

MORPHOMETRIC HISTOLOGICAL, CYTOSKELETAL ANALYSIS OF LUMBAR INTERVERTEBRAL DISC IN HERNIATED AND NON-HERNIATED DISC

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Abstract

Background: The human intervertebral disc is a fibrocartilage between vertebral end plates. They aid in forming the joints between the vertebral bodies, which are of the symphysis kind. Knowledge of the lumbar disc's morphometry, physiology and anatomy is essential for managing disc related ailments. Morphometric measures are significant therapeutically for the operating surgeon and beneficial for creating artificial implants for disc replacement procedures. The study aimed to analyse the morphometry, histology and cytoskeleton of the lumbar intervertebral disc in herniated Vs non-herniated discs. **Materials and Methods:** 50 X-rays of the lumbosacral spine, with an equal percentage of male and female (20-30 years of age and no prior spine surgeries), were obtained for morphometric measures from Govt Kilpauk medical college hospital, Kilpauk, Chennai-10. Distribution of index of Lumbar disc at various regions and histopathological observation were measured. **Result:** The results of the morphometric study demonstrate that the disc height and other indexes are almost identical compared to studies in other Indian populations, with little variance between genders. Histological observations included collagen pattern derangement, neovascularisation, chondrocyte proliferation, and alterations in ground material. **Conclusion:** This study found that histological disc modifications were associated with cytoskeletal and morphometric degeneration, implying that a better knowledge of these changes is critical for developing future therapy options for surgical discectomies.

INTRODUCTION

The intervertebral disc is a highly organised matrix put down by several cells in a precise pattern. Laterally, the core gelatinous nucleus pulposus is enclosed inside the more collagenous annulus fibrosus and the cartilage end plates inferiorly and superiorly.^[1] The human disc contains some vascular supply within the cartilage end plates and the annulus fibrosus at birth. Still, these arteries quickly fade, leaving the disc with minimal direct blood supply in the healthy adult. Water is lost from the matrix with age, and the proteoglycan composition alters and declines. The disc, particularly the nucleus, becomes less gelatinous and more fibrous, forming fractures and fissures.^[2] More blood vessels from the annulus's outer portions expand into the disc. There is an increase in cell proliferation and cluster formation, as

well as an increase in cell death. Cartilage endplate thins, cell density changes, cracks, and subchondral bone sclerosis occur.^[3] These alterations are comparable to those found in degenerative disc disease, prompting debate about whether ageing and degeneration are independent processes occurring on a different timetable. Other illnesses involving the intervertebral disc might cause morphological abnormalities. Patients' discs with spinal abnormalities, such as scoliosis, develop ectopic calcification in the cartilage end plate and, in certain cases, the disc itself. In individuals with spondylolisthesis, discs exhibit very lengthy cell processes.^[4]

Cells in herniated discs appear to have higher cellular senescence and produce more matrix metalloproteinases than cells in non-herniated discs. It is not always evident what role aberrations play in

the etiopathogenesis of many illnesses.^[5] Disorders might be induced by a genetic predisposition or a tissue reaction to an injury or environmental change. Whatever the initial cause, a tissue shape change will likely impact the tissue's physiologic and mechanical functioning.^[6] Intervertebral disc (IVD) degeneration, more prevalent in LBP patients than asymptomatic persons, is influenced by various professional histories and genetic predispositions. Most disc degeneration cases, particularly in the clinical context, are identified using imaging techniques. Still, there are few large-scale studies on the histomorphological alterations in IVD, particularly in clinically well-defined surgical material. To the best of our knowledge, most histomorphological reports are based on postmortem materials.^[7]

When warranted, direct or indirect decompression of neural components, restoration of disc height, sagittal and coronal balance, and solid fusion are the primary aims of spine surgery.^[8] One of the most often utilised ways for attaining these aims is interbody fusion with an intervertebral spacer (cage). However, choosing an oversized cage can increase distractive and compressive forces on the endplates, predisposing the patient to endplate subsidence, vertebral body fracture, loss of correction, and procedure failure with recurrence of symptomatic pathology and the need for complex revision surgery.^[9] Inadequate cage selection may predispose the patient to avoidable future issues; therefore, the most anatomically accurate cage size should be utilised to minimise such possible problems. There is a scarcity of published evidence on normal disc height values in the ageing spine. Determining individual lumbar disc height is challenging because current research depends on plain radiographs.^[7] An anatomical understanding of the intervertebral discs at the lumbar level, particularly L-4 and L-5 and L-5 and S-1, is vital, as these two locations are prone to disc prolapse.

The morphometry of the lumbar intervertebral disc at the L-4 & L-5 and L-5 & S-1 levels is not required since the treatment technique for disc prolapse is continually changing.^[10] Artificial discs are created, inserted in the disc space, and surgically removed due to disc prolapse. Artificial disc design is always based on morphometric examination of the disc. This morphometry varies from location to region and between races. As a result, comprehensive research and data are necessary to create suitable implants. The correlation of cell morphology with disc location might be useful in evaluating how the disc changes with degeneration or illness, as well as whether IVD cells have the potential to modify their phenotype.^[11] Histopathology of the normal intervertebral disc is not well documented in many textbooks, and when it is, it is classified as fibro cartilage. As a result, the histological examination of a normal human intervertebral disc is performed using H & E and specific stains. Abnormal deteriorated disc histology is used to research and illustrate the changes in

degenerated discs and to determine if disc degeneration due to ageing causes disc prolapse or disc prolapse due to other reasons causes degenerative changes. Another purpose of the research is to determine whether cytoskeleton composition, such as vimentin, is present or absent in normal non-herniated and pathological herniated discs using immunohistochemistry.^[12]

Aim

The study aimed to analyse the lumbar intervertebral disc's morphometry, histology and cytoskeleton in herniated vs non-herniated discs.

MATERIALS AND METHODS

This study included 50 X-rays of the lumbosacral spine from a South Indian population. For histological studies of an intervertebral disc, specimens are collected from post-mortem cases for non-herniated discs and from operation theatre for herniated disc from cases undergone discectomy for disc prolapse surgeries at Govt Kilpauk medical college hospital, Kilpauk, Chennai. The study comprised patients aged 20-30 with no prior spine procedures, no current or previous pregnancy, and an unnatural death between 20 and 25 for a non-herniated disc. For herniated disc Patients over 30 had definite diagnoses of lumbar spine pathology, definite clinical spinal abnormalities during clinical examination and on x-rays, a definite diagnosis of rickets, osteoporosis, and diabetes, and long-term medication use such as steroids and anticonvulsants are excluded. The study omitted any long-term morbidity or X-rays demonstrating degenerative changes such as osteophyte production.

Morphometric Analysis

Anteroposterior and lateral images of the lumbosacral spine are acquired for morphometric measures of intervertebral discs (Figure 1). Lateral views are acquired in a lateral recumbent posture with hip and knee flexed at 45 degrees to achieve a well-balanced position on the spine. Both pictures are taken with a 100 cm gap between the spine and the X-ray beam.

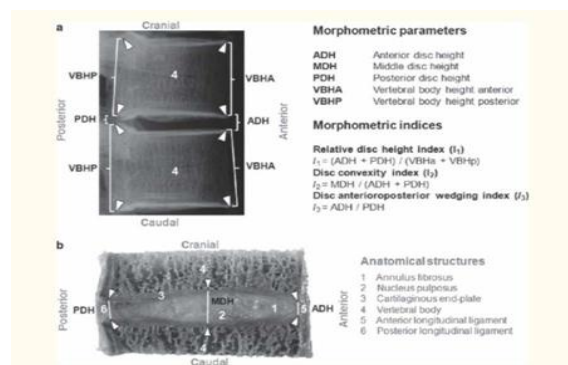


Figure 1: Morphometric parameter analysis

Morphometric indices were calculated using the following formulae

- Relative disc height index-I (1) = (ADH+PDH) / (VBHA+VBHP)
- Disc convexity index-I (2) = MDH / (ADH+PDH)
- Disc anteroposterior wedge index-I (3) = ADH/PD

During the post-mortem, non-herniated discs are taken from autopsies in the forensic section. The disc will be removed from those aged 20 to 30 who died in unnatural ways, such as in car accidents. Special permission is being requested to remove the normal disc. Virchow's approach is commonly used to open all three cavities. Following the evacuation of the abdominal contents, including the aorta, the lumbar vertebral column with its anterior longitudinal ligament is visible.

Tissue preparation for histopathological analysis
For roughly 12 - 16 hours, all surgical samples were promptly preserved in 4-6% buffered formaldehyde, pH 7.4. Patients with visible calcification or remaining bone material were gently decalcified in 0.1 M EDTA, pH 7.4 until completely decalcified. The paraffinembedded specimens were cut into slices (2-4 m) and put on salinised glass slides for routine staining (H&E, Masson-Trichrome) and light microscopy evaluation. A histomorphological differentiation between annular and nuclear disc tissue was made using light microscopic criteria, notably under polarised light, permitting examination of the collagen network organisation.

Statistical Analysis

ANOVA was used to examine the differences between the tested parameters. SPSS software was used to perform correlations, and the significance threshold was chosen at $p < 0.05$.

RESULTS

The study included 50 cases, 25 males and 25 females. The anterior (APH) and posterior (PBH) body height was increased in the L2-L3 lumbar region. The front, medial, and posterior disc heights were lower in the L1-L2 area. The mean body height and disc height are independent of one another. The disc height is higher in the anterior area at all levels than in the middle and posterior regions (Figure 2). The mean value of RDHI and DPWI increases in the L5-S1 area. In the L1-L2 area, the Disc Convexity index value rises. In the L4-L5 and L5-S1 regions, the DPWI and DCI index values are comparable [Figure 3].

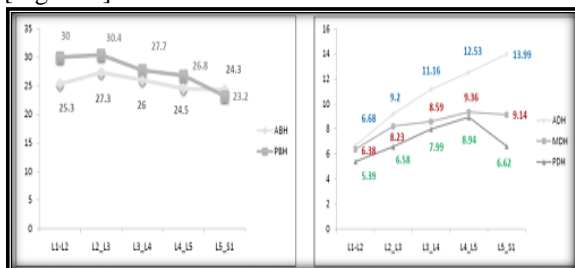


Figure 2: Mean comparison of morphometric parameters at lumbar level A) APH and PBH; B) Disc height of anterior, Medial and Posterior

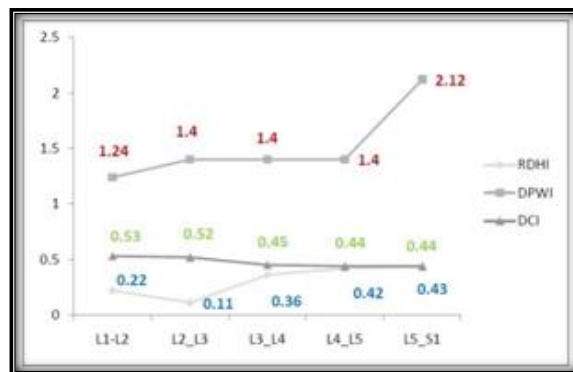


Figure 3: Distribution of index of Lumbar disc at various regions

Male individuals have higher ABH levels in the L2-L3 and L5-S1 regions than female participants, with a significant difference ($p < 0.05$). The male and female individuals have almost identical mean PBH in all lumbar areas except L-2 and L-4 ($p < 0.05$) [Figure 4].

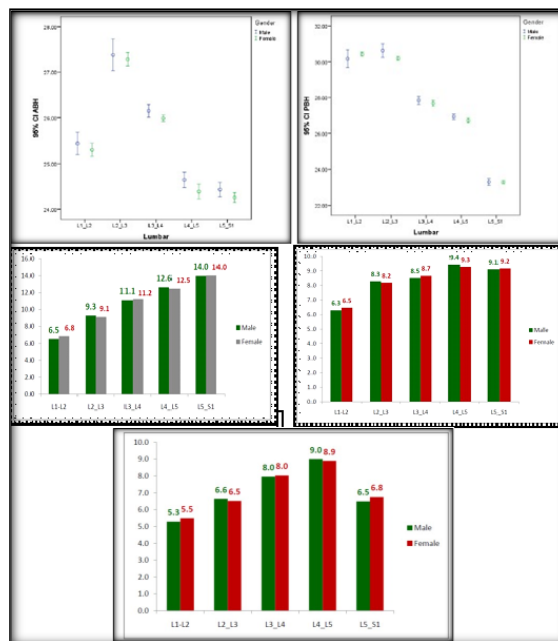


Figure 4: Mean comparison of height among both genders at the various regions A) ABH – Error plot; B) PBH – Error plot; C) Anterior Disc; D) Medial Disc; E) Posterior disc

The male population has an increase in ADH in the lumbar region of L2-L3 and L4-L5, whereas the female population has an increase in L1-L2 and L3-L4. Furthermore, there is a considerable variation in these levels between male and female participants. There was no difference in the L5-S1 level between the genders (Table 1). The middle disc height in the lumbar level of L2-L3 and L4-L5 in the male population and L1-L2, L3-L4, and L5-S1 in the female population is enhanced. In all lumbar levels, there is a considerable mean difference in medial disc height between genders [Table 1].

Compared to the other areas, the RDHI score is relatively low in the L2-L3 region and quite high in

the L5-S1 region. However, there is little variation between male and female participants (Figure 3). The RDHI demonstrates that the female population has higher L5-S1 height than the male population. There is a substantial mean difference in RDHI across genders in L5-S1. In all lumbar levels except L4-L5, there is a substantial difference in mean posterior disc height between genders (Table 1). The PDH in the lumbar level of L2-L3 in the male population and L1-L2, L3-L4, and L5-S1 in the female population is enhanced [Figure 4]

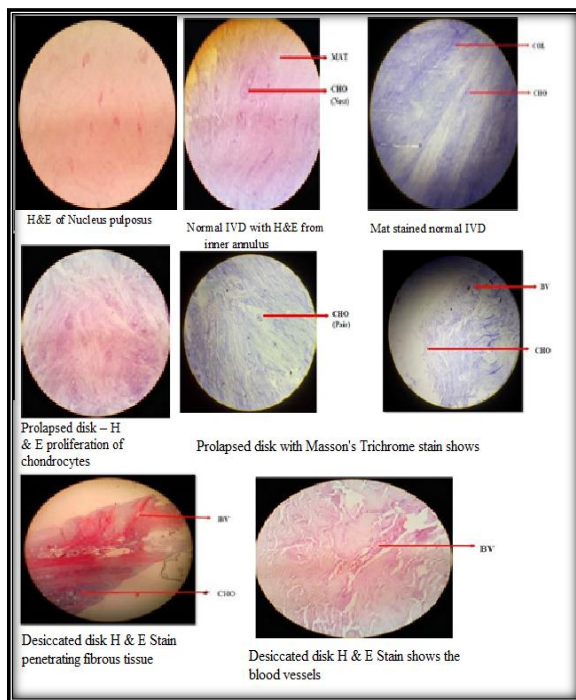


Figure 5: Histochemical staining of prolapsed disk

The DPWI demonstrates that the male population has a higher L5-S1 level. In DPWI, there is a substantial mean difference in L5-S1 level across genders. The disc convexity index is higher in the male population at the L1-L2 level and higher in the female population at the L5-S1 level and L2-L3 region. Males have a substantially higher mean DCI than females, which is extremely obvious [Table 1].

Histochemical Analysis

The image depicts a normal intervertebral disc stained with a specific stain (MAT). Collagen fibres are organised in a wavy pattern and dyed bright blue. Collagen fibres in consecutive lamellae are oriented differently, forming an angle with collagen fibres in subsequent lamellae. Chondrocytes are visible as singles and are sparse [Figure 5].

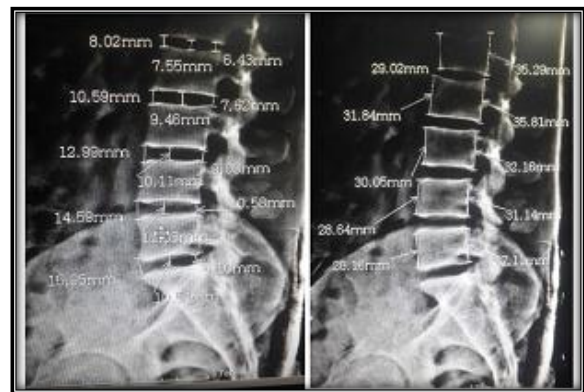


Figure 6: Morphometry of Lumbar Vertebra

This investigation does not reveal abnormal results, such as crystal deposition or cancer. The samples tested for vimentin filament by immunohistochemistry came out negative.

Table 1: Mean comparison of morphometric parameters between genders

| Parameters | Lumbar region | | | | |
|------------|---------------|--------------|--------------|---------------|----------------|
| | L1-L2 | L2-L3 | L3-L4 | L4-L5 | L5-S1 |
| ADH | | | | | |
| Male | 6.519 ±0.191 | 9.292± 0.189 | 11.092±0.178 | 12.614 ±0.213 | 13.967 ± 0.116 |
| Female | 6.848 ±0.227 | 9.118± 0.158 | 11.227±0.193 | 12.455± 0.198 | 14.022 ±0.170 |
| Sig. | 0.000* | 0.001* | 0.013* | 0.025* | 0.188 |
| PDH | | | | | |
| Male | 5.288 ±0.153 | 6.638 ±0.156 | 7.957±0.102 | 8.997± 0.144 | 6.485± 0.204 |
| Female | 5.487 ±0.197 | 6.516 ±0.083 | 8.031± 0.151 | 8.886 ±0.159 | 6.750± 0.113 |
| Sig. | 0.000* | 0.002* | 0.047* | 0.510 | 0.000* |
| MDH | | | | | |
| Male | 6.287 ±0.198 | 8.287± 0.183 | 8.520±0.198 | 9.436± 0.246 | 9.106 ±0.078 |
| Female | 6.466 ±0.204 | 8.180 ±0.113 | 8.665 ±0.217 | 9.274 ±0.251 | 9.174 ±0.093 |
| Sig. | 0.003* | 0.017* | 0.017* | 0.012* | 0.008* |
| RDHI | | | | | |
| Male | 0.222 ±0.011 | 0.115 ±0.004 | 0.355 ±0.006 | 0.419 ±0.008 | 0.430 ±0.009 |
| Female | 0.222± 0.011 | 0.113± 0.002 | 0.357±0.009 | 0.418± 0.007 | 0.435 ±0.008 |
| Sig. | 1.000 | 0.231 | 0.402 | 0.163 | 0.038* |
| DPWI | | | | | |
| Male | 1.233± 0.024 | 1.400± 0.014 | 1.394±0.013 | 1.402± 0.010 | 2.156 ±0.062 |
| Female | 1.248 ±0.032 | 1.399± 0.014 | 1.398 ±0.016 | 1.402 ±0.009 | 2.078± 0.028 |
| Sig. | 0.063 | 0.813 | 0.332 | 0.942 | 0.000* |
| DCI | | | | | |
| Male | 0.532± 0.005 | 0.520 ±0.003 | 0.447 ±0.005 | 0.437± 0.005 | 0.445± 0.004 |
| Female | 0.524 ±0.008 | 0.523 ±0.002 | 0.450 ±0.007 | 0.435 ±0.006 | 0.442 ±0.003 |
| Sig. | 0.008* | 0.001* | 0.120 | 0.163 | 0.001* |

DISCUSSION

Intervertebral disc degeneration (IDD) is a chronic, complicated disease linked with low back pain; the processes behind its development are yet unknown. IDD is best described as a cascade that begins with alterations to the local cellular microenvironment and continues to impair structure and function.^[11] Its progression is accompanied by morphological alterations and systematic changes in its histological features. The relationship between cell morphology and disc location might be important in determining how the disc changes with degeneration or sickness and if IVD cells can change their phenotype.^[7] As a result, the study was conducted to examine lumbar disc morphometry, histological examination of a normal human intervertebral disc, and immunohistochemistry to determine whether cytoskeleton composition, such as vimentin, is present or absent in normal non-herniated and pathological herniated discs.

By carefully examining the intervertebral disc height at the lumbar level, it was discovered that the anterior disc height increased gradually from L-1/L-2 to L-5/S-1 disc level, with the highest anterior height at the L-5/S-1 disc level. In their study on the morphometry of lumbar vertebrae in the Central Indian population, Agichani et al. found that the average anterior height is greater than the posterior height, save at L-5. According to this study, anterior height grows from L-1-L-3 and reduces from L-4-L-5, whereas anterior body height increases from L-1-L-2 and then drops.^[13] This is in contrast to the discovery made by Moon et al. in their radiological research on lumbar disc morphometry, which shows that the maximum disc space is at the L-4-L-5 level in males and the L-5-S-1 level in females.^[14] There is no gender difference in the height of the intervertebral disc space in the current investigation.

In terms of disc height at the middle level, it was discovered that there was a gradual rise to the level of L-4/L-5 and a modest drop at L-5/S-1 when compared to L-4/L-5. When the height of the intervertebral disc is compared posteriorly, there is a gradual reduction from L1/L-2 to L-5/S-1, indicating that the disc at L-5/S-1 is wedge-shaped and contributes to lumbar lordosis. In this study, the height of the intervertebral disc in the centre and posterior end is greatest at the L-4-L-5 disc, whereas the height at the anterior end is greatest at the L5-S-1 disc.

According to our findings, the female population has a higher RDHI index at the L-5/S-1 level than the male population, and there is a significant mean difference between the genders in the L-5/S-1 disc. This was consistent with the findings of Akeda et al.^[15] The DPWI index reveals that the male population's L-5/S-1 level has grown. There is a substantial mean difference in the L-5/S-1 level, consistent with the data described by Pourahmadi et al.^[16] When comparing the disc convexity index, and males have an increase at the L-1/L-2 and L5/S-1

levels. In contrast, females have a minor rise at the L-2/L-3 level. According to Shao et al., the mean disc convexity index in the male population differs considerably from the female population.^[17]

Herniated disc histology is highly diverse, as is the degree of structural damage, which ranges from protrusions (when the outer annular lamellae stay intact) to extrusions (when they rupture) to sequestrations (when the herniation is fully removed from the disc body). Herniated discs are frequently significantly more vascularised (than normal intervertebral discs). The majority of the samples in this investigation are prolapsed or extruded. The most significant discovery shared by all samples is a disorganised pattern of collagen fibres with no evidence of collagen as a bundle or wavy pattern of collagen as also stated by Weller et al.^[18] A considerable body of evidence suggests that systematic histological evaluation of disc specimens is not justified for cost-effectiveness considerations.^[19-22] Considering the current study's findings, we give compelling proof that determining the disc histology provides essential data on the sample's composition. This morphological information may be useful since it may infer disc degeneration in the remaining intervertebral discs. Second, assessing histo-degenerative alterations in removed disc material is a document for medicolegal and quality control purposes.^[18]

CONCLUSION

The Morphometric analysis found similarities in the trend of lumbar vertebral body heights measured by imaging in all-male samples and similarities in female samples. Minor changes exist in both genders' anterior, middle, and posterior disc heights. The morphologic correlate of what is clinically diagnosed as degenerative spine disease is the number of pathologic alterations. Most of these pathologic findings, such as neovascularisation, collagen fibre derangement, and chondrocyte clusters, are of little value to the submitting surgeon, whose primary goal is to rule out any unexpected cancer or inflammatory disease. Chondrocyte clusters, neovascularisation, and synovial metaplasia appear to represent a mistaken reaction to damage. These cellular alterations may lead to disease progression rather than reconstitution by further changing the microenvironment and metabolic balance of the typically avascular disc. A deeper knowledge of these modifications is critical for developing future surgical discectomies options. Growth factor injections and transplanting mature or stem cells are being researched as potential novel therapeutics. Any of these therapeutic alterations might reshape the function of the pathologic evaluation of materials.

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