Basrah Journal Of Surgery

Original Article
Bas J Surg, March, 13, 2007

HISTOLOGICAL DEMONSTRATION OF PAINFUL PROLAPSED INTERVERTEBRAL DISC WITH HISTOCHEMICAL DETECTION OF NERVES INGROWTH

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Abstract

Microscopic study was done on surgically collected samples of prolapsed intervertebral disc. These samples were collected from patients suffered from low back pain associated with right or left leg pain. The study was focused on patients below 40 years. Magnetic resonance imaging (MRI) was used to confirm the diagnosis.

The study demonstrates the degenerative changes that occur early in these patients which may be resulted from any traumatic causes which lead to series of degenerative changes that occur faster and differ from that changes which occur with age progress.

Forty samples of prolapsed intervertebral discs were collected surgically and 5 control intervertebral disc were studied for comparison.

Nerve ingrowths have demonstrated (histological and histochemical) in region of the excised tissue of prolapsed disc. This feature is accompanied with several degenerative changes like cloning of chondrocytes, irregularity of collagen fibers, and invasion of newly formed blood vessels into the disc matrix.

Introduction

The intervertebral disc consist of three basic parts; The central is the nucleus pulposus, the surrounding is the annulus fibrosus and the upper and lower parts are the vertebral end plates[1], the nucleus pulposus consist of unusual fluid[2] formed of supporting tissue, which is the only remnants of embryonic notochord that persist in adult[3]. Annulus fibrosus consist of lamellae of collagen type I these fibers are thinner and fewer posteriorley than interiorly[4]. The annulus has 10% of its dry weight is elastic fibers[5].

The vertebral end plates towards the vertebral body is hyaline cartilage mostly found in younger discs, and fibrocartilage towards the nucleus pulposus which is mostly found in older discs[6]. Kim et al (2003)[7], concluded that the intervertebral disc contains some enzymes that synthesize its matrix and some other enzymes that break it down, he also suggested that there is balance between degradation and synthesis of its matrix.

The disc itself has a low metabolic rate, receives most of its nutrition by diffusion[8], for unknown reason the nucleus pulposus losses much of its vital blood supply during first decades of life[9].

Disc degeneration and herniation are multifactor process and that both mechanical and biochemical derange-ment exist[10].

Histological cloning and edge neovascularization are more specific indicators of degeneration of intervertebral disc[11,12] the healthy adult disc has some nerves, mainly restricted to the outer lamellae of the annulus fibrosus, some of these nerves terminates in propriocepters[13].

Degenerated human intervertebral disc have been shown to contain more nerve tissue and more vascular than normal disc[14-16].

The aim of this study is to investigate the presence of nerve fibers within the prolapsed intervertebral disc tissue and its relation with disc degeneration, back pain, and leg pain duration.

Materials and methods

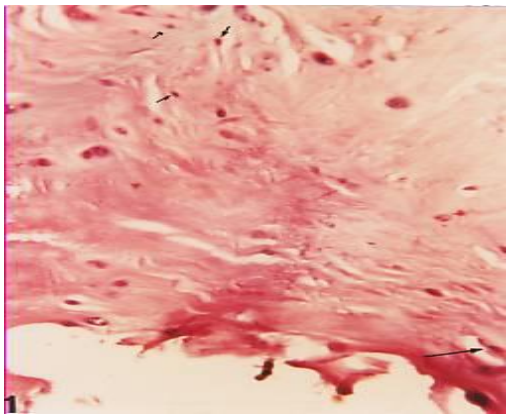
Samples of prolapsed intervertebral disc were obtained after surgical operations carried at Ebn Al-Beetar private hospital from 40 patients with prolapsed intervertebral disc at levels L3-L4, L4-L5 & L5-S1.

Patients age was below 40 years old. Samples were divided into two groups (Tables I&II) according to pain period in patients back pain or legs pain, first group were contained of 15 samples from patients who suffered from pain for one year or more, second group 25 samples from patients who suffered from pain for less than a year.

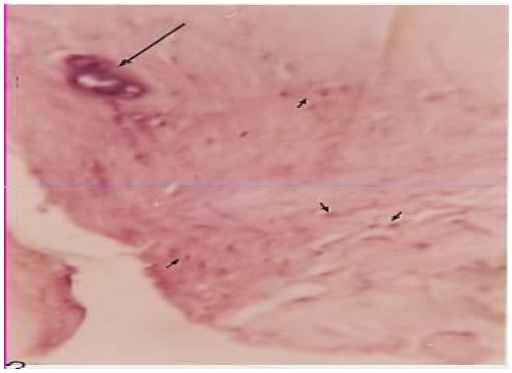
Specimens were collected directly from patients after operation, tissue fixed in 10% neutral formalin for about 12 hours, dehydrated in successive increasing ingredient of ethanol/water mixture concentration 50%, 70% & 90%, then absolute alcohol was put. Tissue cleared with xylene, embedded with melted paraffin. (3-5) μ thick section were cut, mounted over albumenized glass slide. Tissue sections were stained with hematoxylin and eosin to demonstrate the general morphology; some other sections were stained with Periodic acid Schiff (PAS) to illustrate the proteoglycane content of extra cellular matrix. Tissues of prolapsed intervertebral disc were stained with silver nitrate to demonstrate nerve fibers in growth according to[17] H.C. Cook (1974). Tissue sections were examined under light microscope and photographed the selected fields that show our results.

Results

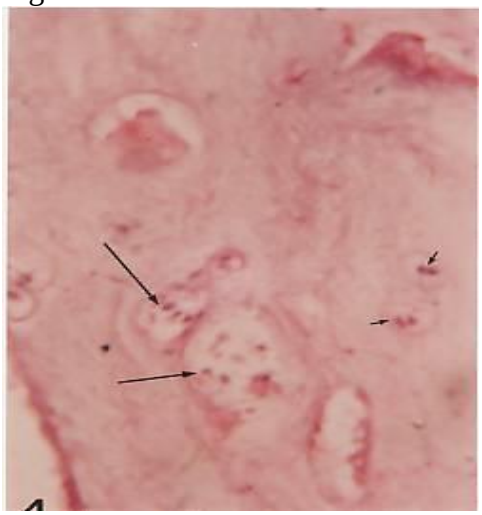
First group: Histological findings of the first group revealed wide spread of chondrocytes in some tissue as shown in Fig.1.



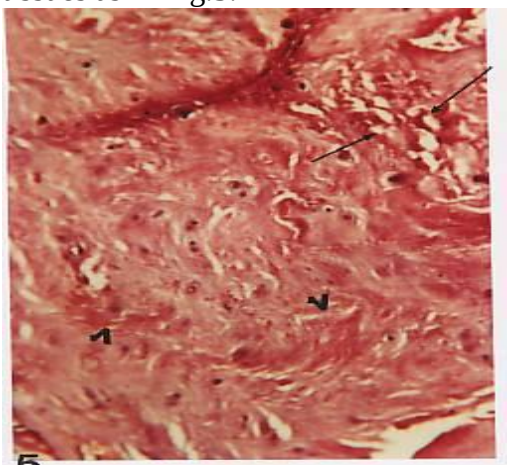
Large number of these chondrocytes showed clusters and cloning. Different histological features of chondrocytes degenerations shown in Fig. 2&3.



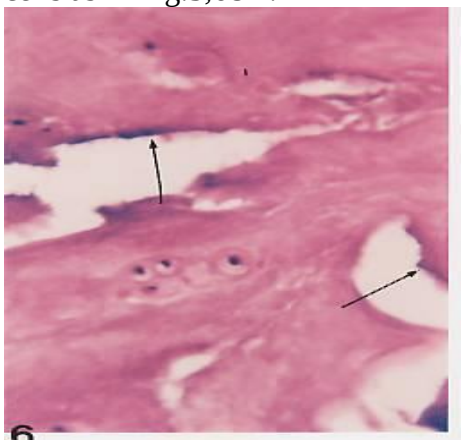
Some of the expanded lacunae contain degenerated chondrocytes, others contain blood cells as demonstrated in Fig. 4.

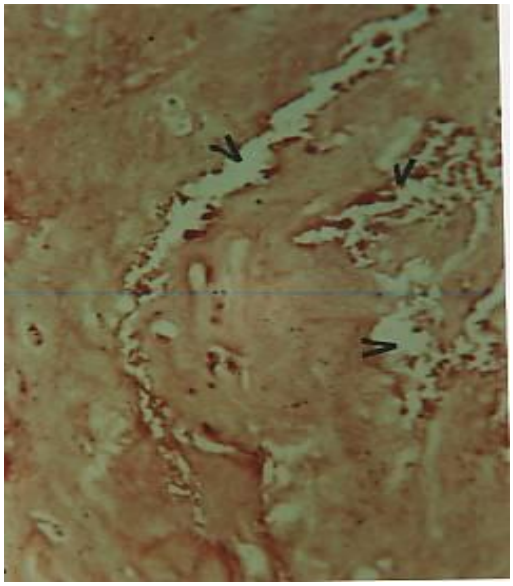


Tissue spaces were noticed clearly accumulated in dead region of necrotic tissues as in Fig.5.

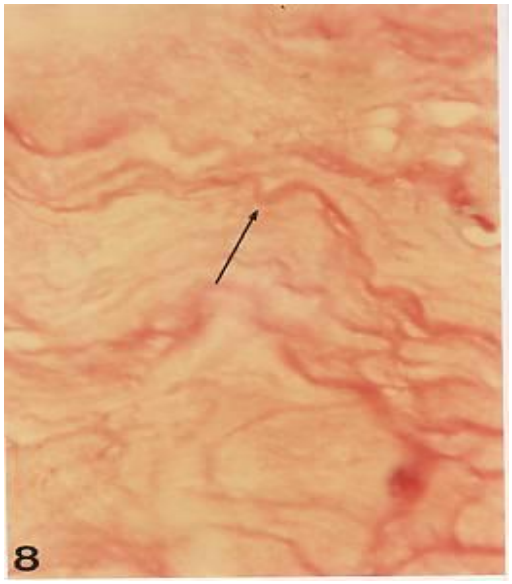


Some of these channels and spaces lined by darkly stained border, and some others were filled with blood cells as in Fig.3,6&7.

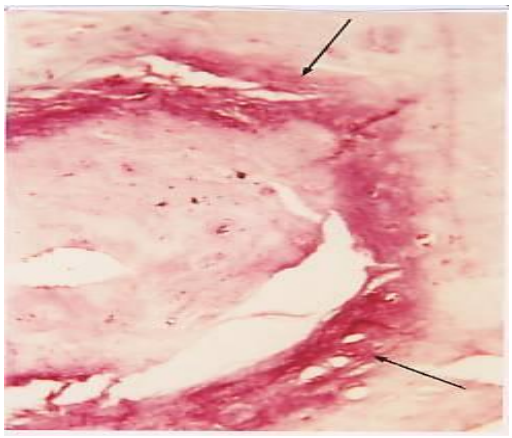




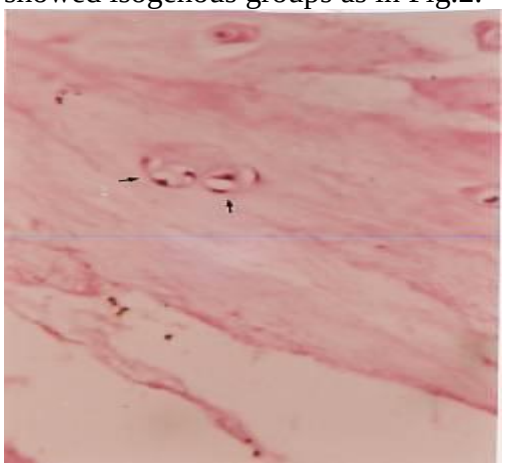
Few fibroblasts and fibrocytes were seen in some cases spread in between chondrocytes specially in between degenerated chondrocytes as in Fig.2.
Tissue fibers appeared normal in some cases and in others appeared slightly disoriented (Fig.5). Large area of dead necrotic tissue present with many spaces and remnants of irregular darkly stained fibers as in Fig.8.



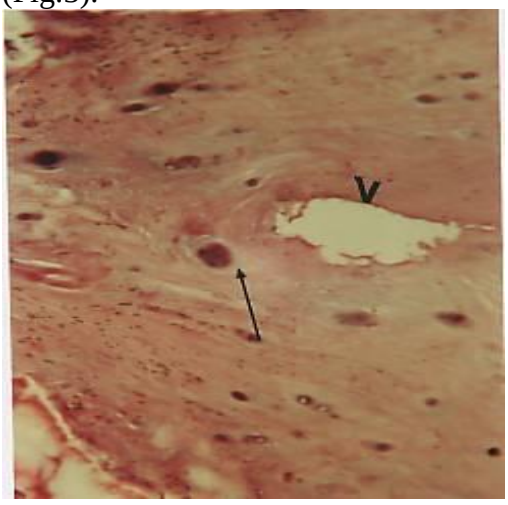
Second group: Histological findings of the second group revealed tissue cracks and tearing, some of these cracks are surrounded by darkly stained area, others showed no such darkness as in Fig.1.



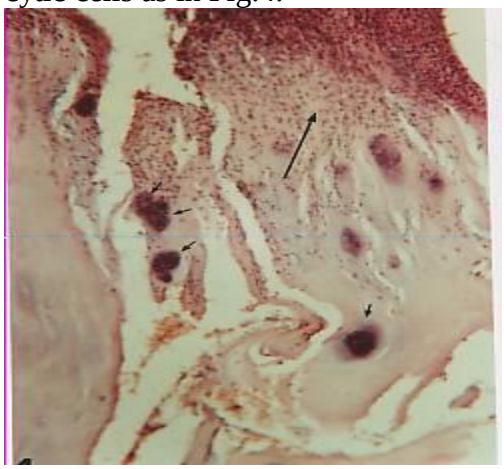
Generally chondrocytes number was few chondrocytes appeared in different histological features, some of them appeared normal, others were either small in size with their lacunae or showed isogenous groups as in Fig.2.



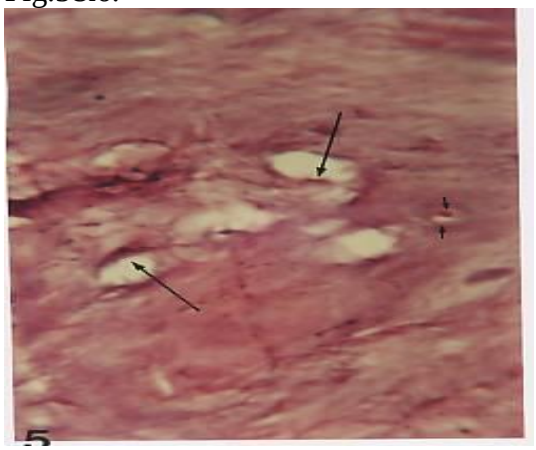
Expanded lacunae were seen in some tissues with darkly stained cells (Fig:3).

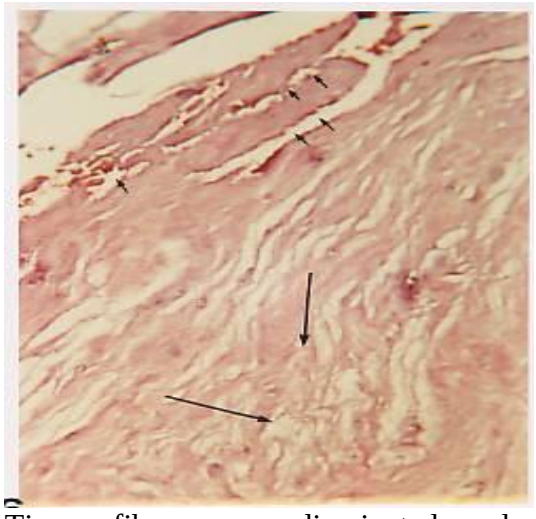


Most of the disc tissues showed granulation tissue at their periphery, which are represented by accumulation of large number of cells like fibroblast, fibrocytes and mononuclear phagocytic cells as in Fig.4.



The results of this groups also showed, appearance of different sized spaces in between tissue fibers, some of these spaces are lined by flat dark cells as in Fig.5&6.

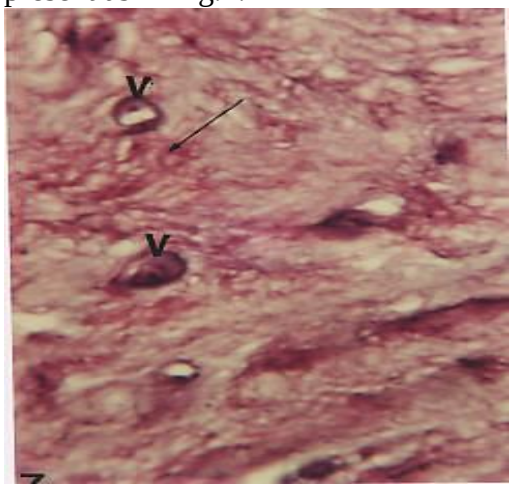




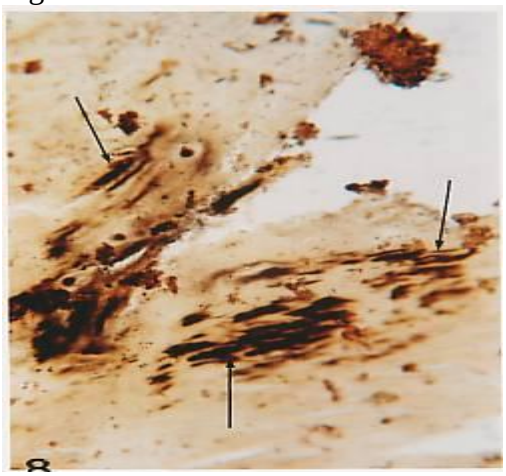
Tissue fibers were disoriented and fragmented and in some tissues of prolapsed disc they were seen with variable staining affinity, some

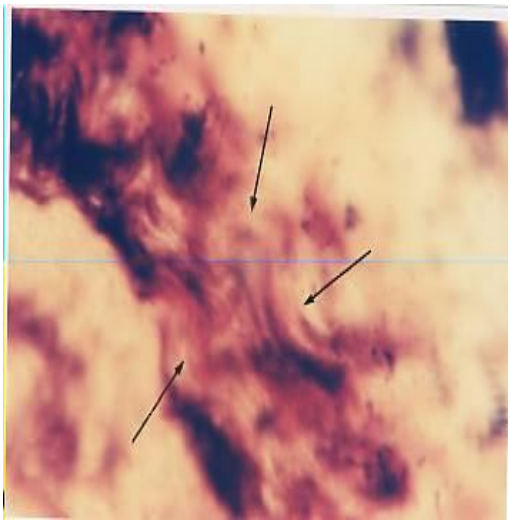
appeared faint acidophilic, others however appeared darker in its acidophilic stain.

In some cases fine irregular network fibers with basophilic stain were present as in Fig.7.



Observation of wavy fibers were clearly detected as nerve fibers as In Fig.8 &9.





Discussion

On the basis of pain duration in back and both legs, the histological demonstration of tissue samples, showed differences between the first and the second group. The first group show slight tissue response as it compared with the second one, this response is demonstrated by slight spread of fibroblastic cells that occur as a result of metaplasia of the tissue of intervertebral disc to another kind of tissue because of affected trauma and injury[18].

Degeneration of tissue is clearly demonstrated by the presence of tissue spaces and cloning of chondrocytes, these findings are in agreement with that of references[10-12].

The morphology of tissue spaces were different from region to another, some of the spaces appeared small and grouped together each one was found adjacent to the other, which may indicates that these are empty lacunae of degenerated chondrocytes in a localized area.

In other cases some spaces appeared large and irregular in their shape like torn areas or cracked regions. Usually the intervertebral disc get its nutrition by diffusion from the outer blood vessels at the antrolateral surface of the vertebral body and capillary tufts in the end plate, Boos[9], explain that obliteration of some of these vessels, early in the first half of the second life decade diminished the nourishment of the disc which initiate tissue breakdown.

Other authors like Mauona[19], evaluate the role of diminished arteriole blood flow in painful disc degeneration.

Decrease in tissue nourishment affect vitality of the cells, some of them degenerated and die others showed reduction in cellular products, and this was clearly indicated by matrix depletion that were clearly seen by using PAS stain. In other cases

however signs of chondrocytes degeneration were seen, which could be due to dehydration as a result of lack in the product of proteoglycane and poorly developed aggrecans that will hold water and give the disc its healthy appearance in MRI and without it look compressed, dark and with sign of tearing and cracking.

In the other hand however, the presence of darkly stained regions that lined some spaces, probably suggest that these spaces are developing endothelial layer through the process of neovascularisation. The results also showed some expanded lacunae containing erythrocytes, degenerated chondrocytes, and mononuclear cells within these lacunae, these findings confirm the results of Rapanti[20], who suggested that the newly developed blood vessels occur in response to trauma are associated with endothelial cells, fibroblast and mononuclear cells.

Our study propose a kind of relationship between those spaces with the darkly stained region, which may represent a lacunae of degenerated chondrocytes and the beginning of newly developed blood vessels.

Weinstein[21], suggested that trauma to the intervertebral disc may result in a focus of inflammation inside the disc, that inflammation involves many events in cells, their products, factors, enzymes all together work trying to repair the damaged parts .

We also proposed that mononuclear cells which are phagocytic cells appear in region of neovascularization to clean up the debris from degenerated tissue they are resident cells from the cells of the disc tissue itself and that confirm the findings of Nerlich[22], because the disc itself is active tissue that containing significance mechanisms for selfrepair[8].

Histological demonstration of tissue sample of the second group showed clear tissue response. At the periphery of most prolapsed disc tissue were granulated tissue, this response start from the periphery where higher concentration of oxygen than internal regions of the disc. The most prominent active cells are the fibroblasts cells which are clearly identified by their pale nuclei and prominent nucleoli, these cells start regeneration process by proliferation, migration and formation of newly formed collagen fibrils and proteoglycane[18]. Variability of the morphology and staining ability of collagen fibers in the same specimen was obvious could be explained that the presence of two generation of collagen fibers, the old one which some of them appeared degenerated, and the newly developed collagen fibers which are appeared immature fibers. Other sign of degeneration was the fragmentation and disorientation of collagen fibers, associated with cloning of chondrocytes, tissue spaces and neovascularization, these findings agree with Duance[23] findings who concluded that the changes in cross link profile of collagen fibers in degenerative disc disease are indicative of increase matrix turnover and tissue remodeling. Porkharna and Philips[24] also suggested that alteration in concentration in some of the crosslink with degeneration of IVD may plays role in pathogenesis of the degenerative process. According to our findings, we conclude that the imbalance in the degeneration and regeneration that occur to the intervertebral disc effect the distribution of these fibers, their arrangement and their biochemical component, therefore these fibers be disorganized and appeared as thread like or thick fibrous tissue mass.

Traumatic effect on the disc accelerate the degenerative process with tissue attempt for healing by different unsuccessful ways of regeneration,

these attempts are the newly developed extracellulr matrix ,new generation of chondrocytes by cloning, neovascularization and mononuclear phagocytic cells that seen adjacent to chondrocytes and vascular ingrowth areas(Nerlich[22]).

On the other hand the appearance of dark basophilic stained areas around the torn or cracked spaces, is probably because of the leakage of enzymes products from the healthy chondrocytes to the surrounding area, these findings were noticed from patients who suffered from pain for short time period (few months) these chemical could irritate the area and cause a focus of inflammation followed by serial of events that accelerate the degenerative process, and that could be the main cause of the pain in the degenerative disc prolapsed Boose[25], and Boden[26], concluded that more than 70% of normal asymptomatic people have disc prolapsed pressurizing nerve roots but with no pain, which is agree with our findings.

Demonstration of wavy nerve fibers which were clearly detected by silver nitrate that run in the regions of some tissue samples of the second group, some were peripherally located as bundles adjacent to the torn area, others were seen separately deep in the tissue of prolapsed IVD which agree with Freemont[15] findings that indicate nerve ingrowth all way into the nucleus pulposus in 30% of discogram positive discs, and these nerve fibers were not traveling with the blood vessels, and look like pain nerve fibers.

Compression of these nerve by twisting bending of its region could generate Pain. Our finding indicate change of intervertebral disc tissue as healing attempt after changes in biochemical and structural component of the tissue in response to hard trauma action.

Conclusion

1. It seems that some people will develop chronic life–long pain as a result of an accelerated form of disc aging which induced traumatically.
2. Not all pain that produced is originated from the prolapsed intervertebral disc, it could be originated from the disc itself, so we are suggesting that the removal of complete prolapsed intervertebral disc in surgery is better to avoid pain recurrence.
3. Granulation tissue is a tissue response for regeneration as a result of trauma and injury which is periphery located in the tissue, whereas neovascularization is a tissue response and attempt for regeneration for tissue from inside where degeneration occur and accumulate tissue debris.
4. Newly formed cracks show darkly stained area around it, probably because of many chemicals that leak for healthy cells and fibers. This kind of cracks induced pain also.
5. Nerve in growth could be part of the process of disturbed repair.

Table: I {First group}

Pain Period	Site of disc Prolapse	No. of sample	♀	♂
1 year	L3 – L4	4	-	4
2 years	L3 –L4 L4 – L5	4 3	2 2	2 1
3 years	L3 –L4 L4 –L5	2 2	2 2	- -

Table: II {second group}

Pain Period	Site of disc prolapse	No. of samples	♀	♂
1 months	L5 –S1	1	-	1
3 months	L3 –L4	5	1	4
4 months	L4 -L5	3	2	1
5 months	L4 – L5 L5 –S1	2 2	- 1	2 1
6 months	L3 –L4 L4 – L5	8 4	4 -	4 4

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