

## NEOVASCULARIZATION IN HERNIATED LUMBAR DISC: A HISTOLOGICAL STUDY

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### ABSTRACT

*Background:* Inter-vertebral disc is the largest avascular structure in human body, which is primarily a load bearing and stabilizing unit of the human spine. Degenerative disorders and disc herniation causes proliferation or in growth of new blood vessels in this structure. Lumbar disc herniated tissues were studied microscopically in comparison with the cadaveric lumbar disc tissue, to evaluate the changes particularly the formation of new blood vessels.

*Methods:* It was a case control study in which 45 lumbar herniated disc tissues ( $L_4 - L_5$  and  $L_5 - S_1$ ) and 45 dissected, fresh cadaveric disc tissues of same level and almost of same age groups were collected and in reference to age were divided into groups. Both sets of tissues were processed, sectioned and stained with Hemotoxyllin / Eosin, to observe the architecture of annuli fibrosis and nuclei pulposus parts of disc and the micro-vessels under light microscope.

*Results:* Cadaveric discs, group A, B and C compared with herniated discs  $A_1$ ,  $B_1$  and  $C_1$ , Annuli fibrosi in herniated discs ( $A_1$ ,  $B_1$ , and  $C_1$ ) showed significant reduction of cells, disorganized lamellar pattern of collagen, formation of cysts, clefts and numerous new micro-vessels as compared to fresh cadaveric disc tissues (A, B and C).

*Conclusion:* Disc degeneration and herniation results in the formation of micro-vessels which may not only serve as source of nutrients but also contribute in the healing process of discal tears.

*Key words:* Lumbar disc herniation, Cadaveric lumbar disc, neo-vascularization, different age groups.

### INTRODUCTION

Human spine forms the central axis of the skelton to which all other moving elements and organ systems are directly or indirectly attached. Efficiency of the human spine in coping with varying demands of load bearing and movement depend on the properties of discal connective tissue. Human spine has got opposing functional attribute, providing a rigid structural support and functional movement. This dual purpose is achieved by the segmented structure of the spine, vertebral bodies and their intervertebral discs.<sup>1</sup> The disc transmit load and provide flexibility to the spine, owing to its extra cellular matrix, collagen and proteoglycans.<sup>2</sup> People whose work involves repeated bending and lifting have a 300% – 600% increased risk of lumbar disc herniation.<sup>3</sup> The disc is an avascular structure composed of an extra cellular matrix embedded with chondrocytes and fibrocyte like cells. The Annulus Fibrosus is densely packed with rings or highly organized collagen type – I fibril sheets or lamellae, in which fibrocytes are distributed. The nucleus pulposus, a gelatinous structure in which water molecules are retained in the matrix by

proteoglycans and collagen type – II among which chonrocytes are suspended. Fibrocytes and chondrocytes produce and maintain the matrix in which they are embedded and in the disc they have good ability under such avascular condition.<sup>4</sup>

Inter-vertebral disc plays a central role in the spinal disorders and earliest start of degenerative process in disc ultimately results in many spinal disorders. Inter-vertebral disc degeneration is a common phenonmenon with an increasing frequency with age, upto 90% of individuals older than 60 years of age have at least one degenerated intervertebral disc.<sup>5</sup> Intervertebral disc disease is a major epidemiological problem as 50 – 70% of adults experience at least one episode of the low back pain in their life time.<sup>6</sup> One specific Patho-anatomic condition related to back syndromes, particularly the sciatic pain, is the lumbar disc herniation.<sup>7</sup> In herniated disc, detached part not only causes the mechanical compression but also produces chemical mediators, responsible for the sciatic pain. Some disc cells e.g. chondrocytes produce cytokines.<sup>8</sup> Either due to degenerative changes or disc injury, the herniated disc becomes

vascularized.<sup>3</sup> The degree of neovascularisation depends on age and the severity of disease.<sup>8</sup>

This study was designed to observe the status and degree of herniated disc neovascularization in different age groups as compared to fresh cadaveric discs.

## MATERIAL AND METHODS

The study was carried out in the Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi. Forty five surgically obtained human herniated lumbar disc tissues and forty five fresh cadaveric lumbar disc tissues of similar age groups and from the same level (L<sub>4</sub> – L<sub>5</sub>, L<sub>5</sub> – S<sub>1</sub>) were studied under light microscope, observations and results of both disc tissues were compared and analysed.

### Collection of Material

In this study Forty five male / female adult cadavers with almost normal lumbar spine approximately ranged between 20 – 65 years of age, selected as control. Only those cadaver who had non-traumatized spine, had no spinal deformity, previously unoperated spine, and had no wound or bedsore on the back, were included in this study. Each Cadaveric lumbar disc was dissected out within 24 – 48 hours after death, preserved in 10% neutral buffered formalin. Those lumbar discs which showed marked changes in colour, irregularities in shape, felt very hard in consistency or showed extra growth over it were not included in this study. Microscopically, disc tissues showing signs of infection, chronic inflammatory lesion or neoplastic changes were excluded from the study. In this study group forty five selected patients for herniated lumbar disc tissues were adult male / female, age ranged between 20 – 65 years from L<sub>4</sub> – L<sub>5</sub> and L<sub>5</sub> – S<sub>1</sub> level. All patients had low backache and radicular pain in their legs on the affected side and the diagnosis were confirmed by magnetic resonance imaging (MRI), before surgery. Surgically obtained herniated disc tissue from each individual patient was preserved in 10% formalin, as soon as it was removed. Those patients who had evidence of scoliosis, congenital or acquired spinal deformity, previously operated spine and had history of chronic systemic disease or malignancy were all excluded from this study. In reference of age both disc tissues were divided into group A, and A<sub>1</sub> (20 to 35 years), group B and B<sub>1</sub> (36 to 50 years) and group C and C<sub>1</sub> (51 to 65 years) each group was comprised fifteen discs. Both sets of disc tissues fixed in 10% formalin for 24 – 48 hours, surgically obtained specimens had brown or black discolouration (due to blood stain) were again fixed in a freshly prepared fixative for another 24 hours, dehydrated, cleared, and paraffin embedded blocks were prepared, four

micron thick sections were taken, stained with haematoxyline and eosin to observe the normal and disrupted / disorganised architecture of the disc. Annulus fibrosus (AF) and nucleus pulposus (NP) and the presence of new blood vessels. Findings were recorded by three anatomists and one histopathologist independently to minimise bias.

## RESULTS

Of the 45 discs from group A<sub>1</sub>, B<sub>1</sub> and C<sub>1</sub>, 32 were found protruded (nucleus pulposus) and 13 were prolapsed (annulus fibrosus) types of herniation. The 13 prolapsed, mostly found in 50 years old and older than 50 years while protruded hernia mostly found in younger adults.

In 13 prolapsed, 7 were complete extrusions, 3 free sequestration and 3 were found incomplete extrusions. Histological findings suggest that changes in the annulus fibrosus were more extensive in prolapsed discs than in the protruded discs. Eleven of 15 discs in group A<sub>1</sub> showed protruded, four of 15 were prolapsed hernia. Thirteen of 15 from group B<sub>1</sub> found protruded and only two were prolapsed type while in group C<sub>1</sub> only two discs were protruded and 13 showed prolapsed hernia. In comparison of A<sub>1</sub>, B<sub>1</sub> and C<sub>1</sub> all discs in group A and most of the disc (eight) in group C showed normal lamellar and wavy pattern of collagen / proteoglycan with abundant distribution of fibrocytes and chondrocytes in normal scattered manner as shown in Figure 1. In Group C, thirteen of fifteen discs showed atrophy of Nucleus Pulposus and myxomatous degeneration (annular collagen fiber swollen and in reversed direction). Seven of 15 in group B and nine of 15 in group C annuli fibrosi showed in-growth of blood capillaries with unusual pairing of cells which is significant.

These new blood vessels usually were found close to the disc cells. In comparison of group A, B and C with group A<sub>1</sub>, B<sub>1</sub> and C<sub>1</sub>.

Annuli fibrosi of A<sub>1</sub>, B<sub>1</sub> and C<sub>1</sub> showed disorganized lamellar pattern cystic cavities and cleft formation and decreased amount and pairing of disc cells. Nine of 15 group A<sub>1</sub>, thirteen of 15 group B<sub>1</sub> and eleven of 15 from group C<sub>1</sub> showed in-growth of new blood vessels or capillaries in outer and inner part of Annulus fibrosus, few capillaries containing blood cells in their lumen. Two of 15 from group B<sub>1</sub> nuclear pulpotic part showed thin walled blood vessels as shown in Figure 5. Most of the tissues in the group C showed prolapsed hernia having large amount of AF than NP. Three discs in this group did not show AF part. Finding local necrosis swollen fibers with cystic cavities, two discs tissue showed small pieces of vertebral end plates in sixty year old and older than sixty years. Histological findings showed similarities as found in group A<sub>1</sub>, two of 15 in this group showed capillaries in surrounding of disc.

**DISCUSSION**

This study on herniated lumbar discs indicates high prevalence of this problem in males which is consistent with the fact that males are usually involved in professions which render their discs more vulnerable to degeneration and herniation. This is quite agreed with the studies by other investigators.<sup>9-11</sup> In this study, we found two types of herniation “prolapsed” and “protrusion” types. In our study prolapsed herniated tissue commonly found in older age group while nucleus pulpotic part mostly found in young adults. These findings are in agreement with the study by Yasuma et al.<sup>12</sup> In their study degenerative process in the nucleus pulposus is first seen during the third decade of life, whereas in the annulus fibrosus, the first change arise as fissures, appears in the fifth decade of life. In our study all herniated tissues in group A<sub>1</sub>, B<sub>1</sub> and C<sub>1</sub>, Annuli fibrosus showed loss of collagen fibrils with dis-organised and disrupted lamellar pattern, result in clefts or fissures formation, across the annulus fibrosus, and clefts may extend to the inner part, “nucleus pulposus”. Annuli fibrosus showed myxomatous degeneration, swollen fibres with cystic cavities. Nucleus pulpotic part mostly showed loss of proteoglycans disrupted wavy pattern, fibers found in crumbling appearance with unusual pairing of cells. These histological findings are in agreement with the study by Buckwalter.<sup>13</sup> They suggest that tears or fissure formation are to be associated with the gradual loss of collagens and proteoglycans produced by the disc cells.

Akmal et al. (2004) suggest that nicotine leads to a reduction in viable cells. Normally Nucleus pulposus consists of type – II collagen with minimal quantity of type – I collagen. In degenerative disc disorder, type – II collagens replaced with the more fibrosus type – I collagen, resulting in a stiffer Nucleus pulposus.<sup>14</sup> In our study the new blood vessels were found in all age groups of herniated discs and were highest in young middle age group B<sub>1</sub> (35 – 50 years). In herniated groups A<sub>1</sub>, B<sub>1</sub> and C<sub>1</sub> these were found upto annulus fibrosus but two out of 15 discs in group B<sub>1</sub> the new blood vessels were even found upto the nucleus pulposus. This is consistent with the study of Virri et al.<sup>3</sup>

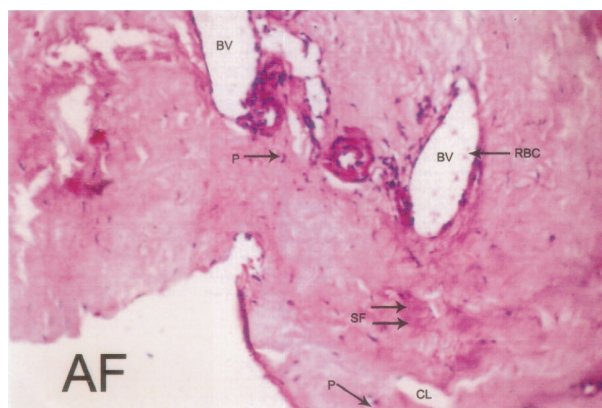
It is possible that pre-existing blood vessels

**Table 1:** Grouping of cadaveric and herniated discs with and without herniation.

Lumbar Disc	Groups	Age (Years)	No. of Discs in Each Group Male / Female	Protosun Hernia	Prolapse Hernia
Cadaveric	A	20 – 35	15 – 13/2	–	–
	B	36 – 50	15 – 11/4	–	2
	C	51 – 65	15 – 15/0	3	7
Herniated	A <sub>1</sub>	20 – 35	15 – 11/4	11	4
	B <sub>1</sub>	36 – 50	15 – 14/2	13	2
	C <sub>1</sub>	51 – 65	15 – 9/6	2	13

**Table 2:** Prevalence of blood vessels in Herniated and cadaveric disc tissues.

Lumbar Disc	Groups	Age in Years	Blood Vessels Found	Protosun Hernia	Prolapse Hernia
Cadaveric	A	20 – 35	15	–	–
	B	36 – 50	4/15	+1	+3
	C	51 – 65	9/15	+2	+7
Herniated	A <sub>1</sub>	20 – 35	9/15	+7	+2
	B <sub>1</sub>	36 – 50	13/35	+11	+2
	C <sub>1</sub>	51 – 65	11/15	+7	+5



**Footnotes:** BV: Blood vessels, CL: Cleft formation, P: Paired chondrocytes, RBC: Red blood cell, SF: Swollen fibres.

**Fig. 1:** Blood vessels in AF (Annulus Fibrosus) of Intervertebral Disc.

within the intervertebral disc were extruded together with the herniated tissue or that the blood vessels developed de novo after the herniation has occurred.

This neovascularisation in disc herniation represents an attempt to repair the injured tissue or one perhaps necessary for the resorption of harmful tissue fragments.<sup>15,16</sup> The topographical study may indicate that at least some of these newly formed capillaries support the nutrition of discal cells that have been dislodged from their native intervertebral disc.

It is **concluded** this study supports the view that lumbar disc herniation involved in degradation of collagens and proteoglycans with significance decrease of cells (fibrocytes / Chondrocytes) result in the formation or in – growth of new blood vessels, this new vascularity represent the collateral pathways which serve not only as source of nutrients but also contribute in the healing process of discal tears.

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