A Clinicopathological Study Of Changes In Intervertebral Discs

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Citation

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Abstract

OBJECTIVES: To identify the most important histomorphological indicators of disc degeneration by comparing with asymptomatic control intervrtebral discs (IVD) from cadavers; highlight the magnitude of symptomatic degenerative disc disease (DDD) compared to other spinal diseases resulting in surgical excision of IVD; study the utility of grading system to analyse the degenerative changes.
DESIGN: Surgically excised intervertebral discs from 100 patients were studied by histomorphology aided with histochemistry. This included patients with prolapsed IVD due to degenerative causes, tuberculosis spine and trauma. Ten control IVD removed at autopsy were studied for comparison. Based on histomorphology and histochemistry, the four features viz. matrix depletion, matrix fibrillation, chondrocyte cloning and edge neovascularisation were graded; the grades were added for study and control cases to represent a score.
RESULTS: Degenerative causes accounted for 67% of cases indicating this as the leading cause of disc excision. Edge neovascularisation and chondrocyte cloning were nearly absent in controls in contrast to study group. Mean scores of the histological features were the highest in the degenerative causes of intervertebral disc prolapse (IVDP).
CONCLUSIONS: Chondrocyte cloning and edge neovascularisation are more specific indicators of degeneration. Edge neovascularisation, though labelled a specific indicator of prolapse due to DDD, has low percentage positivity.

Figure 1

INTRODUCTION

Progressive degenerative changes in the spine start in 3rd decade with first manifestations occurring in the intervertebral disc (IVD) followed by changes in the bone and articular cartilage.[1] The IVD transmits load hydrodynamically from one vertebra to the next. If normal intradisc pressure exists in standing position, a forward flexion or stooping increases it by as much as 400%[2]. In a disc in the young, the water content of nucleus pulposus (NP) is 85% and that of annulus fibrosus (AF) is 78%. Decreased imbibing capability due to loss of negatively charged proteoglycans leads to a fall in water content to 70% in both tissues. The pathoanatomical and biochemical alterations in IVD with aging and/or degeneration, disturb structural integrity and impair function. Symptoms can arise at any unpredictable point along the continuum of degenerative changes. Also symptoms due to senescence versus pathologic processes can be very difficult to differentiate.[3] Excision of IVD is frequently done in symptomatic cases not responding to conservative treatment, and curetted disc material is sent for histopathologic assessment. A macroscopic grading system given by some workers is difficult to apply as we do not have access to the entire disc.[4] Histopathologists do mention about presence of the various morphological parameters, but rarely do they agree on the degree of changes.

In the present study our aim was to study the histological changes in the spectrum of conditions leading to disc excision in 100 consecutive cases, with a focus on degenerative causes of prolapse as this forms a major category. The alterations will be compared to asymptomatic control discs. Histomorphology aided by histochemistry is used to delineate important indicators of disc degeneration. The utility of a grading system to analyse degenerative changes will be discussed so as to assess its predictive value.

MATERIALS AND METHODS

One hundred consecutive specimens of IVD excision done for symptomatic, radiologically evaluated patients and sent for histopathological examination formed the study group. Ten IVDs removed at autopsy from patients dying of causes unrelated to spinal or disc disease formed the control group.

The entire volume of the material sent was processed and fixed in 10% neutral buffered formalin, dehydrated in graded ethanol/water mixtures, cleared in xylene, embedded in paraffin and stained by hematoxylin and eosin to study morphology. In addition representative sections were stained with toluidine blue[5] and safranin O[6] to illustrate the proteoglycan content of extracellular matrix and van Gieson stain to highlight fibrillation. Histologic features selected for grading degeneration in the two groups were: matrix depletion(Md), matrix fibrillation(Mf), chondrocyte cloning(Cc) and edge neovascularisation (En). As grading was based essentially on a subjective assessment of morphological features, in order to validate the results grading was done independently by two pathologists(H.M, S.A.K). Each of the four features was graded as per criteria given in Table I.

Figure 2

Table 1: Grading criteria for histological features

Feature (stain)	Grade 0	Grade 1	Grade 2	Grade 3
Matrix depletion (safranin & toluidine blue)	no change	focal area of pallor	Patchy unstained areas	Confluent unstained patches
Fibrillation (Van Gieson)	no change	very fine fibres (myxoid appearance)	groups of fine wavy fibres	Coarse strands, cleared intervening areas
Chondrocyte cloning (H&E)	normal (6cells/lacuna)	6-12 cells/lacuna	12-20 cells/lacuna	>20 cells/lacuna
Edge neovascularisation (H&E)	No blood vessels along the edge	Occasional capillary	tuft of capillaries	Extensive neovascularisation along the edge

The grades of all the four features were added to obtain a score for study as well as control group which would range from 0 to 12. Scores were analyzed to give a meaningful interpretation of degenerative changes. The grades of histological features of study group were compared to those of control groups by analysis of variance (ANOVA).

RESULTS

There was a wide age range of patients undergoing disc excision ranging from 18 to 75 yrs. (mean age 43.21 yrs.). Maximum patients were in their 4th decade (39 patients, 39%) followed by 5th decade (25 patients, 25%). Very few patients were seen at extremes of age groups i.e. 4% (4 cases) in 2nd decade and 6% (6 cases) in seventh decade. Control cases were equally divided in 4th and 5th decades of life. Out of 100 patients, 62 were males and 38 females. In general, males were predominantly represented in all age groups, with maximum sex difference being in 4th decade (males 29, females 10; M:F =3:1).The commonest presenting symptom was low back ache seen in 40% cases of study group.

Microscopic features were as follows (Table II):

Figure 3

Table 2: Positivity of histologic features in the study and control groups

Histological feature	Study group, n=100 No. & % of cases	Control group, n=10 No. & % of cases
Matrix depletion *(Md)*	96, 96% (2.57)	10, 100% (1.50)
Matrix Fibrillation *(Mf)*	99, 99% (1.63)	8, 80% (0.1)
Chondrocyte cloning *(Cc)*	53, 53% (0.72)	3, 30% (0.24)
Edge neovascularisation *(En)*	41, 41% (1.12)	0, 0% (-)

Figures in parenthesis indicate mean grade of parameters in each group.

Matrix depletion (Md): Safranin stains glycosaminoglycans red[5]; lack of staining was observed in areas depleted of matrix (Fig.1). Toluidine blue stains cartilage matrix metachromatically and highlights matrix alterations[7] which are seen as well defined circular areas of altered staining around chondrocytes called globular matrix alterations[8] (Fig.2).

Figure 4

Figure 1: Photomicrograph showing lightly stained areas of depleted matrix around chondrocytes (Safranin x100).

Figure 5

Figure 2: Photomicrograph depicting chondrocyte cloning i.e. increased number of chondrocytes per lacuna forming rounded clusters (H&E x200).

Matrix fibrillation (Mf):It is seen as fine to coarse wavy collagen threads highlighted as red coloured strands on Van Gieson stain[9]

Chondrocyte cloning (Cc): It is identified as increased number of chondrocytes per lacuna forming rounded clusters (normal upto 6 cells/lacuna[10]). This is a feature depicting intrinsic cartilage regeneration[11] (Fig.2). Some cells of these 'chondrones' showed degenerative changes e.g. pyknotic nuclei and cleared out cytoplasm.

Edge neovascularisation (En): It is characterised by proliferation of endothelial cells forming clusters of capillaries located along the edge of fibrocartilage fragments. In the vicinity of these were present polygonal to spindle cells having high N:C ratio and basophilic cytoplasm. Some lymphomononuclear cells and extravasated RBCs were also present.

Based on clinical profile, the study group was divided into 4 categories: degenerative disc disease (DDD) with intervertebral disc prolapse (IVDP), lumbar canal stenosis (LCS) with IVDP, tuberculosis spine and trauma. The score range and mean calculated in each category are given in Table III.

Figure 6

Table 3: Score range in the four clinicopathological categories of study group

Category	No. of cases(%)	Range of total score (mean)
DDD* with IVDP	59(59%)	2-10(5.84)
LCS **with IVDP	8(8%)	4-9(6.50)
Tuberculosis spine	18(18%)	2-7(3.88)
Trauma	15(15%)	1-5(3.06)

(*DDD- Degenerative disc disease; ** LCS- Lumbar canal stenosis)

Depletion and fibrillation of matrix were seen both in study and control group with no significant difference. Thus it is important to analyse the frequency of the other two microscopic parameters, Cc and En, in the degenerative group (i.e. first two categories) as compared to tuberculosis and trauma group, to emphasise their importance as a degenerative feature leading to IVDP.

Table IV highlights the significantly higher positivity of Cc as well as its higher mean grades in the degenerative categories (DDD and LCS with IVDP). En was observed to be higher in LCS with IVDP as compared to DDD. The fifty percent positivity in the tuberculosis group is attributed to the associated inflammation. Trauma group, on the other hand, had a low positivity.

The IVD excised were from lumbar, dorsal and cervical regions. Correlating clinicopathological diagnosis with spinal levels, IVD from lumbar region is affected maximum (79%). DDD in 93.5% cases affected the IVD from lumbar region. Also LCS with IVDP had all (100%) cases at lumbar levels.

Figure 7

Table 4: Percentage positivity of cc and en in the four categories

Important feature	DDD	LCS with IVDP	Tuberculosis	Trauma
Chondrocyte cloning (Cc)	59.6% (1.0)	75.0% (1.12)	6.1% (0.12)	7.1% (0.07)
Edge neovascularisation (En)	45.1% (0.70)	75.0% (1.0)	50.0% (1.12)	14.2% (0.14)

Figures in parenthesis indicate the mean grade of Cc and En in each category

STATISTICAL ANALYSIS

Both Cc and En were found to be statistically significant in the study group

($p<0.05$, ANOVA test). Total score was the most reliable parameter with p value <0.01.

DISCUSSION

Out of the 100 patients constituting the present study, 67 belonged to the degenerative categories (59 from DDD and 8 from LCS with disc prolapse). Thus degenerative causes of disc excision constituted 67% of study cases, a fairly high percentage to demand focus on this entity. A few previous studies[9],[10] involved purely IVDP caused by degeneration and do not mention the magnitude of DDD vis-à-vis other spinal diseases leading to discectomy. By studying 100 consecutive cases, other causes for IVDP identified were tuberculosis spine and trauma.

Stenosis or narrowing of the vertebral canal with IVDP due to degeneration will cause signs of neurovascular compression earlier in a narrow or asymmetrical canal.

Adult IVD lacks blood supply and blood-borne infections are extremely rare.[12] In India, tuberculosis of the spine is common and surgically excised tissue from the involved spine often includes IVD. Trauma has unequivocally shown to produce acute prolapse; the main cause of disc prolapse being degeneration. In the present study, 15% had antecedent trauma causing prolapse, including 6% having accompanying fracture vertebrae indicating severe intensity of trauma.

Males constituted 62% of total cases and 62.9% of DDD patients. This is explained by higher exposure of males to physical back strain[13]. Butler et al[14] stated that lumbar IVDs are more frequently affected due to their position at lordotic apex. In our study, low back ache (LBA) was the presenting symptom in 40% of study cases. 82% of total cases and 93.5% cases of DDD were from the lumbar region.

The upper value in the range of scores was higher in the degenerative categories i.e. DDD with IVDP and LCS with IVDP. The score was lower in tuberculosis and trauma cases which was largely due to Md and Mf. Further, it is noted that newly formed blood vessels, which happen to be present along the edge in inflammation added significantly to the score in the tuberculosis group.

Analysing the histopathological changes in the disc tissue, it has been emphasized in literature that distinction between the two components of IVD- nucleus pulposus (NP) and annulus fibrosus (AF), is purely histomorphologic i.e. areas showing chondrocytes were in NP and those showing predominantly collagen fibres were in AF. On hematoxylin and eosin, proteoglycan depletion in extracellular matrix can be seen as an area showing pallor of eosinophilic staining of matrix. This can be recognised better as well as graded by the use of specific stains like safranin O or toluidine blue, both of which show lack of staining in the region of matrix depletion. We used both these stains to highlight loss of proteoglycans so that early and subtle changes of degeneration can be identified and graded. Md was present in 96% of study group and 100% of control cases. Mean grade was, however, 2.57 and 1.50 in study and control groups respectively. Distribution of depleted matrix was noteworthy. Depleted matrix zones surrounded clusters of chondrocytes and showed layering of extracellular matrix. These have been aptly referred to as globular matrix modifications[8].

Mf characterized by fine to coarse collagen threads were highlighted by van Gieson's stain. It clearly indicates qualitative and quantitative alterations in collagen as described in literature[15] since young fibrocartilage does not show visible collagen fibres in matrix. Detection of Md and Mf in all categories of study group and control group is interesting and supports the concept that disc degeneration is an age related process and cannot be correlated with symptomatic disease or prolapse [16].

Cc is the next important change studied. The chondrocytes were seen to grow in small rounded groups of an increased number of cells per lacuna sharply demarcated by a rim of adjacent fibrocartilage. These cloned chondrocytes were seen to lie in modified territorial matrix. Cc was present in 53% of study group and 30% of control group cases. Mean grades were 0.72 and 0.24 in study and control groups respectively. Nerlich et al [11] described this feature as a sign of advanced degeneration. However, in our study, age range of patients showing Cc was wide (30-62 years); higher grades were present in older patients.

En has been labelled as the most specific indicator of disc prolapse by Weidner and Rice[10]. Though this was seen in only 50% of IVDP cases in their series, it was significantly absent in the control cases. These authors attributed the low positivity to sampling error as only 1/2 to 1/3rd of curetted material was examined microscopically. To eliminate this factor we processed the entire volume of curetted material sent for histopathological examination. We found En in 43.5% cases of DDD and in 41.0% in the entire study group. Percentage positivity in our study is lower than that reported by Weidner and Rice (50%)[10] as well as by Chitkara (47%)[17].The possible explanation can be `edge' not being included in the sectioned surface. The duration of prolapse is also likely to influence the observation of well developed En. Repanti et al[18] suggested that these blood vessels were newly formed, perhaps through metaplasia of undifferentiated mesenchymal cells. En was present at the convex/concave edges of fibrocartilage fragments in the form of capillaries intermingled with proliferating endothelial cells, fibroblasts and a few mononuclear cells. On scrutinizing these areas, we came across large cells scattered in the stroma. These cells had large nuclei and moderate cytoplasm showing basophilia indicative of proliferative activity and are likely to be mesenchymal cells referred to by Repanti et al [18].

Histological features specific to tuberculosis (i.e. epithelioid cell granulomas and caseation necrosis) were essentially present in the marrow spaces and soft tissue adjoining IVD.

The disc tissue here only showed age-related changes i.e. Md and Mf. Granulation tissue and newly formed blood vessels were present in 50% of tuberculosis cases (mean grade 1.12) while only 6.1% showed Cc.

IVD excised for trauma too showed age related changes. En and Cc were present in a small number of cases (14.2% and 7.1% respectively) with lower mean grades. They were identified especially when surgical excision was not immediate after trauma.

Cc and En were statistically significant morphological indicators of symptomatic degeneration. Md and Mf, on the other hand, were present both in study and control cases. The percentage positivity and mean grades of these were analysed to study their relevance in each group. Cc shows the highest positivity in degenerative categories i.e. 59.6% and 75% in DDD and LCS with IVDP respectively. The mean grades were also higher in these two categories. The percentage positivity for tuberculosis and trauma was far less (6.1% and 7.1% respectively) with lower mean grades.

En, an important feature of prolapse, is seen in 75.0% and 45.1% cases of LCS with IVDP and DDD respectively in our series. It was present in 50% cases of tuberculosis and 14.2% cases of trauma. Neovascularisation, though traditionally called the most important indicator of disc prolapse in literature [10], is also a feature observed in inflammation (tuberculosis) and small percentage of trauma cases. This finding further supports the role of inflammatory mediators, cytokines[19], which are implicated in En in IVDP due to degeneration as these substances are definitely present in tuberculous infection as well as in trauma.

CONCLUSIONS

Cc has emerged as an important and specific indicator of degeneration leading to prolapse, with percentage positivity much higher than that of En. LCS with prolapse also shows higher grades of Cc as compared to En, which emphasizes its importance. En proved to be less significant for two reasons: it has higher positivity and grade in the inflammatory group (tuberculosis) and had lower positivity in DDD inspite of examining the entire disc material. Md and Mf are universal age related changes. Though their mean grades were higher in study group, their presence is not a significant indicator of degeneration causing prolapse.

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