Fibrous cartilage (territorial matrix), inter-territorial with fibrous aspect and small isogenic groups or isolated chondrocytes (AA–PAS, ×40).

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Figure 2 – Cartilaginous matrix with isolated or degenerated chondrocytes disappeared from lacunae, fibrillar disorganization, although maintained in oblique plans to each other. Matrix territories retain metachromasy of the fibers at AT pH 5 (×40).

Histopathological changes – type III, in advanced forms of degenerescence of the intervertebral disk, (Figure 3), seen in 29 cases (57.44%), were:

▪ myxoid degenerescence and intradiskal calcifications;
▪ hyalinized disk cartilage with intradiskal calcification areas and the presence of young cells – fibroblasts and chondroblasts;
▪ cartilaginous tissue with areas of fibrosis and chronic inflammatory infiltrate.

Figure 3 – Fibrous cartilage with multiple fissures, with extensive areas of dystrophic calcification and disappearance of the chondrocytes from lacunae (HE stain, ×40).

Column and paper-adsorption chromatography analysis together with electron microscopy examination of the 47 fragments of intervertebral disks revealed quantitative and qualitative changes of the three anatomical structures. Data obtained were corroborated with the results of the MRI investigation.

Electron microscopy studies performed on intervertebral disk fragments obtained by discectomy revealed qualitative and quantitative changes of all types of collagen in all three anatomical structures of intervertebral disks, which correspond to MRI changes.

The dominant morphological characteristic of the injured chondrocyte from the degenerated intervertebral disk in electron microscopy is the presence of numerous complex lipid vacuoles of unequal sizes, all over the cytoplasm, with a tendency to unification. In many degenerated chondrocytes the autophagic phenomenon includes large cytoplasmic territories or the aspect of residual bodies (autophagosomes) with abundant undigested granular material. On the surface of many chondrocytes there are mixed expansions like microvilli or long digitiforme ones as micro-folds in contact with a reduced intercellular matrix. The sub-plasma membrane is seen in some chondrocytes as fine, granular electron-dense material, representing either a secretion or endocytted product. Territorial cartilage matrix from herniated intervertebral disk reveals in electron microscopy collagen fibers of unequal thickness, sectioned in various incidences and showing a disordered arrangement.

In six cases (12.76%) the following aspects of the intervertebral disk were encountered (Figure 4):

▪ absence/minimal increase of collagen I and II in nucleus pulposus;
▪ increase with a slight change of collagen I, II, III, IV in annulus fibrosus;
▪ absence of collagen I and presence of collagen II in the cartilaginous end-plates.

Figure 4 – Type I and II collagen fibers (TEM).

These cases correspond to the four patients (66.6%) with MRI changes type I and to two patients with MRI changes type II and also to the five cases (83%) with histopathological changes type I and one with type II.

In seven cases (14.89%), electron microscopy revealed the following aspects (Figure 5):

▪ increase of collagen III in nucleus pulposus;
▪ moderate presence of collagen V and increase of collagen III in annulus fibrosus;
▪ presence of collagen III, V, VI in cartilaginous end-plates.

Figure 5 – Fragment from intervertebral disk studied by scanning electron microscopy revealing type II changes.

Disk lesions of these cases corresponded to type II MRI changes in five cases (71.4%) and type II histopathological changes for all cases.
As for the other 34 cases, (72.34%), electron microscopy revealed the following changes (Figures 6 and 7): decrease of all types of collagen in cartilaginous end-plates with the presence of all six types of collagen (I, III, IV, V, VI, X) in nucleus pulposus and in annulus fibrosus, with attenuation and disappearance of proteoglycans. In this stage other changes, such as lipid vacuoles, autophagosomes, collagen degradation and fissures were also noticed. These correspond to all patients with type III MRI changes and to 29 cases (85.1%) with type III histopathological aspect.

Distribution of the cases according to the MRI aspect, histopathology and electron microscopy, are presented in Table 1.

<table>
<thead>
<tr>
<th>Aspect</th>
<th>No. of cases (%)</th>
</tr>
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<tbody>
<tr>
<td>MRI</td>
<td>Type I</td>
</tr>
<tr>
<td>Histopathology</td>
<td>4 (8.5%)</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>6 (12.76%)</td>
</tr>
</tbody>
</table>

**Discussion**

Histopathological aspects seen in less than 1/10 of the analyzed intervertebral disks presented myxoid degenerescence corresponding to the early forms. Moderate forms of degenerescence of the intervertebral disk were encountered in 1/10 of the cases and the observed histopathological changes were: fibrous disk tissue with interfibrillar areas of edema, discal cartilage with chondrocytes in lacunae, papillary aspect and hemorrhage areas, discal cartilage with hyalinization areas. 4/5 of the analyzed disks showed advanced forms of degenerescence of the intervertebral disk: hyalinized discal cartilage +/- intradiskal calcification or ossification areas, chronic inflammatory infiltrate within the discal cartilage. More than half of the subjects presented advanced degenerative lesions of the intervertebral disk revealed by MRI, which were also confirmed by the histological examination.

The intervertebral disk is a highly organized matrix laid down by relatively few cells in a specific manner. The central nucleus pulposus is contained within the more collagenous annulus fibrosus laterally and the cartilage end-plates inferiorly and superiorly [11]. Annulus fibrosus consists of concentric rings or lamellae, with fibers in the outer lamellae continuing into the longitudinal ligaments and vertebral bodies. This arrangement allows disks to facilitate movement and flexibility within the spine [12]. With increasing age, water is lost from the matrix, and the proteoglycan content also changes and diminishes [13]. Thus, the nucleus becomes less gelatinous, more fibrous and fissures eventually occur. There is also an increase in cell proliferation and formation of cell clusters as well as an increase in cell death. The cartilage end-plate undergoes thinning, altered cell density, formation of fissures and sclerosis of the subchondral bone [14]. These changes are similar to those seen in degenerative disc disease, causing discussion as to whether aging and degeneration are separate processes or the same process occurring over a different timescale. Additional disorders in which the intervertebral disk is affected can demonstrate other morphological changes. Thus, disks from patients with spinal deformities such as scoliosis have ectopic calcification in the cartilage end-plate and sometimes in the disk itself. The role that abnormalities play in the etiopathogenesis of different disorders is not always clear [15].

Changes associated with aging progressively affect all structures of the spinal unit. Disk degeneration is associated with biochemical changes followed by macroscopic alterations including tears and fissures, which may lead to disk herniation, the main cause of radiculopathy.

The etiology of disk degeneration still remains incompletely known, despite the advanced research in the field. The idea that the most critical event of this process is the disturbance of the oxygen, nutrients and residual materials diffusion in the disk space is largely accepted. The disk suffers an opening, which can appear as a consequence of some external factors, such as overloading, vibration forces and spine deformities [16]. Alternatively, it can also be the result of some quantitative and qualitative changes of the matrix [17]. As the quantity of water from the disk decreases, the nutrients and residuals diffusion is restrained. This leads to an anaerobic metabolism, which compromises the
A biological staging corresponds to the imaging staging of intervertebral disk lesions. Electronic microscopy studies made on disk fragments obtained by discectomy revealed quantitative and qualitative changes of all types of collagen the three anatomical structures of intervertebral disk that correspond to the MRI changes.

The obtained concordances give us hope for an early diagnosis of disk hernias, by associating data obtained from cell biology of the intervertebral disk with the images provided by MRI.

These correlations are very important from the practical point of view, because the predictive values allow the approximation with a high degree of probability of future results ("disease outcome") in patients with lumbar disk hernia.

References


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