Disparity between MR Imaging and Histochemical Grading in Human Intervertebral Disc Degeneration

June Ho Lee, M.D.,1 Chun Kee Chung, M.D., Ph.D.,1 Hyun-Jib Kim, M.D., Ph.D.2
Department of Neurosurgery,1 Seoul National University College of Medicine, Clinical Research Institute, Neuroscience Research Institute, Medical Research Center, Seoul, Korea
Department of Neurosurgery,2 Seoul National University Bundang Hospital, Seongnam, Korea

Objective: In order to establish the index of degeneration, the authors performed a histochemical study with Safranin-O staining and investigated the occurrence of apoptosis in the human intervertebral disc.

Methods: Eighteen intervertebral disc specimens surgically extracted from the patients and two additional specimens from the autopsied cases were stained with Safranin-O for proteoglycan according to a standard protocol. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) was used to detect the fragmented DNA known to be associated with apoptotic cell death and classification scheme was formulated for categorization of the degree of Safranin-O staining (normal, moderate reduction, faind) by modification of Makin’s histological-histochemical grading. The Kruskal-Wallis H test and Chi-square test were used for statistical analysis.

Results: The statistical results showed a significant difference in the mean age between "normal" Safranin-O staining group and the others (19.3 versus 55, 43.4, p=0.021). However, there was no statistically significant correlation between Safranin-O staining and MR grading of disc degeneration. Only six of eighteen surgical specimens and none in autopsies showed positive apoptotic cells in TUNEL staining.

Conclusion: The determination of the degree of degeneration in surgically obtained disc tissue per se by histochemical staining or by the degree of apoptosis that corresponds to its morphologic change was not feasible.

KEY WORDS: Disc Degeneration · Histochemical grading · Safranin O · Apoptosis.

Introduction

Human intervertebral discs undergo age-related degenerative changes that contribute to some of the most common causes of impairment and disability for middle aged and older persons: back stiffness or pain and neck pain. Various radiologic, biochemical, and structural alterations take place during the process of aging and degeneration of the intervertebral disc and these changes have been used as a scale for the grading system of disc degeneration.1,2,3

The degree of degeneration has widely been documented by the grading systems based on its morphologic change, whether by its gross look or its appearance in the magnetic resonance imaging (MRI), in the evaluation of intervertebral disc degeneration. However, a microscopic or histochemical grading system of disc degeneration that is suitable to match these morphologic changes has not been established yet. As an alternative, the degree of apoptosis has previously been proposed as a scale of disc degeneration by many investigators.

Since Gruber et al. first reported in 1998 apoptotic cell death in the lumbar disc annulus, many authors have used apoptosis as a scale of disc degeneration.4,5 However, an apoptosis as a natural phenomenon, its incidence in the normal undiseased discs, or its degree in the various stages of disc degeneration has not been adequately clarified. In the current study, the authors performed a histochemical study with Safranin O staining and investigated the occurrence of apoptosis to establish the index of degeneration in the human intervertebral disc.

Materials and Methods

Eighteen specimens of intervertebral disc were extracted from the individuals with a herniated disc, spondylolisthesis,
or spinal stenosis after the performance of each surgical procedure. Two additional specimens extracted from autopsied cases were also included. Among the 18 patients, the male to female ratio was 1:1 and the mean age of the patients was 46.7 years (range 17 to 71 years). The specimens from autopsied cases were from a six year-old male and a 30 year-old female. Of the 20 specimens, 10 were obtained from L4-5, 8 from L5-S1, 2 from L3-4 (Table 1).

For the proper preparation of surgical specimens, the authors carefully tried to avoid from mixing the cartilaginous endplate or outer annulus fibrosis with surgically obtained disc tissues. Specimens were fixed in 4% glutaraldehyde and were also cryofrozen in Tissue-Tek O.C.T. Compound (Miles, Elkhart, IN) and kept in a freezer at -70°C until sectioning. Samples were sectioned (10μm thick), and stained with Safranin-O for proteoglycan according to a standard protocol. In brief, hydrated sections were sequentially immersed for 5 minutes in hematoxylin, 5 minutes in running tap water, 4 minutes in 0.02% fast green, 10 seconds in 1% acetic acid, and then 6 minutes in 0.1% Safranin-O. For the routine light microscopic examination, specimens embedded in paraffin were stained with Toluidine blue (Fig. 1).

Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) was used to detect the fragmented DNA known to be associated with apoptotic cell death (Fig. 2). End labeling was performed by use of the Apoptag Plus in situ apoptosis detection kit (ONCOR, Gaithersburg, MD). Counter-staining was performed with Propidium iodide.

The degree of disc degeneration by morphology was evaluated according to the criteria of Pfirrmann et al (Table 2) for MRI classification of disc degeneration. The classification scheme was formulated for the categorization of the degree of Safranin-O staining (normal, moderate reduction, faint) by modification of Mankin's histological-histochemical grading (Fig. 3).

The Kruskal-Wallis H test and Chi-square test were used for statistical analysis and P-value less than 0.05 was accepted as statistically significant.

Results

The results are summarized in Table 3 to 5. Three specimens were classified as "normal" for Safranin-O staining. Seven were classified as "moderate reduction" and 10 as "faint". The Kruskal-Wallis test results showed a significant difference.
Table 1. Demographic data of surgical specimens

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Level</th>
<th>Diagnosis*</th>
<th>MR grading</th>
<th>Safranin O staining</th>
<th>TUNEL staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>F</td>
<td>L5-S1</td>
<td>HVD</td>
<td>3</td>
<td>faint</td>
<td>6/17(35%)</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>M</td>
<td>L4-S5</td>
<td>HVD</td>
<td>3</td>
<td>faint</td>
<td>8/19(42%)</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>M</td>
<td>L5-S1</td>
<td>HVD</td>
<td>4</td>
<td>faint</td>
<td>0/14</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>M</td>
<td>L4-S5</td>
<td>HVD</td>
<td>4</td>
<td>faint</td>
<td>12/26(46%)</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>M</td>
<td>L5-S1</td>
<td>Spondylothesis</td>
<td>5</td>
<td>moderate</td>
<td>0/21</td>
</tr>
<tr>
<td>6</td>
<td>55</td>
<td>M</td>
<td>L4-S5</td>
<td>HVD</td>
<td>4</td>
<td>moderate</td>
<td>0/4</td>
</tr>
<tr>
<td>7</td>
<td>68</td>
<td>F</td>
<td>L4-S5</td>
<td>HVD</td>
<td>5</td>
<td>faint</td>
<td>4/6(67%)</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>F</td>
<td>L3-S4</td>
<td>HVD</td>
<td>4</td>
<td>moderate</td>
<td>0/20</td>
</tr>
<tr>
<td>9</td>
<td>71</td>
<td>M</td>
<td>L5-S1</td>
<td>Spinal stenosis</td>
<td>4</td>
<td>moderate</td>
<td>0/33</td>
</tr>
<tr>
<td>10</td>
<td>49</td>
<td>F</td>
<td>L4-S5</td>
<td>HVD</td>
<td>4</td>
<td>moderate</td>
<td>0/29</td>
</tr>
<tr>
<td>11</td>
<td>57</td>
<td>F</td>
<td>L4-S5</td>
<td>Spondylothesis</td>
<td>5</td>
<td>faint</td>
<td>6/32(19%)</td>
</tr>
<tr>
<td>12</td>
<td>57</td>
<td>M</td>
<td>L4-S5</td>
<td>HVD</td>
<td>3</td>
<td>moderate</td>
<td>0/16</td>
</tr>
<tr>
<td>13</td>
<td>31</td>
<td>F</td>
<td>L5-S1</td>
<td>HVD</td>
<td>4</td>
<td>faint</td>
<td>0/23</td>
</tr>
<tr>
<td>14</td>
<td>22</td>
<td>M</td>
<td>L5-S1</td>
<td>HVD</td>
<td>3</td>
<td>normal</td>
<td>0/18</td>
</tr>
<tr>
<td>15</td>
<td>51</td>
<td>M</td>
<td>L5-S1</td>
<td>HVD</td>
<td>3</td>
<td>faint</td>
<td>0/12</td>
</tr>
<tr>
<td>16</td>
<td>48</td>
<td>F</td>
<td>L4-S5</td>
<td>Spondylothesis</td>
<td>4</td>
<td>moderate</td>
<td>0/11</td>
</tr>
<tr>
<td>17</td>
<td>41</td>
<td>F</td>
<td>L3-S4</td>
<td>HVD</td>
<td>4</td>
<td>faint</td>
<td>8/15(53%)</td>
</tr>
<tr>
<td>18</td>
<td>48</td>
<td>F</td>
<td>L5-S1</td>
<td>HVD</td>
<td>5</td>
<td>faint</td>
<td>0/11</td>
</tr>
<tr>
<td>19</td>
<td>30</td>
<td>F</td>
<td>L4-S5</td>
<td>Autopsy</td>
<td>–</td>
<td>normal</td>
<td>0/28</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>M</td>
<td>L4-S5</td>
<td>Autopsy</td>
<td>–</td>
<td>normal</td>
<td>0/20</td>
</tr>
</tbody>
</table>

*HVD: herniated intervertebral disc, † TUNEL staining: Number of positive cells/Number of total cells in 30 high power fields

Table 2. Classification of disc degeneration on MRI

<table>
<thead>
<tr>
<th>Grade</th>
<th>Structure</th>
<th>Distinction of Nucleus and Anulus</th>
<th>Signal Intensity</th>
<th>Height of Disc</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Homogeneous, bright white</td>
<td>Clear</td>
<td>Hyperintense, isointense to CSF</td>
<td>Normal</td>
</tr>
<tr>
<td>II</td>
<td>Inhomogeneous with or without horizontal bands</td>
<td>Unclear</td>
<td>Intermediate to hypointense</td>
<td>Moderately decreased</td>
</tr>
<tr>
<td>III</td>
<td>Inhomogeneous, gray</td>
<td>Unclear</td>
<td>Intermediate to hypointense</td>
<td>Slightly decreased</td>
</tr>
<tr>
<td>IV</td>
<td>Inhomogeneous, gray to black</td>
<td>Unclear</td>
<td>Intermediate to hypointense</td>
<td>Normal</td>
</tr>
<tr>
<td>V</td>
<td>Inhomogeneous, black</td>
<td>Unclear</td>
<td>Hypointense</td>
<td>Collapsed</td>
</tr>
</tbody>
</table>

Table 3. Comparison of mean age among three different Safranin O staining groups

<table>
<thead>
<tr>
<th>Safranin-O staining</th>
<th>N</th>
<th>Mean age</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3</td>
<td>19.3</td>
<td>12.2</td>
</tr>
<tr>
<td>Moderate reduction</td>
<td>7</td>
<td>55.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Faint</td>
<td>10</td>
<td>43.4</td>
<td>16.9</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>43.9</td>
<td>17.2</td>
</tr>
</tbody>
</table>

The Kruskal–Wallis test results showed a significant difference in the mean age between "normal" Safranin O staining group and the other two groups (p=0.021; Table 3). However, there was no statistical correlation between the degree of Safranin-O staining and MR grading of disc degeneration (Chisquare test, p = 0.417; Table 3). There was no statistically significant difference in the category of age and the distribution of MR grade between the cell cloning positive and negative group (Table 5).

Only six of eighteen surgical specimens and none in autopsied cases showed positive apoptotic cells in TUNEL staining. Therefore, a statistical analysis for the correlation between TUNEL staining and disc degeneration was not performed.

Discussion

The degree of degeneration has widely been documented by the grading systems based on its morphologic change in the evaluation of intervertebral disc degeneration. A comprehensive knowledge of the changes in the biologic behavior of the cells and in the matrix component of the disc is a new challenging area in the research of pathophysiologic mechanism of the disc degeneration. Therefore, a histochemical grading system of disc degeneration that could explain and pair with the degree of changes in the disc morphology, whether by its gross look or its appearance in the magnetic resonance imaging(MRI), is required. However, a microscopic or histochemical grading system of disc degeneration that is suitable to match these morphologic changes has not been established yet. As an alternative, the degree of apoptosis has previously been proposed as a scale of disc degeneration by many investigators. In the sequence of these proposals, some authors have reported correlation between the degree of apoptosis and the age. However, they found no direct correlation between the degree of apoptosis and the degree of disc degeneration.

Collagens and proteoglycans are the primary structural components of the intervertebral disc macromolecular framework. The matrices of disc components differ significantly in their relative amounts of these two structural macromolecules. Proteoglycan and water concentration decrease with aging. Safranin-O is a cationic dye that binds specifically to polymers, one dye molecule to each negatively charged group of either chondroitin 6-sulphate or keratan sulphate. Due to these properties, the intensity of Safranin-O staining correlates positively with the fixed charge density in the cartilage matrix.
Table 4. Relation between Safranin-O staining and MR grading

<table>
<thead>
<tr>
<th></th>
<th>MR grading</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safranin - O</td>
<td>Normal</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Staining</td>
<td>Moderate reduction</td>
<td>5</td>
<td>1</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Faint</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>18</td>
</tr>
</tbody>
</table>

There was no statistical significance between the degree of Safranin-O staining and MR grading of disc degeneration (Chi-square test, P>0.05)

Table 5. The degree of correlation between age, MR grading, the degree of staining by Safranin-O, and cell cloning determined by p-value

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>MR grade</th>
<th>Safranin - O</th>
<th>Cloning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.164</td>
<td>0.021</td>
<td>0.141</td>
</tr>
<tr>
<td>MR grade</td>
<td>0.164</td>
<td>0.021</td>
<td>0.141</td>
</tr>
<tr>
<td>Safranin - O</td>
<td>0.021</td>
<td>0.417</td>
<td>0.892</td>
</tr>
<tr>
<td>Cloning</td>
<td>0.141</td>
<td>0.892</td>
<td>0.531</td>
</tr>
</tbody>
</table>

and has been used for semiquantitative estimation of proteoglycans in cartilage tissue. The Safranin-O staining was included in histological-histochemical grading system of articular cartilage by Mankin et al.18.

Since Kerr et al.17 first reported in 1972 that the apoptosis is a form of cell death that is distinct from necrosis, it has been considered as a final common pathway of cell death in various conditions such as cancer, ischemia, and spinal cord injury. Gruber et al.14 pioneered the study of apoptosis in disc degeneration in 1998. They quantified the incidence of apoptotic cell death in the annulus and compared its quantity between the tissues from the diseased subjects and normal control. Their findings revealed that there is a high incidence of apoptosis in the intervertebral disc. In their another study.10 they even reported that insulin-like growth factor-1 (IGF-1) and platelet-derived growth factor (PDGF) had anti-apoptotic effects on human intervertebral disc cells in vitro.

Since Gruber's report, the estimated degree of apoptosis has been considered and reflected as a scale of disc degeneration by several authors. Lotz et al.15,20 loaded mouse-tail discs in vivo with an external compression device and demonstrated that apoptotic cell death was proportional to the compressive stress and the time of loading. Although mechanical stress has been regarded as an important modulator of the degeneration, the underlying molecular mechanism remains unclear.

Some pathologic experiment reported the induction of apoptosis by increased caspase-9 activity and decreased mitochondrial membrane potential after mechanical overload21 while others reported the mere increase of the number of apoptotic cells depending on the weight of the load.22 Other report demonstrated that apoptosis in the cartilaginous endplate of mouse spondylosis model increased with age and resulted in a marked decrease in cell density.23 However, to the authors' knowledge, an apoptosis as a natural phenomenon, its incidence in the normal discs, or its degree in the various stages of disc degeneration has not been adequately clarified.14,17,18

In the present study, apoptotic cells were detected in only six of eighteen (33%) surgical specimens and none in autopsied cases. This finding may suggest that apoptotic cell death is not a wide spread phenomenon in normal or even in the degenerated disc tissue. Although the TUNEL detection method preferentially identifies apoptotic cells, it can also label necrotic cells during the late period as well as viable cells with a high transcriptionsal activity.14,24,25 Therefore, the TUNEL method is vulnerable to a high probability of false-positive results. The demonstration of the typical apoptotic morphology of fragmented, shrunk nuclei with condensed chromatin is required, in addition to TUNEL, to identify apoptosis.24

Whether the apoptosis can be used as an index of disc degeneration, especially during the early stage of degeneration when its morphologic change is not evident, is still a debate. Aigner T et al.10 previously had reported negatively to this suggestion in their study with human articular knee cartilage. On the contrary, there are some in vivo animal experiments that indicate the autocrine or paracrine fashion of disc tissue's response to apoptosis-triggering substances (i.e. Fas/ Fas-L, bcl2, P53), suggesting a potential mechanism for apoptosis of notochordal cells in normal animal nucleus pulposus.6,13 In the authors' opinion, TUNEL method combined with electron microscopic study, other immunohistochemical assay (i.e. poly (ADP-ribose) polymerase (PARP), p85 immunohistochemistry) of apoptosis-triggering substances, or assay of apoptosis-inhibiting substances (i.e. Two mitogen-activated protein kinase (MAPK))14 may contribute to solve that question.

The present study demonstrated significant differences only in the category of the mean age between "normal" Safranin-O staining group and the other two groups. This could be interpreted as a by-product from several pitfalls in the present study. Most of the patients in the current study had advanced degree of disc degeneration on the magnetic resonance images and therefore the range of disc degeneration included in the statistical analysis consists of only three grades, from Grade 3 to 5. Consequently, this biased distribution of the subjects may have affected the statistical results. Moreover, the degree of Safranin-O staining was categorized by an arbitrary classification scheme.

As previously noted, we could not draw any conclusion about the possible parity between the MR imaging and histochemical grading in human intervertebral disc degeneration. Further studies that is based on the standardized quantitative Safranin-O or TUNEL staining estimation method and that
include earlier stages of disc degeneration are mandatory to
draw more reliable conclusions regarding the effect of age and
disc degeneration on the Safranin-O or TUNEL staining of
disc tissue.

Conclusion

The present study suggests that the determination of the
degree of degeneration in surgically obtained disc tissue
per se by histochemical staining or by the degree of apoptosis
was not feasible. Further studies that is based on the stand-
ardized quantitative histochemical staining estimation method
and that include more diverse stages of disc degeneration are
mandatory to draw more reliable conclusions regarding the
effect of age and disc degeneration on the histologic staining
pattern of disc tissue.

* Acknowledgement
This work was supported by a grant from Seoul National University
Hospital (grant No. 419990259).

References

potic cell death is not a widespread phenomenon in normal aging and
osteoarthritis human articular cartilage: a study of proliferation, pro-
gressed phase of disc degeneration and viability of chondrocytes in normal

2. Akazoe L, Oriz A: The study of apoptosis in spine pathogenesis. Spine 29:
500, 1999

3. Ariga K, Miyamoto S, Nakase T, Okuda S, Meng W, Yonenobu K, et al: The re-
ationship between apoptosis of endplate chondrocytes and aging and

chanical stress-induced apoptosis of endplate chondrocytes in organ-
cultured mouse intervertebral discs: an in vivo study. Spine 28: 1528-1533,
2003

Spine 20: 1307-1314, 1995

regional distribution of apoptosis in osteoarthritis disc. Spine 30: 519-524,
2005

7. Fraser RD, Bleed JF, Moskowitz RW: Spinal degeneration: pathogenesis
and medical management. The Adult Spine. Philadelphia: Lippincott-
Raven Publishers, 1997, pp 735-759

W, Schulte-Hermann R: In situ detection of fragmented DNA (TUNEL
assay) fails to discriminate among apoptosis, necrosis, and autolytic cell death:

9. Gruber HE, Hanley EN: Analysis of aging and degeneration of the

10. Gruber HE, Norton HH, Hanley EN: Anti-apoptotic effect of 1G-1 and
PDGF on human intervertebral disc cells in vitro. Spine 25: 2153-2157,
2000

Cells long-term compressive loading on the intervertebral disc cause apo-

with wide-ranging implications in tissue kinetics. Br J Cancer 26: 239-257,
1972

paracrine Fas-mediated counterattack: a potential mechanism for apoptosis
of notochordal cells in intact rat nucleus pulposus. Spine 30: 1247-1251,
2005

14. Kooit MM, Muhling J, Knaapen MW, de Meyer GR: RNA synthesis and
splicing interfaces with DNA in situ end labeling techniques used to

15. Lott JC, Chin JR: Intervertebral disc cell death is dependent on the

16. Mankin HJ, Dielmann H, Lippold L, Zarins A: Biochemical and
degmentational abnormalities in articular cartilage from osteoarthritic human

17. Park JB, Kim KW, Han CW, Chang H: Expression of Fas Receptor on
nucleus pulposus cells in herniated lumbar disc tissue. Spine 26: 142-146,
2001

18. Park JB, Chang H, Kim KW: Expression of Fas ligand and apoptosis of
disc cells in herniated lumbar disc tissue. Spine 26: 618-621, 2001

resonance classification of lumbar intervertebral disc degeneration. Spine
26: 1873-1878, 2001

20. Raininko R, Manninen H, Barrie MC, Gibbons LE, Gill K, Fisher LD:
Observer variability in the assessment of disc degeneration on magnetic
resonance images of the lumbar and thoracic spine. Spine 29: 1029-1035,
1995

et al: Intervertebral disc degeneration: the role of the mitochondrial pathway
in annulus fibrosus cell apoptosis induced by overload. Am J Pathol 164:
915-924, 2004

22. Schiebler ML, Camino VE, Julfen MD, Zlatkin MB, Grenier N, Kessel
HY: In vivo and ex vivo magnetic resonance imaging evaluation of early
disc degeneration with histopathologic correlation. Spine 16: 635-640,
1991

degeneration in magnetic resonance imaging: a comparative biochemical,
histologic, and radiologic study in cadaver spines. Spine 16: 629-634,
1991

24. Thompson JP, Parce RH, Seibert MT, Adams ME, Tsay IKY, Bishop
PB: Preliminary evaluation of a scheme for grading the gross morphology

25. Tumusvikula DRZ, Schmidt RE, Roth KA: Simultaneous detection of
TdT-mediated BUDP-tobin nick end-labeling (TUNEL)-positive cells and
multiple immunohistochemical markers in single tissue sections. Biotechniques
19: 800-805, 1995

26. Wahb Alzaz, Lor JC: Biological response of the intervertebral disc to

Commentary

The biologic basis for healthy disc tissue is appropriate disc
cell function but also the cell biology and the cell pathologic
of the disc are as yet poorly understood. Various radiologic,
biochemical, and structural alteration take place during the
process of aging and degeneration of the intervertebral disc
and these changes have been used as a scale for the grading
of disc degeneration.

The role of apoptosis in the lumbar disc degeneration has been
reported by investigators but it is still controversial be-
cause apoptosis is present in the tissue not only of
normal healthy discs but also in various stages of disc degeneration.
The authors studied the disc samples with Safranin O
and used TUNEL staining to detect the fragmented DNA which
is known to be associated with apoptotic cell death for the
investigation of apoptosis to establish the index of degener-
ation in the human intervertebral disc that would match the
morphologic changes of the degenerated lumbar disc on MR
imaging, and they suggested that there is no significant corr-
elation between the extent of apoptosis and the various gradings of MR imaging of the degenerated lumbar discs.

In this study, the numbers of disc samples were too small to draw a documented result from. As the authors pointed out, more specific staining techniques have to be developed to demonstrate the apoptotic cells in the degenerated disc tissue as there is a comprehensive understanding about the role of apoptotic cell death in disc degeneration, a new challenging area for many investigators.

Soo Han Kim, M.D.
Department of Neurosurgery, Chonnam University