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Comparison of the biochemical and radiological criteria for lumbar disc degeneration

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ABSTRACT

Background: The relationship between radiological degeneration criteria on lumbar magnetic resonance imaging (MRI) and both the keratan sulfate (KS) and chondroitin sulfate (ChS) levels was examined in disc material taken from patients undergoing lumbar disc herniation (LDH) surgery. To examine whether the biochemical and radiological degeneration criteria testing the reliability of radiological degeneration findings agreed and to evaluate the contribution of the KS/ChS ratio to disc form (protruding or extruding).

Methods: This was a prospective experimental cohort study. Using enzyme-linked immunosorbent assay, KS and ChS levels were measured in the degenerate nucleus pulposus taken from 71 patients with a diagnosis of LDH who underwent surgery. The degeneration levels and disc form (protruding or extruding) were determined according to the Pfirrmann five-stage grading system on preoperative T2-weighted lumbar MRIs. According to the Pfirrmann system, 28 patients were grade III and 43 were grade IV. The relationship between radiological criteria and the KS/ChS ratio was statistically evaluated.

Results: The KS levels ($p = 0.046$) and the KS/ChS ratio ($p = 0.001$) were significantly higher in grade IV patients than in grade III patients. However, there was no difference between the KS and ChS levels and the KS/ChS ratio when patients were classified as protruding or extruding according to their disc structure. Disc structure and biochemical degeneration indicators were not correlated.

Conclusions: The KS level and the KS/ChS ratio were high in patients with marked radiological degeneration on lumbar MRI, demonstrating the sensitivity and reliability of the Pfirrmann five-stage grading system for showing radiological degeneration.

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1. Introduction

The intervertebral disc (IVD) consists of two main structures: the gelatinous, water-binding nucleus pulposus and a layered circular structure called the annulus fibrosus. One of the first signs of disc degeneration is the loss of proteoglycans from the nucleus pulposus [1]. It has been hypothesized that the subsequent loss of water results in a loss of pressure, leading to the collapse of IVD and clinical signs, such as decreased disc height and decreased signal intensity on T2-weighted magnetic resonance imaging (MRI) [2]. Several morphologic grading systems for lumbar disc degeneration have been described. One of the most used grading systems is Pfirrmann et al. classification and grading system for degeneration of lumbar disc herniation [3]. Pfirrmann grading system is based on T2-weighted MRI features of IVD; disc structure, signal intensity, distinction between nucleus and anulus, and disc height. The latter feature is important for distinguishing between Grades IV and V discs. For Grades III and IV, the disc height is not a discriminative feature. The discriminative feature between Grades III and IV is distinction between nucleus and anulus. If it was lost, the radiologist of MRI can classify it as Grade IV. Homogeneous disc structure is the discriminative feature for Grade I discs [3].

The amount of degeneration in IVD can be biochemically quantified by determining the amount of collagen present in the nucleus pulposus, the number of collagen cross-links, the proportions of proteoglycan and collagen, and the keratan sulfate (KS)/chondroitin sulfate (ChS) ratio.

This study evaluated the reliability of the Pfirrmann five-stage classification by comparing the preoperative MRI with the KS/ChS ratio in disc material (degenerate nucleus pulposus) obtained perioperatively and investigated the effects of biochemical degeneration on the type of disc herniation (protruding or extruding).

2. Material and methods

This study was conducted at the Neurosurgery Clinic of our between March 2016 and September 2016. Seventy-one patients diagnosed with lumbar disc herniation (LDH) were included in this study after obtaining their verbal and written consent. Patients with cervical or thoracic disc degeneration, active malignancy, pregnancy, prior back surgery, spine fractures, sacroiliac arthritis, metabolic bone disease, spinal infection, or rheumatoid arthritis and patients having abdominal trauma within 7 days of admission were excluded.

The patients included in this study were classified as grade III ($n = 28$) or IV ($n = 43$) according to Pfirrmann's radiological classification system (Fig. 1a and b). According to Pfirrmann grading system for degeneration of lumbar discs; Grade III was described as inhomogeneous with gray colored discs, in which unclear distinction of nucleus and anulus with normal or slightly decreased disc height. For Grade IV, the distinction of nucleus and anulus was lost with normal or moderately decreased disc height [3]. According to the appearance of the disc herniation on MRI, 28 patients had the protruding form and 43 had the extruding form. Surgery was performed at levels L2-3, L3-4, L4-5, and L5-S1 in 5, 9, 33, and 24 patients, respectively (Table 1).

Disc material collected during surgery was stored at −80 °C until it was sent for biochemical analysis to analyze their KS and ChS content. The tissue samples were homogenized in phosphate-buffered saline (pH 7.4) (FastPrep 24; MP Biomedicals, Santa Ana, CA, USA). Total protein levels in all homogenized tissue samples were spectrophotometrically measured using the Bradford method [4]. The KS and ChS levels in the homogenates were measured with the sandwich enzyme-linked immunosorbent assay (ELISA) using commercial kits (lot no: E1459/Hu-E1895/Hu; Bioassay Technology Laboratory, Shanghai, China) and an ELISA reader (Thermo Fisher Scientific, Waltham, MA, USA). The ELISA result of each sample was divided by the amount of protein found. The results were expressed in ng/mg protein.

The radiological degeneration was evaluated by three independent radiologists using the five-stage Pfirrmann classification system based on T2-weighted mid-sagittal images obtained using contemporary MRI techniques. All three radiologists independently evaluated by three certified radiologists who were blinded to the classification groups of this study (radiologists did participate in any part of operation or biochemical analysis). The interobserver agreement was acceptable. Complete agreement was achieved in a range from

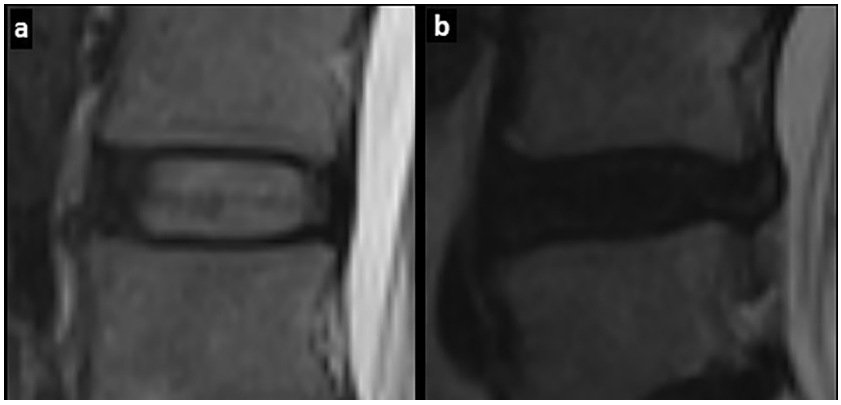

Fig. 1 – Sagittal T2-weighted MRI images. They are evaluated as Pfirrmann grade III (a) and grade IV (b). *MRI: magnetic resonance imaging.

58 of all 71 patients (81.7%). A difference of one grade occurred in 11 (15.5%) assessments of the patients, a difference of two grades in two patients (2.8%). The differences were solved by argument and the final evaluation was done after consensus among all three radiologists about the grade.

To calculate intervertebral disc height index (IDH); lateral lumbar spine X-rays of each patients were centered on the L3 vertebrae with the patients in the left lateral recumbent position. Anterior (A) and posterior disc heights (P) and IVD depth (D) were independently measured by three certified neurosurgeons (MHS, SK and AA). After calculated the mean of three values, IDH was calculated as this formula; IDH = (A + P)/100%.

This prospective study was approved by the local Research Ethics Committee and Institutional Review Board (no.: 20/15-04.11.2015).

2.1. *Statistical analysis*

The data were analyzed using SPSS 20 (IBM, Armonk, NY, USA). The Kolmogorov–Smirnov test was used to determine the distribution of the data. The results are reported as mean ± standard deviation, and the independent Student's *t*-test was used to detect inter-group differences in the mean values. The Chi-square test was used to compare categorical variables. A value of $p < 0.05$ was considered to be statistically significant.

3. Results

In total, 71 patients were evaluated in this study. Differences observed between groups with respect to age, gender and intervertebral disc height index (IDH) were not significant ($p > 0.05$). Patient demographics and characteristics are summarized in Tables 1 and 2.

Table 1 – Patient characteristics.

Pfirrmann radiological grade	Grade I	$n = 0$
	Grade II	$n = 0$
	Grade III	$n = 28$
	Grade IV	$n = 43$
	Grade V	$n = 0$
Disc herniation form on MRI	Protrusion	$n = 28$
	Extrusion	$n = 43$
Surgical level	L1-2	$n = 0$
	L2-3	$n = 5$
	L3-4	$n = 9$
	L4-5	$n = 33$
	L5-S1	$n = 24$

Table 2 – Patient demographics.

	Grade III ($n = 28$)	Grade IV ($n = 43$)	p
Age	47.5 ± 11.7	52.9 ± 13.9	>0.05
Gender (female/male)	15/13	23/20	>0.05
IDH[a]	0.435 ± 0.086	0.384 ± 0.102	>0.05

[a] IDH: intervertebral disc height index (%).

The mean KS levels in the grades III and IV discs were 11.09 ± 2.10 and 10.37 ± 1.53 ng/mg protein, respectively, and the mean ChS concentrations were 882.48 ± 172.54 and 969.78 ± 179.49 ng/mg protein, respectively. The KS/ChS ratio in the material of grade III and grade IV discs was 80.72 ± 14.05 and 95.25 ± 21.83, respectively (Table 3). The KS levels ($p = 0.046$) and the KS/ChS ratio ($p = 0.011$) were significantly higher in Pfirrmann grade IV patients than in grade III patients (Table 3 and Figs. 2 and 3). Disc structure (protrude vs. extrude) and biochemical degeneration of the disc were not statistically significantly correlated.

4. Discussion

The complex relationship between water and cells in cartilage tissue results in a highly resistant, shock-absorbing material with a very limited capacity for repair. For hyaline cartilage to be biomechanically functional, it has to be resistant to microscopic water circulation within the tissue [5]. This microlevel resistance results from the complex function of

Table 3 – ChS and KS levels and KS/ChS ratio in disc materials.

	Grade III $n = 28$	Grade IV $n = 43$	p
ChS (ng/mg protein)	11.09 ± 2.10	10.37 ± 1.53	0.098
KS (ng/mg protein)	882.48 ± 172.54	969.78 ± 179.49	0.046*
KS/ChS ratio	80.72 ± 14.05	95.25 ± 21.83	0.001*

Data were expressed as mean ± standard deviation.
* $p < 0.05$.
KS: keratan sulfate; ChS: chondroitin sulfate.

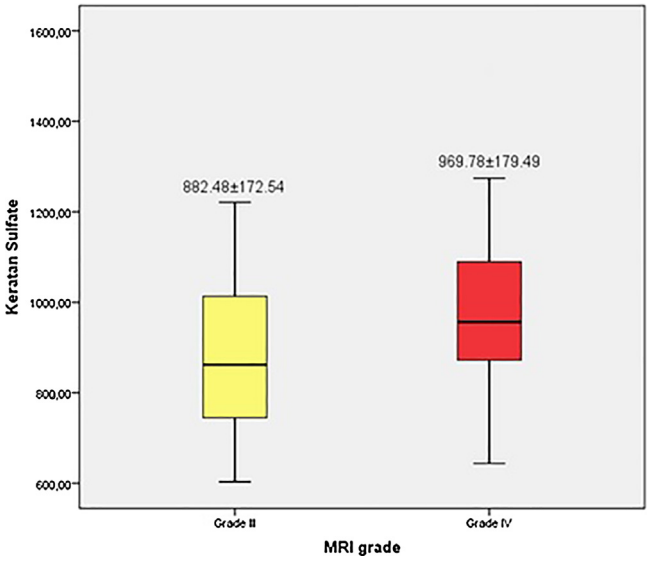

Fig. 2 – Correlation between the keratan sulfate levels and MRI grade. Boxplot showing the median and 25th and 75th quartiles of the mean keratan sulfate levels of grade III and IV patients. Significant differences between the groups are marked with an asterisk (*) ($p = 0.001$). *MRI: magnetic resonance imaging.

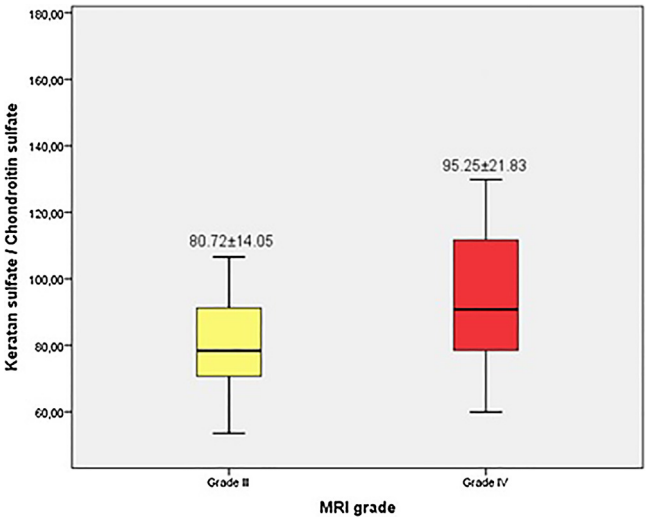

Fig. 3 – Correlation of the KS/ChS ratio and MRI grade. Boxplot showing the median and 25th and 75th quartiles of the mean KS/ChS ratio of grade III and IV patients. Significant differences between the groups are marked with an asterisk (*) ($p = 0.001$). *MRI: magnetic resonance imaging, KS: keratan sulfate; ChS: chondroitin sulfate.

hydrophilic proteoglycans. Mucopolysaccharides, also called glycosaminoglycans, form macromolecules known as ''core proteins'' (e.g. aggrecan). Most core proteins possess ChS and KS chains [3,6]. ChS has three primary functions: inhibiting harmful enzymes (proteases); increasing the glycosaminoglycan pool; and preventing synovial thrombi. In contrast with the role of ChS in the regeneration process, an increase in KS in the tissue is an indicator of biochemical degeneration [7].

Intervertebral disc degeneration can be graded using lumbar MRI with the fast-spin echo technique; the reliability across evaluators is high. The Pfirrmann classification differentiates disc degeneration into five grades based on MRI signal intensity, disc structure, difference between nucleus and annulus, and disc height [3,4].

The relationship between disc signal intensity and chemical composition and histological changes has been examined in several studies [8,9]. Progressive changes in the intervertebral disc have been suggested to be correlated with the loss of disc signal seen on T2-weighted MRIs [10]. The brightness of the nucleus is directly related to the proteoglycan concentration, rather than its fluid and collagen contents [11]. Changes in the hydration and composition of the disc can be seen with sufficient accuracy on MRIs [12].

Many systems for grading lumbar disc degeneration are available [13]. Most studies have focused on the posterior section of the disc and whether its shape is bulging, protruding, or extruding [14]. Few studies have examined the nature of the disc's structure [15]. In the Pfirrmann five-stage grading system, the most important differentiation in the algorithm is between disc height, separating grades IV and V. The difference in disc height does not distinguish grades III and IV [3]. As the algorithm does not take changes to the bone marrow in adjacent vertebrae into account, Modic et al. separated these into three types [10,16].

The earliest degenerative changes are in the nucleus pulposus, which contains dense proteoglycans. Spine-lock MRI techniques, such as quantitative T1ρ measurements, are a non-invasive method for determining the proteoglycan level in the disc. This allows degeneration to be identified at an early stage. However, current grading systems including the Pfirrmann five-stage grading system are somewhat deficient in terms of showing local degenerative changes [17].

Although quantitative T1ρ measurements provide information on the level of degeneration as reflected by the proteoglycan level in the disc, the most important parameter is not the total proteoglycan level, but the KS/ChS ratio [7]. A shift in the ratio toward KS shows actual degeneration because an increase in KS in the tissue is a biochemical indicator of degeneration [7,9]. This study compared the KS/ChS ratio in the disc material taken from patients during LDH surgery with the degree of radiological change. The relationship between this parameter and the structural form of the hernia was also evaluated.

In general, studies till date were conducted by taking samples from cadavers. The advantage of our study is that we used material taken from the living body during surgery, and thus the loss of water and the possibility of changes to the content can be disregarded.

The results showed that the KS/ChS ratios and KS levels in Pfirrmann grade IV patients were significantly higher ($p = 0.001$ and $p = 0.046$, respectively). These results suggest that Pfirrmann's degeneration grades, which were defined early in 2001 [3], are quite accurate and precise in terms of reflecting structural degeneration. In addition, the absence of a statistically significant relationship between disc structure and the KS/ChS ratio and KS levels suggests that the structure of the discs does not effect the level of biochemical degeneration in extruding, protruding, and fragmenting forms of LDH.

Three limitations to this study should be taken into consideration. First, according to the Pfirrmann's classification system, patients who underwent surgery were classified as grade III or IV. This can explain by that grade I and II discs have chance to be recovered without surgery because the discs have sufficient protein and water, grade V discs are collapsed and asymptomatic (no press on neural rootlets) discs. Second, the sample size was quite small. Third, the values of KS and ChS were not assessed in the blood of patients undergoing surgery.

5. Conclusions

In lumbar disc degeneration, the sensitivity of the currently used Pfirrmann five-stage radiological degeneration evaluation system is sufficient, except for showing local degeneration. Biochemical examination of degeneration showed that the radiological degeneration evaluation was satisfactory and the sensitivity and reliability of the Pfirrmann classification were sufficient. Future in vivo imaging studies prioritizing the level of biochemical markers will enable more sensitive interpretation on the subject of degeneration.

Conflict of interest

None declared.

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