Original Article

Lumbar disc herniation: Is there an association between histological and magnetic resonance imaging findings?

Shiju A Majeed, N Arun Kumar Seshadrinath, Kavitha Ravi Binoy[1], Laila Raji[1]

Abstract

Background: Although validated radiological scoring systems and histological scoring system of surgically removed degenerated disc are used in assessment of progression of intervertebral disc degeneration, there have not been many studies that integrate these two aspects of assessments. The data available in this respect are very limited. This clinical study was designed to find the correlation between quantitative radiological score (Pfirmann grading system and Modic changes [MC]) and quantitative histological degeneration score (HDS).

Materials and Methods: A cohort of 77 patients (45 males, 32 females; mean age of 38 years [range 18–58 years]) who presented with complaints of discogenic pain or radiculopathy at single level were assessed radiologically. They were graded according to the radiological pattern. The surgically excised disc specimen was graded according to HDS. The degree of radiological changes were correlated with the degree of histological changes.

Results: Though the overall HDS (0–15) did not show statistically significant correlation with Pfirmann grading system, there were positive association found between mucoid degeneration, chondrocyte proliferation with the Pfirmann grading and mucoid degeneration, which were statistically significant. Female sex also had a higher association with instability pattern.

Conclusion: The study shows that the Pfirmann grading system, MCs and HDS can reliably be used as scoring systems for assessing lumbar disc degeneration. The radiological assessment can be used as a noninvasive tool to assess the probable change in content rather than the microstructure of a disc undergoing degeneration.

Key words: Histological degeneration score, Modic changes, Pfirmann grading, surgical discectomy, lumbar disc herniation
MeSH terms: Interventional disc, disc, herniated, IV Disc displacement, magnetic resonance imaging

Introduction

Low back pain is a common clinical problem affecting millions of people. Intervertebral disc (IVD) degeneration is attributed as a common cause of low back pain. However, patients with varying degrees of IVD degeneration exist and their clinical picture is variable. In addition, there is a section of patients who have predominant radicular symptoms in their legs and tolerable or minimal back pain. The association between disc degeneration and clinical symptom profile of the patient is not clear. Age related changes in the IVDs are also similar to disc degeneration except for the diffusion delay across the endplates in aged discs as proved by Rajasekaran *et al.*,[1] change in the type of proteoglycans, decrease in type 2 collagen networks, endplate fractures, fissures in the annulus have all been seen in degenerate IVDs.[2-4] Magnetic resonance imaging (MRI) is currently used to assess the degeneration of IVDs.[5] MRI provides excellent anatomic detail of spinal tissues, but fails to provide the type of information that permits a definitive diagnosis in many patients with back pain.[5] Anatomic imaging of the spine is

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highly accurate in the evaluation of non mechanical causes of back pain and less beneficial in the evaluation of back pain, that is, due to mechanical causes.[5] MRI helps in noting the signal changes in the discs, which have resulted from changes in the biochemical alterations in the disc while micro structural changes are less appreciated. MRI also helps to note the changes in endplates of the vertebra known as Modic changes (MC).[6] It can structurally classify a disc herniation to bulge, protrusion, extrusion and sequestration. Pfirmann[7] has graded the disc degeneration seen in T2 sagittal sequence of fast spin echo MRI into five grades. A normal well hydrated disc with a clear demarcation between nucleus pulposus and the annulus is graded as Grade 1 whereas a completely collapsed disc with absent nucleo-annular differentiation is considered as Grade 5. However, this grading is an arbitrary grading based on MRI characteristics alone. Though the histological changes in composition were taken into account while making the scoring system, it is also not clear whether the structural histopathological changes in the disc will be reflected in the routine MRI T2 protocols. MCs[6] have been graded into type 1–3 depending on the signal intensity in T1 and T2 sequences.

Boos *et al*.[8] categorized the histopathological changes in the degenerating IVDs in cadavers. They found that chondrocyte proliferation, granular changes, tears or cleft formation, mucoid degeneration, rim lesions and edge neovascularization to be the common findings in the degenerated IVDs. Based on this study, they developed a histological degeneration score (HDS) for the pathological grading of the degenerated discs. Weiler *et al*.[9] in his study validated this in surgically removed specimens in Caucasian patients undergoing discectomies and found that the first 4 variables proposed by Boos *et al*.[8] remained true for the surgically removed specimens [Table 1].

We studied the histological changes in herniated disc tissue and to find out if there is any kind of correlation between this and MRI findings of disc degeneration and end plate changes.

Materials and Methods

77 consecutive patients undergoing lumbar discectomies for lumbar disc herniation (LDH) were studied for HDS. All patients were of Indian origin. Ethical clearance from Institutional Ethics Committee was obtained and all patients gave valid informed consent. All patients underwent plain radiographs of lumbosacral spine with flexion and extension views and MRI assessment with 1.5 Tesla machine. Our criteria for inclusion were patients who had single level LDH, who had failed a period of atleast 2 months of conservative care. We excluded chronic low back pain patients, patients with multilevel spinal canal stenosis and patients with evidence of higher grades of instability (spondylolisthesis of Graded 2 or above) in plain radiographs. Thus, we had 14 patients with Grade 1 instability at a single level in the cohort of 77 patients as evidenced in flexion extension lateral views of lumbosacral spine.[10] (>3.5 mm of anterior translation).

Table 1: Modified parameters collected for the histologic assessment of disc degeneration and scoring (Boos *et al*. 2002)

Criteria	Grading
Cell density (chondrocyte proliferation): Multiple chondrocytes growing in small rounded groups or clusters sharply demarcated by a rim of territorial matrix	0=no proliferation 1=increased cell density 2=connection of two chondrocytes 3=small size clones (several chondrocytes grouped together, 3-7 cells) 4=moderate size clones (8-15 cells) 5=huge clones (>15 cells)
Structural alterations (tears and clefts): Concentric tears following the collagen fiber bundle orientation in the annulus fibrosus or radiating defects extending from the nucleus pulposus to the outer annulus lamellae parallel or oblique to the end-plate (clefts)	0=absent 1=rarely present 2=present in intermediate amounts between 1 and 3 3=abundantly present 4=scar/tissue defects
Granular changes: Eosinophilic-staining amorphous granules within the fibrocartilage matrix	0=absent 1=rarely present 2=present in intermediate amounts between 1 and 3 3=abundantly present
Mucous degeneration: Cystic, oval or irregular areas with intense deposition of acid mucopolysaccharides (i.e., sulfated glycosaminoglycans) staining dark blue with alcian-PAS	0=absent 1=rarely present 2=present in intermediate amounts between 1 and 3 3=abundantly present
HDS	0-15 points

HDS=Histologic degeneration score, PAS=Periodic acid–Schiff

The Pfirmann grading[7] of the concerned disc was noted according to the criteria suggested by Pfirmann by two experienced radiologists. The presence of MCs[7] in the endplates was also noted. MRI assessment of the grade of disc protrusion was also done.

During surgical discectomy, the entire removed disc fragment was collected and stored in 10% formaldehyde, decalcified for 72 h, fixed in paraffin and thin sections of 2–4 μm was made. At least 5 sections were made from each specimen and analyzed by 2 experienced pathologists. Hematoxylin and eosin staining were performed and HDS was assessed. Alcian blue staining in acidic pH[11] was done to assess the mucoid changes. As per Boos *et al*. criteria, the HDS of the discectomy specimen was calculated taking into account four parameters: (1) Chondrocyte proliferation, (2) Tears or clefts, (3) Granular changes and (4) Mucoid changes [Figures 1-4]. The maximum HDS was 15. The CD34

immunohistochemistry was used to identify blood vessels in the inner annulus/nucleus and S100 immunohistochemistry was used to identify Schwann cells in the inner annulus/nucleus[12] [Figure 5a and b]. A histomorphological distinction between annular and nuclear disc tissue was performed by the use of light microscopic criteria particularly under polarized light, allowing the evaluation of the organization of the collagen network. Three kinds of tissue were identified in the discectomy specimens. (a) Annulus fibrosus: Fibrocartilage that includes large crimped collagen fibers organized in lamellae, with elongated fibroblast like cells lying between lamellae. (b) Nucleus pulposus: Abundant irregular matrix is containing clefts, with a sparse population of rounded cells. (c) Cartilage endplate: Typical hyaline cartilage, with an amorphous matrix and with chondrocyte-like cells exhibiting clearly visible perinuclear halo.

Alcian blue stains[10] the acidic mucopolysaccharide matrix blue. It is suggested that there is a relative increase in the more acidic keratin sulfate in degenerated discs.[11] The intensity of the alcian blue staining of the specimen is graded from 0 to 3 (0 being absent alcian blue staining and 3 being intense homogenous blue staining).

The following parameters were statistically analyzed: Sex versus type of disc herniation, sex versus instability, sex versus MCs, MCs versus HDS, Pfirmann grades versus HDS, Pfirmann grades versus alcian blue, MC versus HDS and chondrocyte proliferation versus MCs.

The Chi-square test was used for the analysis (SPSS software, Ver. 16.0, by SPSS Inc., IBM, Armonk, New York, USA). $P < 0.05$ was taken as significant.

Results

There were 45 males and 32 females in this series. The mean age was 38 years (range 18-58 years). Forty eight

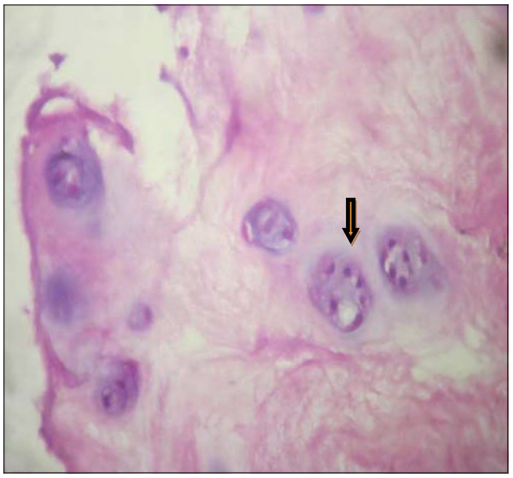

Figure 1: Histopathological slide showing chondrocyte proliferation (black arrow) seen in herniated disc material. Haematoxylin and Eosin stain. ×100 magnification

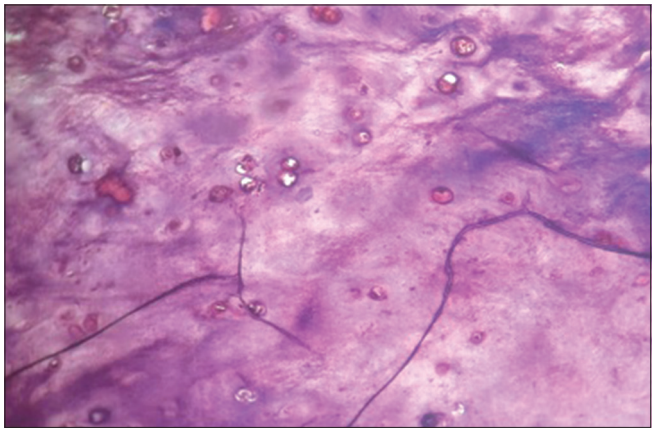

Figure 3: Photomicrograph of hyaline cartilage seen in herniated disc specimen showing chondrocytes and cartilage matrix

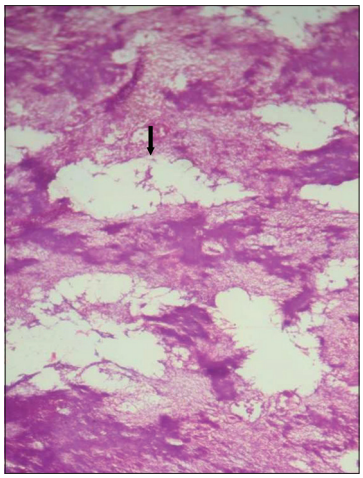

Figure 2: Granular changes, tears and clefts marked by black arrow. ×100 magnification. H and E stains

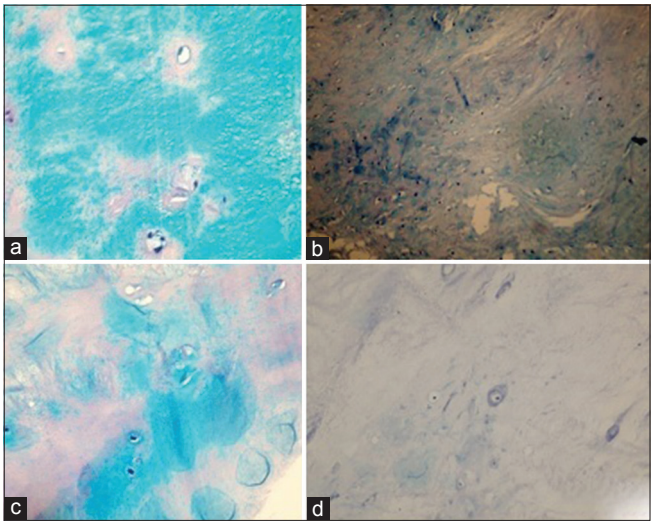

Figure 4: (a-d) Alcian blue staining of Herniated nucleus pulposus showing different grades

discs were protruded, 23 were extruded and six were sequestrated in the entire series. Sex wise distribution of the type of herniation is shown in Table 2 and Figure 6. There was no association between the type of disc herniation and sex ($P = 0.198$).

Sex versus instability

We looked at whether there was any association between sex and instability seen in plain radiographs 34.4% of females showed radiographic evidence of instability while only 6.7% of males showed instability. Thus, female sex has higher incidence of instability ($P = 0.002$, odds ratio 7.333, [1.845–29.146]) [Table 2 and Figure 7].

MC versus sex; MC versus Pfirmann Grades; MC versus histological degeneration score; MC versus chondrocyte proliferation

MC (either 1 or 2) was present in 28.2% of females and 26.7% of males. No significant association was found between sex and MCs ($P = 0.917$) [Table 2 and Figure 8]. About 27.27% of cases showed MC. No significant association was found between Pfirmann grades and MC. The analysis of variance was done to compare mean HDS between the three Modic groups. No statistically significant difference was seen in mean HDS of different Modic groups [Table 3 and Figure 9].

However, following findings were notable. Among cases that showed no MC, 15 out of 56 had evidence of hyaline cartilage and in the set with MC, 16 out of 21 had the presence of hyaline cartilage. Fisher two-tailed *t*-test was done and this was found to be statistically significant ($P = 0.0002$). We categorized the degree of chondrocyte proliferation, low grade being Grade 0–3 and high grade being 4 and 5 and compared with the presence or absence of MCs, there was a statistically significant association ($P = 0.02$) [Table 4 and Figure 10]. No other parameter used in the HDS had a significant association between MCs. There was no association between the presence of CD34 and MCs, in our study ($P = 0.989$).

Pfirmann grades versus sex, Pfirmann grades versus histological degeneration score, Pfirmann grades versus alcian blue score

The predominant grading of Pfirmann seen in the group was Grade 3 amounting to 51.1% in males and 53.1% in females. There was no significant association between grades of Pfirmann and sex [Table 5 and Figure 11]. Again when grades of Pfirmann were compared with HDS, there was no association ($P = 0.123$) [Table 6 and Figure 12]. This shows that a single Pfirmann grade can comprise a wide varying degree of histological changes. The MRI grading of disc degeneration by Pfirmann does not completely reflect the pathological changes going on in the herniated discs. Pfirmann *et al.*[7] have rightly commented in their paper that this grading system can reflect the changes in chemical composition (histologically this being

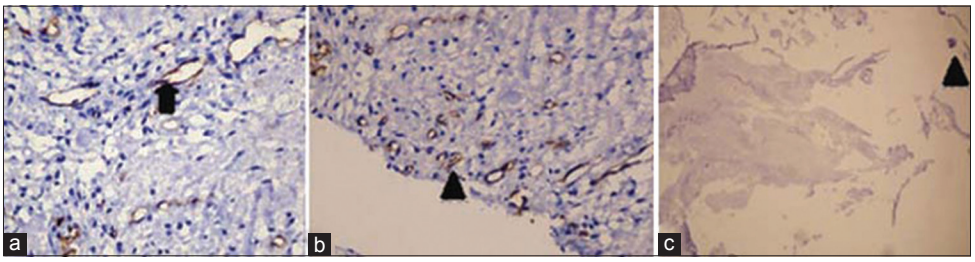

Figure 5: Immunohistochemistry showing CD34 positivity (a and b) and S100 positivity (c)

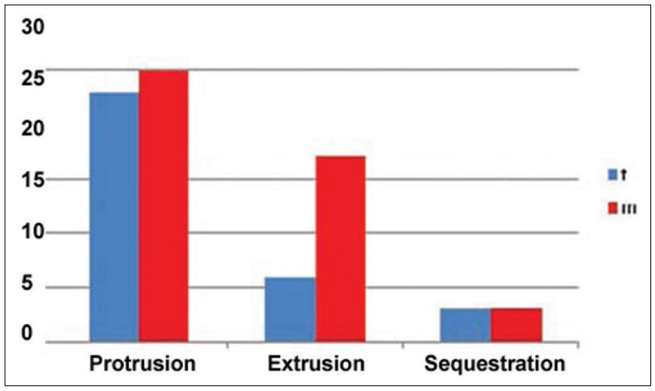

Figure 6: Bar diagram showing the sex wise distribution of various types of disc herniation

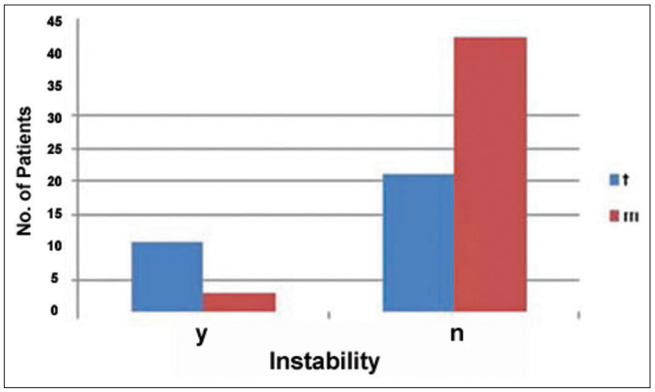

Figure 7: Bar diagram showing the relationship between sex and instability

Table 2: Sex versus disc type, instability and MC

Sex	Type of herniation (%)			Instability (%)			MC (%)		
	Protrusion	Extrusion	Sequestration	Yes	No		0	1	2
Female	23 (71.9)	6 (18.8)	3 (9.4)	11 (34.4)	21 (65.6)		23 (71.9)	6 (18.8)	3 (9.4)
Male	25 (55.6)	17 (37.8)	3 (6.7)	3 (6.7)	42 (93.3)		33 (73.3)	7 (15.6)	5 (11.1)
	χ^2	Df	*P*	χ^2	df	*P*	χ^2	Df	*P*
Pearson Chi-square	3.242 (<5.991)	2	0.198 (>0.05)	9.652 (>3.841)	1	0.002 (<0.05)	0.173 (<5.991)	2	0.917 (>0.05)

Odds Ratio=7.333 (1.845-29.146) 95% CI. MC=Modic changes

the amount of proteoglycans) rather than the alteration in micro structure.

There was a statistically significant association between the alcian blue scoring and the Pfirmann grading ($P = 0.000$). There is an increased association between alcian blue grades with Pfirmann 2 and 3 while alcian blue scores are found to decrease in Pfirmann 4. The graphs show a positive trend in Pfirmann 2 and 3 and a negative trend in Pfirmann 4 [Table 6 and Figure 13].

Pfirmann grades versus MC

No significant association was found between varying grades of Pfirmann and MC on analysis with Chi-square test ($P = 0.416$) [Table 6 and Figure 14].

Discussion

Degenerated intervertebral discs show increased chondrocyte proliferation into clones as opposed to the scanty presence of discal cells in a normal disc.[13] Discal cells are metabolically less active cells[13] which depend on diffusion of oxygen and solutes across endplates for their nutrition.[14] The changed biomechanical environment during degeneration might result in loss of apoptotic mechanism and result in cell proliferation.[15] The relative proportion of acidic keratin sulfate to chondroitin sulfate increases in disc degeneration[11] possibly due to secretion of altered form of glycosaminoglycans by the chondrocytes. Large chain aggrecan molecules become fragmented resulting in decreased water imbibing capacity.

MCs have been suggested to be due to endplate micro fractures (type 1) and their replacement by fibro-fatty tissue (type 2). Albert and Manniche[16,17] proposed two theories for their occurrence: An infective possibility where the endplate changes are due to anaerobic bacterial invasion through the ruptured disc and a mechanical theory where endplate changes are due to micro fractures due to compressive loads in the vertebrae. Shan *et al.*,[18] proved that presence of MCs in adjacent endplates of herniated discs are associated with cartilaginous herniations that are less resorbed than the nucleus rich herniations, Presence of MCs in LDH may indicate a subset of patients who are less likely to benefit by conservative care. Our study also had significantly higher proportion of hyaline cartilage and chondrocyte proliferation in discs with MCs.

Presence of blood vessels was seen in 56% of cases in outer annulus while in 20% of cases they were seen extending to inner annulus and nucleus. Uncontained disc herniations had higher presence of blood vessels which was in concurrence with Yasuma *et al*.[19] In a healthy adult disc, only the outermost regions have sparse blood vessels.[20] The tear in the annulus, which precedes disc prolapse, could be acting like an angiogenic stimulator for the blood vessels.[21]

Table 3: Frequency table MC versus HDS

MC	*n*	Minimum HDS	Maximum HDS	Mean HDS	SD
0	44	3	14	8.70	2.502
1	10	6	13	9.90	2.079
2	7	6	12	9.00	2.449

ANOVA

HDS	Sum of squares	df	Mean square	*F*	*P*
Between groups	11.679	2	5.839	0.984	0.380
Within groups	344.059	58	5.932		
Total	355.738	60			

HDS=Histologic degeneration score, SD=Standard deviation, MC=Modic changes, ANOVA= Analysis of variance

Table 4: Frequency table chondrocyte proliferation versus MC

Chondrocyte proliferation	MCs	
	No	Yes
Low	45	11
High	11	10

Chi-square tests

	χ^2	df	*P*
Pearson Chi-square	6.026538 (>3.841)	1	0.014 (<0.05)

Risk estimate

	Value	95% CI	
		Lower	Upper
OR	3.719	1.262	10.959

OR=Odds ratio, CI=Confidence interval, MC=Modic changes

Table 5: Frequency table sex versus grades of Pfirmann

Sex	Pfirmann grade		
	2	3	4
Female	8 (25)	17 (53.1)	7 (21.9)
Male	11 (24.4)	23 (51.1)	11 (24.4)

Chi-square tests

	χ^2	df	*P*
Pearson Chi-square	0.070 (<5.991)	2	0.966 (>0.05)

No significant association with Chi-square test

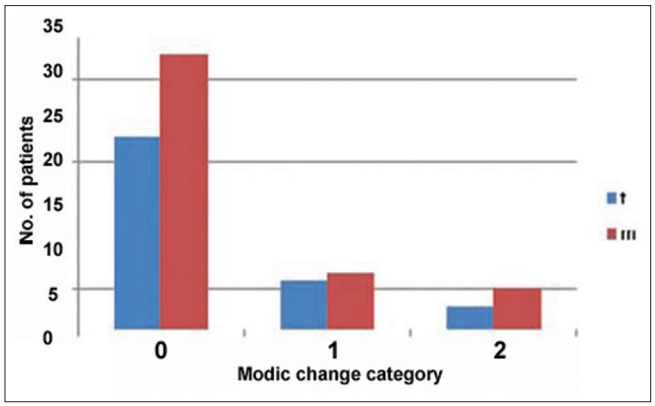

Figure 8: Bar diagram showing the distribution of MC in both sexes

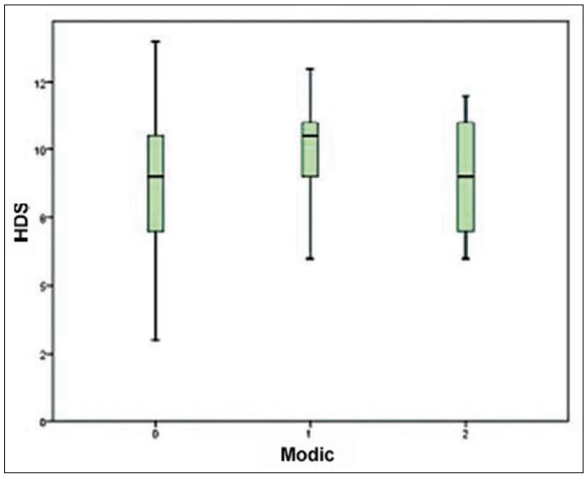

Figure 9: A diagram showing histological degeneration score distribution in various MC

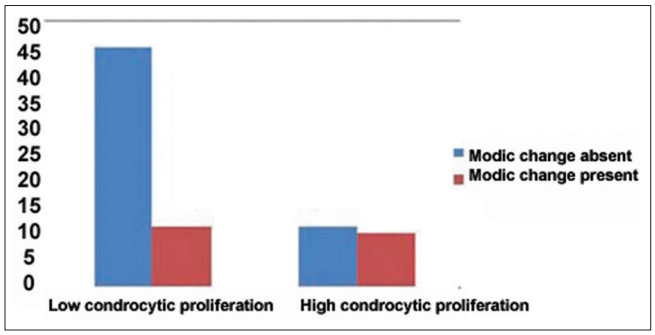

Figure 10: Bar diagram showing the relationship between chondrocyte proliferation and presence of MC

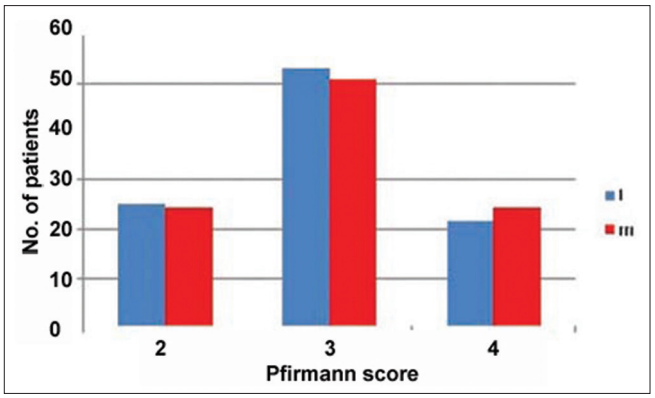

Figure 11: Bar diagram showing the distribution of Pfirmann grades in both sexes

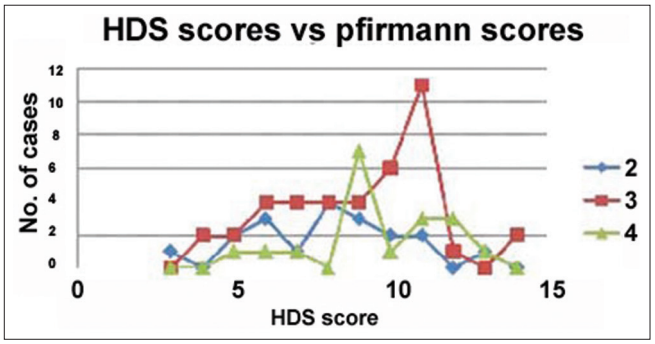

Figure 12: Graph showing distribution of histological degeneration score in various Pfirmann grades

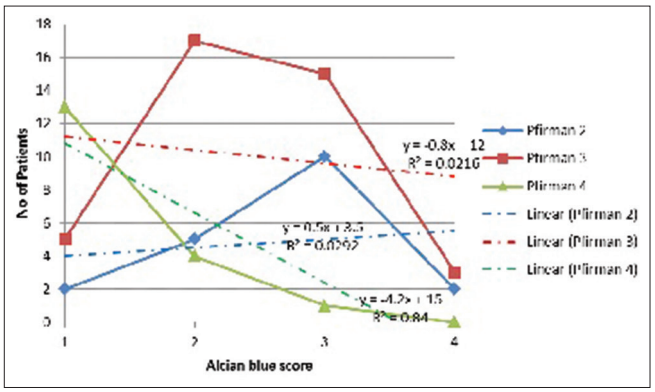

Figure 13: Graph showing the relation between Pfirmann grades and alcian blue scores (note the inverse association between Pfirmann grade 4 and alcian blue scores)

Koike *et al.*,[22] also identified the presence of vascular proliferation and macrophage infiltration in herniated tissue and suggested that the purpose and mechanism of angiogenesis could be different from the small number of blood vessels seen in the outer annulus during aging.

S100 positivity as a marker of Schwann cells[23] was present only in cases, which had blood vessels. Hence, the presence of blood vessels might help in neuronal migration into the herniated nucleus. Studies that have used glial fibrillary acid protein, calcitonin gene related peptide and substance *P* staining have been able to demonstrate varying types of nerve fibers in the inner annulus and nucleus of degenerated discs.[24-28]

The nucleus pulposus contains water (87%),[19] high molecular weight aggrecan and collagen. The annulus contains more of type 2 collagen, elastin and sparse long chain proteoglycans.[29,30] The quantity of high

Table 6 : Frequency table Pfirmann grade versus HDS, alcian blue score and MC

Pfirmann grade	HDS out of 15												Alcian blue scoring				MC		
	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	0	1	2
2	1	0	2	3	1	4	3	2	2	0	1	0	2	5	10	2	15	1	3
3	0	2	2	4	4	4	4	6	11	1	0	2	5	17	15	3	29	7	4
4	0	0	1	1	1	0	7	1	3	3	1	0	13	4	1	0	12	5	1
			χ^2			df			P				χ^2		df	P	χ^2	df	P
Pearson Chi-square	29.814 (<33.924)					22			0.123 (>0.05)				29.408 (>12.592)		6	0	3.930 (<9.488)	4	0.416 (>0.05)

HDS=Histologic degeneration score, MC=Modic Changes

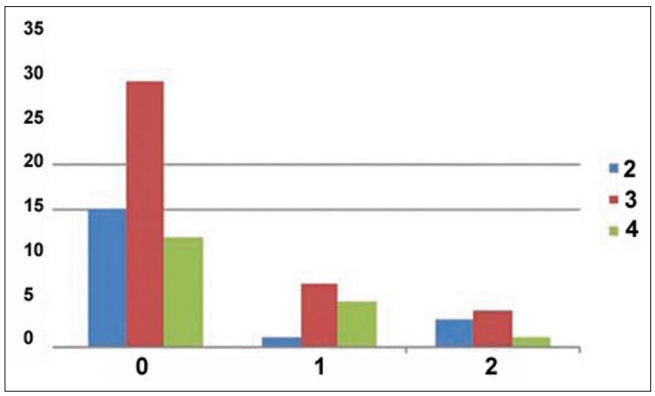

Figure 14: Bar diagram showing distribution of Pfirmann grades and MC

molecular weight proteoglycans and water decrease centrifugally in a disc. As degeneration starts, the water and proteoglycan content of the nucleus decreases, nucleus fissures and extends into the annulus. Later the degradation of type 2 collagen results in fissuring of the lamellar layers of annulus through which nucleus is prone to prolapse. These events lead to altered mechanical properties of the IVD.[3,4,27-30] As the discs degenerate, the differentiation between the nucleus and annulus is lost to varying degrees.

The composition of a disc herniation is clinically important. Nucleus pulposus has high proteoglycan content and only a loose collagen network, therefore when it escapes from the pressurized confines of a loaded disc it swells rapidly in tissue fluid, before shrinking again as much of its proteoglycans leach out. This probably explains the typical "resorption" of many disc herniations, leading to spontaneous resolution of symptoms. Herniated annulus also can swell, but by a lesser amount and its high collagen content leads to rapid invasion by blood vessels and inflammatory cells, leading to some tissue resorption. Less is known about the significance of hyaline cartilage within a herniation. Articular cartilage (a type of hyaline cartilage) does not swell greatly, even when physically disrupted because its collagen type 2 fibrils form a dense three-dimensional network.[31] A lack of swelling restricts proteoglycan loss and subsequent resorption. This could possibly explain why a "hard" disc herniation containing hyaline cartilage can give rise to more severe and persisting signs of nerve root compression.[32,33]

Prior to herniation, the intervertebral disc goes through phases of degeneration.[34] Pfirmann grading of disc degeneration in MRI is based on the nucleo annular differentiation seen in T2 sequencing. This reflects the amount of water imbibed in the two compartments of the disc. It would be worthwhile to know whether the Pfirmann grading has a correlation with the actual proteoglycan content in the disc material. Our results show that in Pfirmann Grades 2 and 3, there is a positive correlation between glycoprotein content and Pfirmann grade. However, higher Grades 4 and 5 show an inverse correlation to the glycoprotein content. Thus, it is possible to postulate that the lack of nucleo-annular differentiation seen in advance Pfirmann grades is due to poor water content consequent to the lack of adequate glycoprotein in the degenerate discs. Pfirmann 4 and 5 have more annular content in the herniation as the amount of glycoprotein is less. Patients with more annular content and cartilaginous content could have a more protracted period of pain while a herniation with predominant nucleus pulposus content can expect to become better in a shorter period of time.[33] This postulate needs to be further studied.

Discal cells in degenerated discs secrete catabolic enzymes like metalloproteinase 3 which break down aggrecan molecules in smaller chains which are inferior in their capacity to imbibe water. In addition, it is postulated that increase in metalloproteinase 3 can positively regulate metalloproteinase 1, which are responsible for lysis of collagen fibers.[35-37]

The histological changes in the degenerative disc are well known and have been extensively described in the literature. However, a well-validated histological grading system for IVD degeneration is currently lacking. Since many translational studies are using histological changes as the primary outcome variable, a comprehensive and validated histological grading for IVD degeneration is essential to the field of IVD research. In IVD regeneration research, we need to look into the histology of the disc degeneration to bring in clues that might help in their regeneration.

Again, female sex has a higher propensity for instability as shown in our series. It is also an established fact that degenerative spondylolisthesis is much more common in

females than males. The role of sex hormones in discal degeneration needs to be studied further.

Conclusion

Lumbar disc herniation comprises a spectrum of histomorphological changes in the disc tissue. The herniated disc contains varying amounts of nucleus, annulus and hyaline cartilage. Present MRI grading of disc degeneration correlates only with the quantitative change in glycoproteins in the disc tissue and does not show the microstructural alterations in a degenerated disc. Presence of modic changes in the endplates adjacent to the herniated disc level suggests significant presence of hyaline cartilage in the prolapsed disc tissue.

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Conflicts of interest

There are no conflicts of interest.

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