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Correlation of Matrix Metalloproteinase–3 Expression with Patient Age, Magnetic Resonance Imaging and Histopathological Grade in Lumbar Disc Degeneration

Lomber Disk Dejenerasyonunda Matriks Metalloproteinaz-3 Ekspresyonunun Hastanın Yaşı, MR ve Histopatolojik Derecesi ile İlişkisi

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ABSTRACT

AIM: The purpose of the present study is to analyze the expression of matrix metalloproteinase-3 (MMP-3), magnetic resonance imaging (MRI) grading and histopathological alterations of the intervertebral disc (IVD) for correlations with each other and with the age, gender and low back pain duration of the patients who had undergone operations for lumbar disc herniation (LDH).

MATERIAL and METHODS: Forty-two patients were admitted to our clinic with signs of LDH and underwent surgery for LDH at 48 IVD levels. In all cases, specimens for histological and immunohistochemical analyses were removed from the IVD space. Lumbar IVD degeneration on MRI of the 48 IVDs from which surgical specimens had been obtained was classified into five grades using the Pfirrmann classification**.**

RESULTS: In the degenerated IVD, the expression of MMP-3, MRI grading and histopathological alterations of the IVD displayed significant correlation. Increased age is closely related with aforementioned alterations. There was no correlation between MMP-3 expression and age, gender and duration of the pain.

CONCLUSION: For evaluating and treating IVD degeneration, MRI is a good and non-invasive diagnostic tool to determine the severity of degeneration. MMP-3 may be a therapeutic target of the degenerated IVD.

KEYWORDS: Disc degeneration, Histopathological changes, Matrix metalloproteinase-3, Magnetic resonance imaging

ÖZ

AMAÇ: Bu çalışmanın amacı lomber disk hernisi (LDH) tanısı ile ameliyat edilen hastalarda matriks metaloproteinaz-3 (MMP-3) ekspresyonunun manyetik rezonans (MR) görüntüleme bulguları, diskdeki histopatolojik değişiklikler, hasta yaşı, cinsiyeti ve ağrının süresi ile olan ilişkisini araştırmaktır.

YÖNTEM ve GEREÇLER: Kırkiki adet LDH tanısı konulmuş olan hasta kliniğimize müracaat etmiş ve toplam 48 adet intervertebral disk seviyesinden opere edilmiştir. Tüm olgularda intervertebral disk aralığından alınan örnekler histolojik ve immünhistokimyasal incelemeye tabii tutulmuştur. MR görüntülerindeki lomber disk dejenerasyonu Pfirrmann sınıflandırmasına gore 5 dereceye ayrılmıştır.

BULGULAR: Dejenere intervertebral disk örneklerinde MMP-3 ekspresyonu, MR derecesi ve histopatolojik değişiklikler arasında anlamlı ilişkili tesbit edilmiştir. Yaş arttıkça bu ilişki daha belirgin hale gelmiştir. MMP-3 ekspresyonu ile hasta yaşı, cinsiyeti ve ağrı süresi arasında anlamlı bir ilişki yoktur.

SONUÇ: İntervertebral disk dejenerasyonunun değerlendirilmesi ve tedavi edilmesi için MR çok iyi ve non-invaziv bir tanı metodudur. Bu aynı zamanda disk dejenerasyonunun ciddiyetini de gösterir. MMP-3 dejenere intervertebral disk hernilerinde tedavinin hedefi olabilir.

ANAHTAR SÖZCÜKLER: Disk dejenerasyonu, Histopatolojik değişiklikler, Matriks metaloproteinaz-3, Manyetik rezonans görüntüleme

INTRODUCTION

The intervertebral disc (IVD) has a unique structure composed of the annulus fibrosus and the nucleus pulposus and possesses elastic properties enabling resistance to compressive loads and the distribution of the loads on the spinal column. The molecular components of the IVD, which include collagen, proteoglycans, and water, undergo considerable degenerative alterations with age (3, 20). The integrity of the IVD relies mostly on the balance between these contents (25). The disintegration of the IVD is primarily executed by proteinases (9, 16). Among these proteinases, matrix metalloproteinase 3 (MMP-3, stromelysin-1) has been found in increased concentrations and has been evaluated as an important element in IVD degeneration (1, 27).MMP-3 is efficient at degrading non-collagenous extracellular matrix including; proteoglycans, gelatin, fibronectin and laminin; additionally, MMP-3 is capable of activating latent collagenases (matrix metalloproteinase 1) (5, 9, 23).

Magnetic resonance imaging (MRI) is the most important diagnostic device for clinical evaluation of IVD degeneration, for it is capable of non-invasively analyzing the water content and biochemical environment of the IVD, and grading the degree of IVD degeneration (2, 19).

The purpose of the present study is to analyze the expression of MMP-3, MRI grading and histopathological alterations of the IVD for correlations with each other and with the age, gender and low back pain duration of the patients who had undergone operations for lumbar disc herniation (LDH).

MATERIAL and METHODS

Between 2010 and 2011, 42 consecutive patients were admitted to our clinic with signs of LDH and underwent surgery for LDH at 48 IVD levels. Patients with a history of inflammatory joint disease were excluded from the study. Surgery was planned based on clinical signs, physical examination findings, MRI results, patient's decision and agreement of two neurosurgeons in terms of indication. A micro-discectomy procedure was performed on all of the patients. In all cases, specimens for histological and immunohistochemical analyses were removed from the IVD space. No extruded or sequestrated part of the IVD was used for analyses. All of the operations were performed under general anesthesia. Prophylactic antibiotic treatment with a first generation of cephalosporin (cefazoline 1 gr, im) was administered at 30 minutes prior and 6 hours after the procedure for every patient. All of the patients were mobilized six hours after the surgery and discharged home one day after the surgery. There were no complications related to the surgery.

One pathologist, who was blinded to the study subjects, performed all histomorphological examinations independently to avoid observer bias. The material was immediately fixed in buffered 10 % formaldehyde. All samples were embedded in paraffin, sectioned by microtome into slices (2–4 µm) and placed on silanized glass slides both for histochemical stainings with hematoxylin and eosin, alcian blue, and additionally for immunohistochemistry. Histomorphological analyses of the disc specimens were performed for the nucleus pulposus using a recently developed grading system (3). Briefly, this grading system takes into account the cell density, structural alterations (tears and clefts), mucoid degeneration, granular matrix changes and cell death at varying scores. The sum of scores for each criterion ranged between 0 and 18 points.

Immunohistochemical staining procedures were done with mouse monoclonal antibodies specifically reacting with MMP-3 with a dilution of 1/10 for 60 seconds (GeneTex, (SPM293), catalog number: GTX17790). Next, using a micrometer under an optical microscope, five portions, each measuring 0.5 x 0.5 mm, were randomly selected from each section after immunohistological staining, and the number of MMP-3 positive cartilaginous cells was calculated as the positive cell ratio (number of positive cartilaginous cells/total number of cartilaginous cells x 100[%]). The evaluation of the immunohistochemical staining procedure was performed independently of the histomorphology examination, to exclude any bias.

Lumbar IVD degeneration on MRIof the 48 IVDs from which surgical specimens had been obtained was classified into five grades using Pfirrmann classification (19). Briefly, this grading system takes into account the structure of the IVD, distinction of nucleus pulposus and annulus fibrosus, signal intensity and height of IVD at varying degrees. Three observers with different levels of experience analyzing spinal MRIs (i.e., a neurosurgeon and two radiologists) graded each of the 48 lumbar IVDs on the T2-weighted sagittal images. In all, 48 selected MRIs were randomly ordered in three sets of 16 MRIs and interpreted independently by the three observers. When disagreement arose, the images were viewed in conference and a consensus was reached.

General linear models along with Duncan multiple compression tests were used for comparisons of gender, age, low back pain duration, MRI grading, histological and immunohistochemical scores. Correlation analyses were done for pairwise correlation between age, pain duration, MR grading, histological and immunohistochemical scores (6). PROC GLM, and PROC CORR procedures in SAS (SAS Institute, Inc.) were used for analyses of obtained data. Statistical significance was set at the $P < 0.05$ level and post-hoc pairwise comparisons were performed when significance reached $P < 0.05$.

RESULTS

General properties of the patients are summarized on Table I.

Patient Population

Thirty-six patients underwent single disc level and 6 patients underwent two-level procedures (18 females and 24 males). The average patient age was 46.35 (±12.97) years. Comparing the age for each gender showed a high homogeneity (p=0.87). The average duration of the low back pain was 19.19 (±8.09)

Table I: General Properties of the Patients

No	Age-Gender	Level	Annular rupture	Low back pain duration (month)	Pfirrmann grade	Histological degeneration score	MMP-3s
1	32- M	L5-1	+	12	4	A:5 B:3 C:3 D:3 Total score: 14	70/100
2	59-M	L4-5	+	24	4	A:5 B:3 C:3 D:3 Total score: 14	60/100
3	59-F	L5-1	-	12	3	A:4 B:2 C:2 D:2 Total score: 10	55/100
4	41-M	L4-5	+	18	4	A:5 B:3 C:3 D:3 Total score: 14	70/100
5	31-M	L4-5	-	18	4	A:4 B:2 C:2 D:3 Total score: 11	70/100
		L5-1	+		4	A:4 B:2 C:3 D:3 Total score: 12	70/100
6	60-M	L4-5	-	30	4	A:4 B:3 C:3 D:3 Total score: 13	70/100
7	40-M	L4-5	-	24	4	A:4 B:3 C:2 D:2 Total score: 11	40/100
		L5-1	-		3	A:5 B:2 C:2 D:2 Total score: 11	40/100
8	31-F	L4-5	+	24	4	A:4 B:2 C:2 D:2 Total score: 10	70/100
		L5-1	+		5	A:5 B:2 C:2 D:2 Total score: 11	80/100
9	61-M	L4-5	-	30	4	A:5 B:3 C:3 D:3 Total score:14	70/100
10	42-F	L4-5	+	18	4	A:3 B:2 C:2 D:2 Total score: 9	45/100
		L5-1	+		3	A:3 B:3 C:2 D:2 Total score: 10	50/100
11	63-F	L5-1	-	30	5	A:6 B:4 C:4 D:4 Total score: 18	80/100
12	35-M	L2-3	+	18	4	A:4 B:3 C:2 D:3 Total score: 12	60/100
13	62-F	L4-5	-	30	5	A:5 B:3 C:4 D:4 Total score: 16	70/100
14	28-M	L5-1	-	12	4	A:3 B:3 C:2 D:2 Total score: 10	60/100
15	35-F	L5-1	-	18	4	A:6 B:4 C:4 D:4 Total score: 18	70/100
16	62-F	L4-5	+	30	4	A:6 B:3 C:4 D:4 Total score: 17	80/100
		L5-1	+		5	A:5 B:3 C:3 D:3 Total score: 14	80/100
17	34-F	L4-5	+	6	3	A:5 B:3 C:4 D:3 Total score: 15	80/100
18	59-F	L4-5	-	24	4	A:6 B:3 C:4 D:3 Total score: 16	80/100
19	64-M	L3-4	-	30	4	A:6 B:3 C:4 D:4 Total score: 17	60/100
20	31-M	L4-5	-	8	3	A:3 B:3 C:4 D:3 Total score: 13	50/100

21	39-M	L4-5	-	10	3	A:4 B:3 C:4 D:4 Total score: 15	60/100
22	52-M	L4-5	-	24	4	A:6 B:3 C:3 D:3 Total score: 15	60/100
23	58-M	L5-1	-	12	3	A:3 B:3 C:2 D:2 Total score: 10	40/100
24	36-F	L5-1	-	18	4	A:6 B:3 C:2 D:3 Total score: 14	50/100
25	38-M	L4-5	-	8	3	A:4 B:3 C:4 D:4 Total score: 15	70/100
26	26-M	L4-5	-	6	3	A:4 B:3 C:2 D:2 Total score: 11	70/100
27	31-M	L5-1 L4-5	+ -	6	3 3	A:3 B:3 C:1 D:2 Total score: 9 A:3 B:3 C:1 D:2 Total score: 9	70/100 70/100
28	51-F	L4-5	+	12	4	A:4 B:3 C:1 D:2 Total score: 10	80/100
29	68-F	L4-5	+	18	4	A:5 B:4 C:4 D:3 Total score: 16	60/100
30	53-F	L4-5	-	30	5	A:6 B:3 C:4 D:4 Total score: 17	70/100
31	31-F	L4-5	-	18	4	A:3 B:3 C:2 D:2 Total score: 10	60/100
32	42-M	L4-5	-	18	4	A:6 B:3 C:4 D:3 Total score: 16	80/100
33	54-M	L4-5	-	24	4	A:5 B:2 C:3 D:3 Total score: 13	60/100
34	36-F	L5-1	-	18	4	A:4 B:3 C:3 D:3 Total score: 13	50/100
35	51-M	L5-1	-	30	4	A:5 B:3 C:4 D:4 Total score: 16	80/100
36	43-M	L5-1	-	18	4	A:3 B:3 C:4 D:3 Total score: 13	60/100
37	65-M	L4-5	-	30	5	A:5 B:3 C:4 D:3 Total score: 15	80/100
38	69-M	L3-4	-	30	5	A:6 B:4 C:4 D:4 Total score: 18	90/100
39	56-M	L4-5	-	18	3	A:4 B:2 C:3 D:2 Total score: 11	40/100
40	33-F	L4-5	-	12	3	A:4 B:3 C:2 D:3 Total score: 12	80/100
41	42-F	L4-5	-	24	5	A:5 B:3 C:3 D:3 Total score: 14	70/100
42	44-F	L5-1	+	6	3	A:4 B:2 C:2 D:2 Total score: 10	50/100

months and showed a high homogeneity among the patients (p=0.92). Fifteen levels (31.2%) showed protrusion, 23 (47.9%) showed extrusion, and 10 (20.9%) showed sequestration. The distribution of the levels was: 1 patient for L2-L3, 2 patients for L3-L4 level, 28 for L4-L5 level and 17 patients were L5-S1 level.

Histological Changes

There was no statistically significant difference in the histopathological degenerative scores when comparing both with gender and duration of the pain (p>0.05). Age, MMP-3 scores and MRI grades displayed statistically significant correlation between histopathological degenerative scores (p=0.001) (Figure 1A-D).

MRI revealed annular rupture at 18 levels where as 30 levels did not show this feature.

Immunohistochemical Analyses

There was no statistical difference in MMP-3 expression when compared in terms of gender, age, and duration of the pain (p>0.05). There was a significant correlation between MMP-3 expression and both histopathological degenerative scores and MRI grades (p=0.001) (Figure 2A-D).

MRI Grades

There was no statistically significant difference in the MRI grades when compared in terms of both gender and duration of the pain (p>0.05). There was a significant correlation between MRI grades and age, histopathological degenerative scores, and MMP-3 expression (p=0.01, p=0.001, and p=0.001 respectively).

DISCUSSION

Degeneration of the IVD is an irreversible and progressive process. Proposed factors triggering this process include advanced age, mechanical pressures, genetic factors, inflammatory changes and biochemical alterations of the matrix components in the IVD (4, 5, 9, 21, 25, 26). For the evaluation of the IVD degeneration and its severity, various techniques such as histopathological staining, immunohistochemical scores, and MRI grading studies are available (1, 4, 7, 19, 23). To the best of our knowledge, this is the first study to analyze the correlation between these methods in conjunction with the patients' age, duration of the low back pain and the gender of the patients.

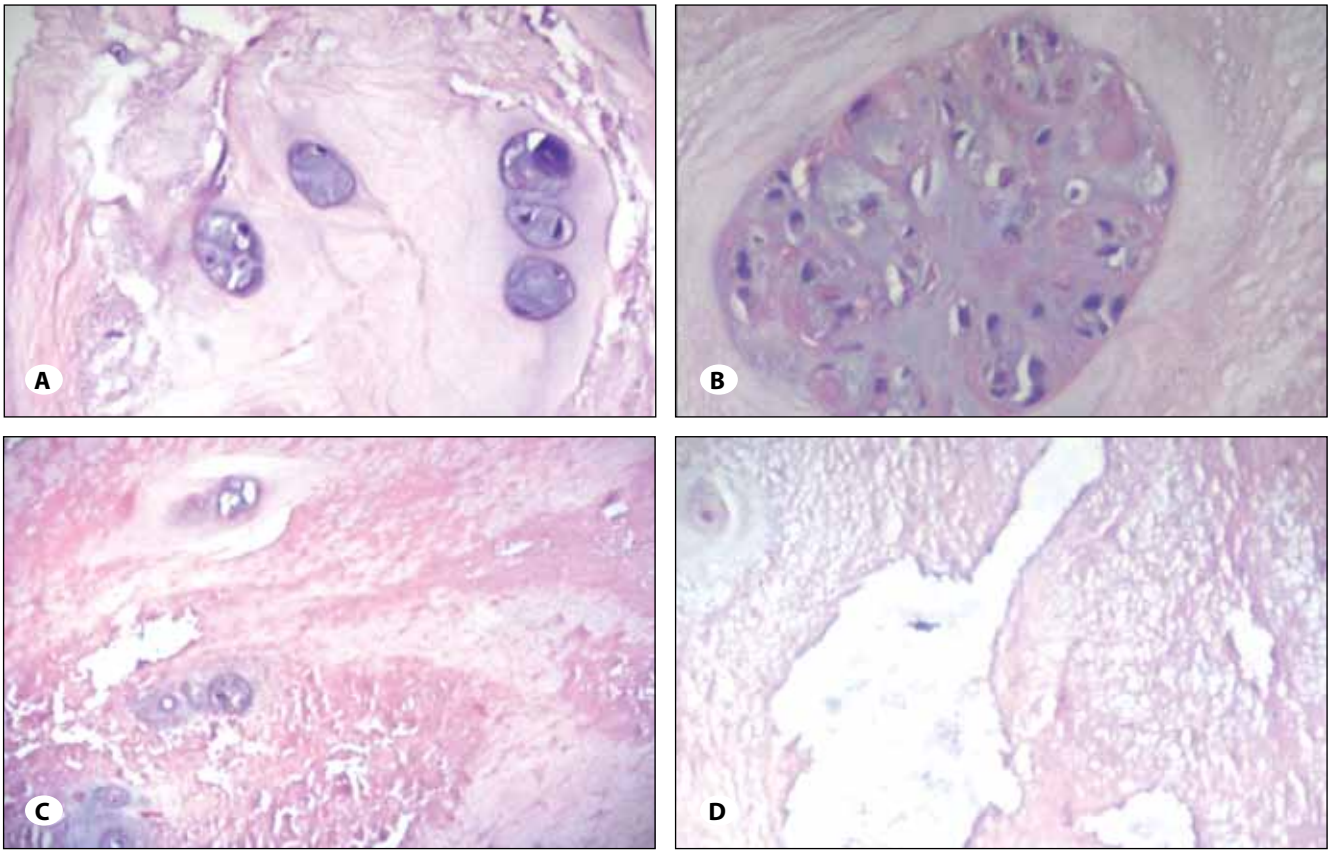

Figure 1: Histomorphological signs of disc degeneration: **(A)** focal cell proliferation; **(B)** largec hondrocytes with large and expanded lacunae; **(C)** granular changes, namely eosinophilic staining and amorphous granules within the fibrocartilage matrix; and **(D)** the appearance of a cleft (H&E, x400).

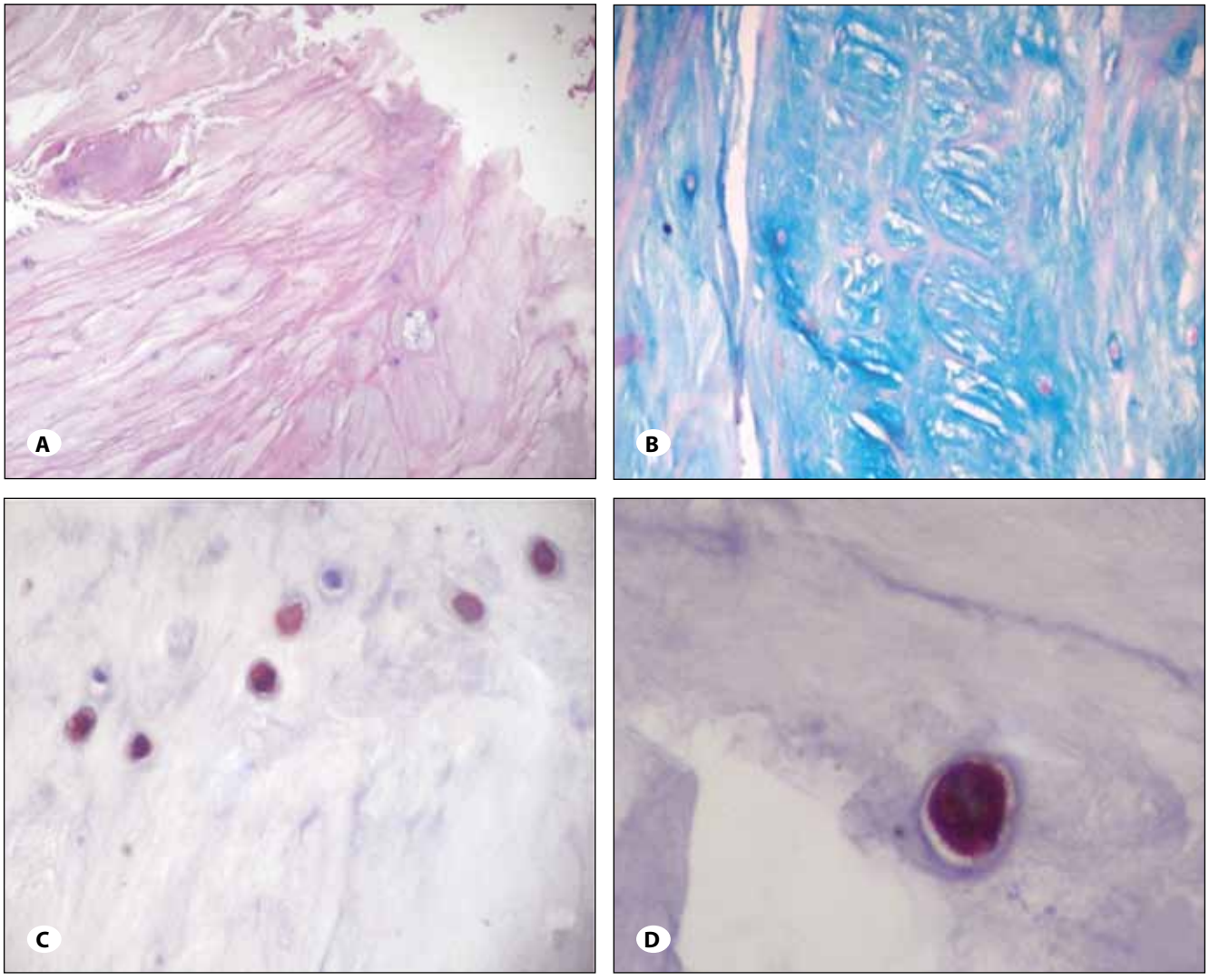

Figure 2: (A) The occurrence of mucoid material. (H&E, x200); **(B)** mucosal degeneration as visualized using the Alcian blue stain (x200); and **(C, D)** immunohistochemical features of MMP-3 expression. Intracellular MMP-3 staining was visible in a significant proportion of disc cells. (c, x200; d, x400).

Although the extruded or sequestrated portion of the IVD always involves material from the nucleus pulposus, in the present study, we obtained samples for histopathological study from the nucleus pulposus itself for each level (14). Weiler classified a system for grading age-related histopathological changes in the IVD by scoring chondrocyte proliferation, structural alterations (e.g., tears and clefts), granular changes, mucous degeneration and cell death, on a scale of minimum 0 to maximum 18 points(25). These histopathological alterations were observed in all disc specimens in our study. Boos and Buckwalter reported a significant correlation between the histopathological score and patient age. On the other hand, Zigouris et al. found only a tendency but no statistically significant correlation between the two (3, 4, 27).An increased correlation between the histopathological grade and patient age was also observed in our study (p=0.001). Moreover, MRI grades and MMP-3 staining of the degenerated IVD displayed a significant correlation (p=0.001), between histopathological score. Duration of the pain and the gender of the patients did not show a significant correlation (p>0.05).

The signal intensity of the IVD on MRI correlates with the chemical composition and histological changes (22, 24). The reduction or loss of signal on T2-W MRI is in an associated with progressive degenerative alterations of the IVD (13). Pfirrmann developed a 5-step MRI grading system based on signal intensity, disc structure, distinction between nucleus pulposus and annulus fibrosus, and disc height(19). In the present study, patient age, histopathological degenerative scores, and MMP-3 expression of the degenerated IVD displayed a significant correlation (p=0.01, p=0.001, and p=0.001 respectively), between MRI grades. Duration of the pain and the gender of the patients did not show a significant correlation (p>0.05).

MMPs are a family of proteolytic enzymes that is associated with breakdown of the IVD's matrix components, particularly collagen and proteoglycans(1, 9, 11, 16). MMP-3 (stromelysin-1) is capable of both degrading the proteoglycans and activating other MMPs(9, 20). In the present study, expression of MMP-3 correlated with patient age, histopathological degenerative scores and MRI grades (p=0.001). Duration of the pain and the gender of the patients did not show a significant correlation (p>0.05).

CONCLUSIONS

In the degenerated IVD, the expression of MMP-3, MRI grading and histopathological alterations of the IVD displayed significant correlation. Additionally, increased age is closely related with aforementioned alterations. For evaluating and treating the IVD degeneration, MRI is a good non-invasive diagnostic tool to determine the severity of degeneration. MMP-3 may be a therapeutic target of the degenerated IVD (7, 9).

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