HISTOLOGICAL AND STRUCTURAL STUDY OF PROLAPSED INTERVERTEBRAL DISC

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Abstract

The research of the etiology of low back pain and right leg pain has been focused on the study of histological degenerative changes of human intervertebral disc prolapse. In patients with low back pain & right leg pain and disc prolapsed according to magnetic resonance imaging (MRI), histological and histochemical studies have demonstrated several histological degenerative changes in the structure of the prolapsed intervertebral disc. Surgically excised intervertebral disc from 105 patients with lumbar disc prolapsed were studied by histomorphology aided with histochemistry this include patients with prolapsed disc due to several causes. 5 control intervertebral discs were studied for comparison. Our result indicated that their was a degenerative structural changes of the intervertebral disc prolapse. Chondrocytes cloning, invasion of blood vessels into the disc matrix, disorganisation and disorientation of collagen fibers, matrix depletion and many other changes were observed.

Introduction

The intervertebral disc is located between two adjacent vertebrae in vertebral column. The histological structure of the adult disc has been extensively described by1-7 which shows that it consist of three basic components; a: Hyaline cartilage plates capping the epiphyseal ends of apposing vertebrae to the annulus fibrosus, b: a superficial highly collagenous sleeve of lamellated connective tissue that connects the hyaline cartilage plates and adjacent compact bone of the vertebral epiphysis, c: The nucleus pulposus a deep gelatinous of fibrocartilage that is confined superiorly and inferiorly by the hyaline cartilage plates and superficially by the annulus fibrosus which consist of elastic fibers and connective tissue.

In the region where the ligament join hyaline cartilage there is a transition zone of fibrocartilage which contains rows of chondrocytes between these aligned chondrocytes are parallel strands of collagen fibers8. Disease of intervertebral disc is a common health problem, according to our knowledge after searching the internet, the present work has not attempted before on a wide bases so, the study of the histological structural changes of the diseased disc should be well determined. The disc degenerate earlier in the life than any other tissue of human body9,10. Due to ageing, normal intradisc stooping, pressure, patients weight, occupation and many other factors can cause prolapsed of the disc which in tern leads to several degenerative histological changes which are more dramatic in the intervertebral disc than in any other components of the spine9. In the prolapsed intervertebral disc, the vascularization
pattern alters as a result of changes in nutritional supply. The structure of cellular and extra-cellular matrix also alters, consequently there are changes in the tensile and shock absorbing function of the intervertebral disc. These changes have been widely demonstrated in our present study.

The aim of our present study is to study the extent of histologically observes degenerative changes in the prolapsed intervertebral disc with a focus on degenerative causes of prolapses as this form major category.

**Materials and methods**

Samples of prolapsed intervertebral disc were obtained after surgical operations carried at Ebn-AlBeetar private hospital from 105 consecutive patients with a lumbar intervertebral disc prolapsed. Prolapsed disc was located at level L3–L4 in 33 patients, at level L4-L5 in 46 patients, at level L5-S1 in 19 patients and L1-L2 in 5 patients, L2-L3 two patients.

Patients with disc prolapse included 68 adult male and 37 female aged 14-72 years old (mean 45) .Specimens were collected directly after operation and tissue samples were fixed in 10% neutral buffered formalin as fixative solution for about 12 hrs. or overnight, tissue dehydrated in a successive increasing gradients of ethanol / water mixtures concentrations (50,70,90, 100).Clearing with xylene, embedding in melted paraffin. (3-5 µ) thick sections where cut, mounted over albumenized glass slides tissue sections where stained with hematoxylin and eosin to study the general morphology, in addition representative sections were stained with PAS ( Periodic acid Schiff teqnique) to illustrate the proteoglycan content of the extracellular matrix.and Masson Trichom for collagen fibers demonstration .

Tissue samples were examined under light microscope and selected fields were photographed by using special light microscope provided with Photographic microscope camera.

**Results**

There was a wide range of patients undergoing disc prolapsed ranging from 14 to 72 years old, mean age 45 years. Maximum patients were in their, decades as shown 4 and 5 decades as shown in table I. a &b, very few patients were seen at extremes of age (2 cases in 2nd decade and 2 cases in 7th decade out of 105 patients), 68 were male and 37 were female. (Table Ia & b). In general males were pre-dominantly represented in all groups with maximum sex difference being in 5 decade (male 21 and female 9).The commonest presenting symptom was low back pain and left leg pain in most cases of the study group.

**Histological findings**

Our patients were classified according to the age into three groups as shown in (table I.b.), first group with prolapsed disc included young aged patients (10, 20 & 30) total number 30 cases, 63 % were males, and 37% were females, 26 cases out of thirty show histological structural changes of the prolapsed disc to both cartilages cells and collagen fibers which are represented by increment in the numbers of chondrocytes, aggregation of chondrocytes in circular manner forming rounded cluster or column or as cloning which may reach up to 10 cells per lacuna (Fig 1. a, b). There are also mitotic division of chondrocytes cells and it is indicated by the presence of cell nests (Fig 2), in some areas large chondrocytes with large expanded lacuna also appear (Fig 3) and small newly formed chondrocyte in this group are seen which are indicated by their small size and small nuclei.(Fig 4). In this group of patients the section of prolapsed disc show no sign of chondrocytes degeneration.
Matrix appeared unaffected, almost normal, no significant changes are noticed, their reaction to PAS is positive, (Fig 5).

The collagen fibers stimulated also together with the chondrocytes, they appeared isolated, irregular, disoriented teased with newly formed blood vessels at the periphery (Fig 6), also appeared in some cases large accumulation of fibroblasts and fibrocytes were clearly noticed (Fig 7), mononuclear phagocytic cells also seen in some sections (Fig 8 a,b).

In the remaining 4 cases out of 30, examination of the prolapsed disc sections showed histological changes to the chondrocytes only, which appeared similar to the above results, represented by proliferation, aggregation hypertrophy cell nests, clusters. No changes have been noticed to the collagen fibers or the matrix of the disc.

Results of the second group of patients with prolapsed disc included, middle aged group in ( 40 & 50 ) years old, total number 57 patients 38♂ (67%) and 19♀ (33%). Histological observations of the tissue of prolapsed disc show that the degree of the changes were found grater in middle aged group rather than young aged group, 53 patients out of 57 show degenerative changes to the cartilages cells, matrix and the collagen fibers. Large aggregation of proliferated chondrocytes as clusters or isogenic groups, hypertrophy of the cells were noticed (Fig 9) but, among these cells large number of chondrocytes appeared degenerated, unhealthy, and this was indicated by the presence of chondrocytes with scanty cytoplasm, pyknotic flattened or fragmented nucleus (Fig 10), some large empty expanded lacunae (Fig 11) were usually seen. Mononuclear phagocytic cells were frequently observed in this area (Fig 12). Extracellular matrix in this group of patients also show sign of degeneration, it appeared depleted around the chondrocytes, faint and unhealthy with numerous spaces (Fig 13). Bundles of collagen fibers appeared irregular, short, interwoven, disubted, disorganized isolated, loosely arranged, over growth (Fig 14), in some places deposition of lipid and calcified materials also seen (Fig 15).

In some areas a proliferation of endothelial cells forming clusters of blood capillaries at the periphery of the prolapsed disc was also noticed (Fig 16).

In the remaining 4 cases ,severe coagulative degeneration occurred to the whole disc tissues, the majority of chondrocytes were in very bad condition, collagen fibers also show high degree of degeneration, matrix depleted, empty spaces & calcification. In some areas large number of fibroblasts and fibrocytes were seen indicating by proliferation and over growth of the collagen fibers, numerous blood spaces, were seen in the edge of the disc, mononuclear phagocytic cells are found (Fig 17).

Third age group from (60-70), including 18 cases 17♂ (61%) and 7♀ (39%). In this group of patients with prolapsed disc, histological examination of the tissues demonstrated that high degree of degeneration to the tissue of prolapsed disc, the degeneration was coagulatve, represented by, first; few individual enlarged chondrocytes were found in large expanded lacunae but appeared unhealthy and degenerated, almost dead.(Fig 18). Cartilage matrix appeared poor faint depleted its show little proteoglycane content, numerous empty expanded lacunae and spaces were found (Fig 19). Collagen fibers loosely arranged, coarse interwoven in seperated short bundles, in most cases overgrowth of collagen fibers replacing the cartilegenous tissue extend through the outer annulus induces ingrowth of granulation tissue was noticed.
Discussion

Analysis of histological changes in prolapsed intervertebral disc tissue is purely histomorphologic, that means the tissue of (IVDP) showing variable degree of degenerative changes in young, middle and advanced aged groups. In young and middle aged groups stimulation of intrvertebral disc tissue, which were represented by proliferation, division, hypertrophy, cloning, or clusters of chondrocytes, as well as changes to the collagen fibers of the disc were found to occur together with the changes in chondrocytes.

In young aged group degeneration of chondrocytes were not commonly seen, but it was found to be more at middle aged and greater at advanced aged groups. This can explain that at young aged patients, cell and tissue are very active so it can resist any external pressure by proliferation, division, hypertrophy of chondrocytes and by regeneration of the damaged cells and tissue. In middle aged group although these changes are exist but chondrocytes degeneration, and death, were very common, that means, regeneration and substitution of cell and tissues is harder at this age, but it also depends on the condition and the history of the patient illness. However this study apparently indicated that age range of patients showing overgrowth of chondrocytes which was wide higher grade were present in young aged and middle aged patients. Using PAS stain showing matrix depletion around the clusters of chondrocytes. In middle aged group, but this was not the case at young aged group, this findings agree with the findings of, Nerlich (1998) how also noticed matrix depletion around aggregated chondrocytes, he referred this to as globular matrix modification. The process of chondrocytes degeneration at middle and advanced aged reduced the ability of matrix synthesis by the chondrocytes.

Since young aged fibrocartilage patients shows growing of chondrocytes in small rounded groups of an increased number of cell per single lacuna, sharply demarcated by a rim of adjacent fibrocartilage matrix, these crowded chondrocytes were seen lie in modified territorial matrix which were noticed in about 75% of the study group. Nerlich described this features as a sign of advanced degeneration.

In young aged patients, stimulation of collagen fibers also occurred at the same time with the chondrocytes, but as our results showed that this stimulation was found to be greater at middle and advanced aged groups, with overgrowth of coarse collagen fibers threads, indicating qualitative and quantitative alteration in collagen, a case which also described in literatures.

The formation of newly developed blood vessels at the periphery of the prolapsed disc invading the fibrocartilage fragments of the disc is important features of prolapses which are seen in most cases. Nerlich also observed this phenomenon he concluded that this is also the features observed in inflammation and small percentage in trauma.

Rapanti et al 1998 suggested that these blood vessels were newly formed, may be through metaplasia of undifferentiated mesenchymal cells. It should be noted that the undifferentiated mesenchymal cell are usually concentrated around the outer blood vessels adjacent to the disc tissue, and because the tissue of the fibrocartilage is degenerated, weaken and loose so the undifferentiated mesenchymal cell can penetrate easily and proliferate to form endothelial cells forming new blood vessels.

Chondrocytes, cloning, vascularizations are significance morphology indicate Symptomatic degeneration. Matrix depletion and fibrillation on the other hand are also another symptom for degeneration which is also indicated by Rapinti et al.
Erdal et al 2000 hypothesized that, the disc composed mainly of three components, Nucleus polposus, annulus fibrosus and end plate, damage in any one of these components result in degeneration of intervertebral disc function.

In degenerated prolapsed human disc, annular lamellar rupture are common and may be associated with spinal pain. Some mononuclear phagocytic cells were seen commonly in close association to degenerated chondrocytes, may be as a part of autoimmune system, or to clear up the tissues from cellular debris. (Weinder, 1998. Pai, et al 1999. and Twomy, 1985) findings shown that there was loss of water of the disc tissue which decreases the amount of hyaluronic acid and increase in collagen fibers, this may considered as a symptom of accelerate aging of the disc.

Our results suggested that histological degenerative changes of the intervertebral disc tissue are probably representative of primary degenerative process independent of age related changes in disc.

Nerlich et al, confirmed that the disc degeneration starts as early as in the second decade of life a case which also shown in the present findings. Therefore only prevention of disc changes inhibits disc degeneration. He also suggested that the disc undergoes an accumulative stress, possibly leading to altered properties of the collagen fibers and thereby tissue destruction. Male constitute about 65% of the total cases this explain by the higher exposure of males to physical break strain. Lumber intervertebral disc are more frequently affected Bilter et al stated that Lumber IVD are affected to their positional at lordotic apex. Our findings indicated that age is not the solely reason behinds disc prolepses, but many other factors can accelerate the degenerative changes in human IVD beside age. Pressure, body weight, patients occupation, social and economic condition of the patients and immobilization are additional factors. Heavy body weight causes an overload on the intervertebral disc leads to pressure on the disc and compression of the disc which in tern leads to lacking of blood supply (oxygen and nutrient) to the disc, causing crowdy and swelling of chondrocytes as a results of increasing in absorption of water because of an increase in the concentration of Na inside the cells. Advanced age on the other hand causes weakness of the muscles and this cause reduction in the activity of the tissue of the disc by reducing the activity of chondrocytes which make the disc more fragile and unable to bear heavy weight. Thus old aged patient's disc show coagulative degenerative changes in the tissue of the disc with the replacement of the hondrocytes by collagen fibers.

Conclusion
1. Appearance of overgrowth of chondrocytes as cloning or clusteres of cells is an important and spesific indicatore of primary degenerative changes leading to prolapse.
2. Development of newly formed blood vessels proved to be the second sign of degenerative (prolapsed) intervertebral disc.
4. Other changes however like matrix depletion, overgrowth of collagen fibers are universal age related changes, their mean grades were higher in middle aged and advancesd age groups, there presens is not significant of degeneration causing prolapse.
5. This study also indicated that, age is not a sole reason behind disc prolapse It is most common in men, and the most effected level was of the spine was L4 – L5 lumber.
Table Ia

<table>
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<th>Age year</th>
<th>Total no.</th>
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<th>Sex Female</th>
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<th>Sex % Female</th>
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<td>3</td>
<td>1</td>
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<td>25%</td>
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<tr>
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<td>14</td>
<td>10</td>
<td>58%</td>
<td>42%</td>
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<tr>
<td>40 - 49</td>
<td>27</td>
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<td>10</td>
<td>36%</td>
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<tr>
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<td>9</td>
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<tr>
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<td>Total</td>
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<td>68</td>
<td>37</td>
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Table Ib

1. Young age group 30 case 29% → male 19 (63%) ↓ female 10 (37%)
2. Middle age group 57 case 54% → male 38 (67%) ↓ female 19 (33%)
3. Old age group 18 case 17% → male 11 (61%)

Fig. 1&2
Histological and structural study of prolapsed Intervertebral Disc

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Fig. 3, 4, 5

Fig. 6, 7, 8a & b

Fig. 9 & 10
Fig. 11 & 12

Fig. 13 & 14

Fig. 15 & 16

Fig. 17 & 18

Fig. 19
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References