ORIGINAL ARTICLE

ANNALS OF PUNJAB MEDICAL COLLEGE

Multimodal Assessment of Spinal Degeneration: Correlation among Radiological, Macroscopic and Microscopic Evaluation Scores

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Submitted for Publication: 01-01-2025
Accepted for Publication 19-08-2025

How to Cite: Salman A, Riaz M, Rahman R, Faisal N, Jan F, Aftab L. Multimodal Assessment of Spinal Degeneration: Correlation among Radiological, Macroscopic and Microscopic Evaluation Scores. APMC 2025;19(3):214-219. **DOI: 10.29054/APMC/2025.1715**

ABSTRACT

Background: Spinal degeneration is a complicated and multifactorial process that is associated with chronic pain and disability. To understand its exact pathogenesis, a comprehensive multimodal (radiological, macroscopic, and microscopic) approach is required that integrates evaluations to elucidate the underlying pathophysiology. **Objective:** To assess lumbar spinal degeneration through radiological, macroscopic, and microscopic approaches, analyze age- and level-related variations, and establish correlations among modalities to refine diagnosis and guide targeted therapies. **Study Design:** Cross-sectional analytical study. **Settings:** Department of Anatomy, University of Health Sciences, Lahore Pakistan. **Duration:** Twelve months from March 12, 2022 to March 11, 2023. **Methods:** 72 lumbar human endplate and intervertebral disc composites were taken from adult male cadavers which were free from spine deformity, fracture, infection, or metastasis. After X-ray imaging, the individual motion segments were separated and were cut mid-sagittally (Fig. 1), and both endplates and IVDs were assessed for macroscopic grades and microscopic scores and grades. **Results:** Significant increase in the mean radiological, macroscopic, and microscopic degeneration scores with age and spinal level. Sclerosis increased significantly with age while osteophytes increased with spinal levels. Significant correlations were observed among radiological, macroscopic, and microscopic scores ($p<0.001$). Macroscopic findings include fissures, fibrosis, brown discoloration, and calcification. Histological evaluation revealed advanced degeneration characterized by fissures, herniation, calcification, and matrix disorganization. **Conclusion:** Multimodal assessment offers a robust framework for understanding spinal degeneration, facilitating accurate diagnosis, and the development of targeted therapeutic strategies.

Keywords: *Radiology, Spinal degeneration, Multimodal, Sclerosis, Osteophytes.*

INTRODUCTION

Spinal degeneration is a complex, multifactorial process with significant implications for global health, contributing to chronic pain, functional disability, and diminished quality of life.[1] The rising prevalence[2] of spinal disorders, fueled by aging populations and sedentary lifestyles,[3] underscores the need to understand the mechanisms driving this degeneration.[4] Central to this pathology is intervertebral disc (IVD) degeneration, accompanied by structural changes in the cartilaginous and bony endplates.[5] These degenerative changes impair spinal biomechanics, leading to pain and disability,[6,7] thus emphasizing the clinical importance of accurate assessment for advancing diagnostic, therapeutic, and preventative strategies.

Degeneration is evaluated through multiple modalities, including radiological imaging, macroscopic examination, microscopic histology, and ultramicroscopic techniques. Radiological imaging provides non-invasive insights into structural alterations such as disc space narrowing, sclerosis, osteophyte formation, and Modic changes.[8] Macroscopic examination reveals morphological features like resorption, fissures, herniation, and calcification,[9] while

microscopic analysis offers a detailed view of cellular and matrix-level disruptions.[10] Despite the wealth of data from these methods, an integrative approach that correlates findings across scales remains underexplored, creating a gap in understanding how structural, cellular, and molecular changes interrelate.

The endplate (EP) and IVD play critical roles in spinal biomechanics and metabolism.[11] EP degeneration, marked by structural disruptions and calcification, accelerates IVD degeneration, characterized by nuclear herniation, annular fissures, and proteoglycan loss.[6,12] Advanced imaging techniques, such as MRI and CT, have enhanced the detection of degenerative changes, yet correlating these with macroscopic and microscopic findings poses a significant challenge.[13,14]

This study aims to bridge this gap by investigating correlations among radiological, macroscopic, and microscopic degeneration scores of the EP and IVD. The findings promise to inform diagnostic criteria, identify biomarkers, and refine therapeutic interventions, ultimately improving patient outcomes and advancing the understanding of spinal degeneration.

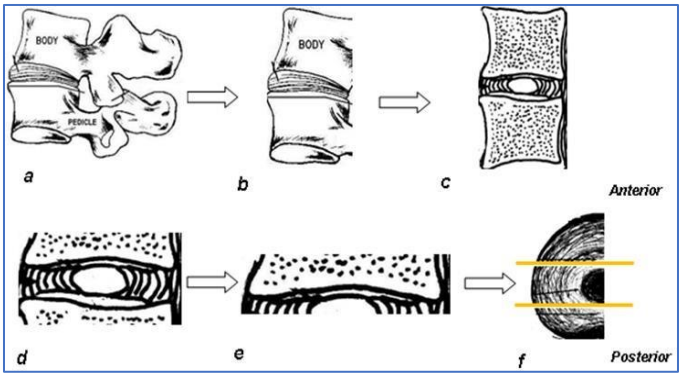
METHODS

Following the University of Health Sciences, Lahore, ethical committee approval (Letter No: UHS/REG-21/ERC/6602), a cross-sectional analytical study was conducted on lumbar segments obtained from human cadavers. Cadavers were obtained from the Anatomy Department, Faisalabad Medical University (FMU), Faisalabad, with permission from relevant authorities. The sample size of 72 vertebral endplates & intervertebral disc composite was calculated to be examined while doing microscopy (Formula for sample size calculator for designing clinical research, keeping the power of study equal to 80% and level of significance/margin of error will be equal to 5%).

Lumbar segments without any obvious spine deformity, fracture, infection, or metastasis were included in the study. After X-ray imaging, the individual motion segments were separated and were cut mid-sagittally (Fig. 1) and both endplates and IVDs were assessed for macroscopic grades using the protocol described by Zehra *et al* 2019.[22]

For microscopic assessment, 72 IVD, cartilaginous, and bony endplate composite was taken from the center of the midsagittal section (Fig.1) and were fixed in 10 % neutral buffered formalin and decalcified with 0.5M EDTA at pH 8 before getting paraffin sections of 5µm. These sections were stained with H& E and grading of both endplate and disc degeneration was performed using the protocol described by zehra *et al* 2019 and were assigned from grade I to grade IV.[22]

Fig.1: Diagrammatic representation showing how the specimen will be cut for subsequent sectioning



a) motion segment, b) neural arch removed, c) specimen cut in mid-sagittal plane, d) specimen with 5mm of bone above and below the disc, e) mid-transverse section cut through the disc, f) final sectioning of specimen in the coronal plane.

RESULTS

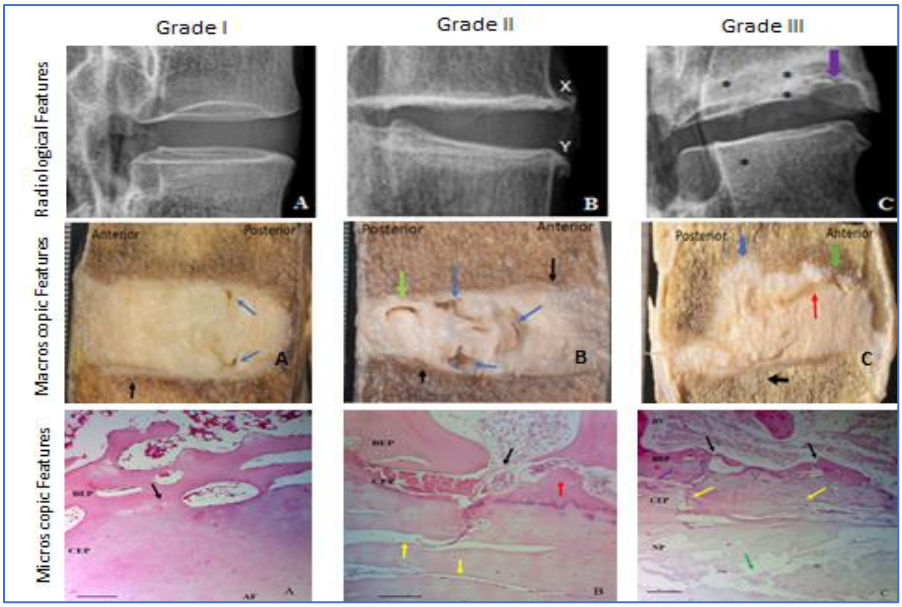
This study evaluated the radiographic, macroscopic, and microscopic grades of degeneration of BEP, CEP and IVD and their correlation with each other in the human lumbar segment. A total of 72 endplates and intervertebral discs from adult male cadaveric specimens from L1-L5, and aged 21-80 years, were evaluated by means of radiography, macroscopic and microscopic observation. On lateral radiographs, sclerosis was observed as whitish hyperintensity at upper and lower endplates, while osteophytes were identified as bony projections hanging from vertebral margins in both anteroposterior and lateral views (Fig.2). The x rays of the young spines were well aligned with no signs of sclerosis and no osteophytes while they were most obvious in samples of older individuals. Macroscopic examination showed features like fissures, fibrosis, calcification, discoloration, or herniation in the intervertebral disc while resorption, fracture, or herniation in endplates (Fig. 2). H & E stained tissue sections showing microscopic features of intervertebral discs and endplate degeneration for different grades. Most of the samples had well-organized and intact BV, BEP, and CEP. But degenerated sample revealed fissures, disorganized matrix, areas of hypo or hypercellularity, fractured BEP or CEP, herniating CEP or IVD into the BEP, and calcification (Figure 2).

Only 6 %, 7%, and 15% of the discs were classified as non-degenerated (Grade I) based on radiological, macroscopic, and microscopic assessments, respectively. Meanwhile, 59%, 61% and 58% showed mild degeneration (Grade II), and 35%, 32% and 27% exhibited moderate degeneration (Grade III). Notably, none of the discs demonstrated severe degeneration (Grade IV) on any assessment modality.

Mean sclerosis, osteophyte, and overall radiological degeneration scores, along with mean macroscopic and microscopic degeneration scores of the endplate, disc, and overall degeneration and their correlation, are presented for age groups (A, B, & C) and each spinal level (L1-L5) in Table 1.

Significant correlations were observed between the mean sclerosis, osteophyte, and overall radiological degeneration scores, along with mean macroscopic and microscopic degeneration scores of the endplate, disc, and overall degeneration with each other (Table 2).

Figure 2: Lumbar segments showing radiological, macroscopic, and microscopic features



(A) Grade I: Radiograph shows no sclerosis or osteophytes. Macroscopically, non-uniform cartilaginous endplate (black arrow) and nucleus fissures (blue arrows) are observed. Microscopically, intact BV, BEP, and CEP are seen. (B) Grade II: Radiograph shows mild sclerosis and osteophytes (X, Y). Macroscopic features include nucleus fissures (blue arrow), radial fissure in the annulus (green arrow), and cartilaginous endplate resorption (black arrow). Microscopy reveals fractured BEP (black arrows), CEP protruding into BV (red arrow) and a disorganized matrix (yellow arrow). (C) Grade III: Radiograph shows sclerosis (black asterisks), a large osteophyte, and Schmorl's node (purple arrow). Macroscopically, indistinguishable nucleus and annulus, anterior annulus fissures (red arrow), fractures (green arrow), and annulus herniation (black arrow) are noted. Microscopically, fractured BEP (black arrow), CEP protrusion (red arrow), calcification (purple arrows), granular tissue, and disorganized matrix in CEP (yellow arrows) and NP (green arrows) are observed.
Scale bars: Microscopy - 200X, 50 µm.

Table 1: Radiological, macroscopic, and microscopic degeneration scores by age group and spinal Level, with correlation analysis

Group	Radiological Degeneration Scores			Macroscopic Degeneration Scores			Microscopic Degeneration Scores		
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age	Sclerosis	Osteophytes	Overall	EP	IVD	Overall	EP	IVD	Overall
A	2.0(0.92)	1.47(0.5)	3.5(1.0)	13.9(4.4)	21.8(5.3)	35.7(9.2)	16.4(5.8)	9.1(3.1)	25.4(8.5)
B	2.1(0.6)	1.5(0.5)	3.6(0.8)	14.7(3.5)	22.5(4.6)	37.2(7.3)	17.2(4.6)	9.6(2.3)	26.4(6.1)
C	2.5(0.8)	2.5(0.5)	4.9(1.2)	23.8(4.1)	34.7(5.5)	58.5(8.9)	29.4(7.7	14.6(4.1)	44.5(7.1)
Correlation with Age Spinal Level	0.23	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**
L1	1.7(0.6)	1.5(0.5)	3.1(0.8)	13.1(5.1)	20.6(5.1)	33.7(09.7)	16.6(6.6)	8.7(2.9)	24.1(9.1)
L2	2.1(0.9)	1.8(0.6)	3.9(0.9)	15.3(0.9)	24.4(6.9)	39.7(11.2)	18.8(6.8)	9.9(2.7)	27.9(9.3)
L3	2.5(0.7)	2.0(0.7)	4.5(1.1)	18.7(5.2)	26.0(6.8)	44.8(11.7)	21.8(7.9)	11.1(3.2)	33.1(10.1)
L4	2.3(0.7)	1.9(0.8)	4.2(1.2)	19.5(5.5)	30.2(7.8)	49.7(13.3)	23.3(7.1)	13.0(4.5)	37.3(10.2)
L5	2.7(0.8)	2.0(0.8)	4.7(1.4)	22.2(6.6)	32.2(8.7)	54.4(15.1)	26.8(8.3)	14.4(5.6)	41(13.1)
Correlation with spinal level	0.04*	0.09	0.01*	0.003**	0.002**	0.002**	0.03*	0.007**	0.005**

(Group A=21-40 years, Group B= 41-60 years and Group C= 61-80 years). The values shown are the mean (SD). EP: endplate, IVD: intervertebral disc.

Table 2: Pearson correlation coefficient (r) between each of the radiological, macroscopic and microscopic degeneration scores

Assessment mode		Macroscopic degeneration scores			Microscopic degeneration scores		
		EP	IVD	Total	EP	IVD	Total
Radiological degeneration scores	Sclerosis	0.55***	0.42***	0.51**	0.53***	0.45***	0.53***
	Osteo-phytes	0.67***	0.76***	0.76**	0.69***	0.73***	0.72***
	Total	0.79***	0.75***	0.81**	0.78***	0.76***	0.81***
Macroscopic degeneration scores	EP				0.89***	0.79***	0.93***
	IVD				0.88***	0.83***	0.93***
	Total				0.92***	0.86***	0.97***
Microscopic degeneration scores	EP						
	IVD						
	Total						

*(EP, IVD and overall) with each other. ***p<0.001, **p<0.01 and* p<0.05.*

DISCUSSION

This study assessed degenerative changes in the cartilaginous endplate (CEP), bony endplate (BEP), and intervertebral disc (IVD) in human lumbar segments, exploring correlations across radiological, macroscopic, and microscopic levels. A total of 72 samples from adult male cadaveric specimens aged 21-80 years, spanning lumbar levels L1-L5, were examined using multi-modal evaluation techniques.

The results revealed that the majority of samples exhibited mild to moderate degeneration, with no samples showing severe degeneration (Grade IV) in any modality. This absence of severe degeneration may be attributed to the cohort being derived from the normal local population, with cadavers representing typical anatomical variations and degenerative processes.

Radiological assessments primarily focused on sclerosis and osteophyte formation, which are common markers of degeneration.[15] A significant increase in overall radiological degeneration scores with age was seen, which has also been reported in many previous studies done on different populations.[16,17] Sclerosis, indicative of bone remodeling and altered mechanics, and osteophytes, reflective of vertebral adaptation to mechanical stress, were observed in increasing frequency with age.[18] Significant correlations were found between osteophyte formation and age, while sclerosis increased insignificantly with age. However, sclerosis exhibited a strong correlation with spinal level, with higher sclerosis observed in lower spinal segments. This finding supports the hypothesis that altered biomechanical loading at lower lumbar levels contributes to accelerated degeneration. Lower spinal segments, particularly L4/5 and L5/S1, are subject to greater mechanical forces and, therefore, more prone to degenerative changes.[17,19]

Interestingly, while osteophytes and sclerosis were correlated with age, no significant correlation was found between these two factors, suggesting that they may develop independently in response to mechanical and biochemical stressors. This could indicate that different degenerative pathways are at play, with sclerosis potentially reflecting a regenerative process and osteophyte formation signaling adaptive changes to loading conditions.[20] The role of Modic changes, which reflect regenerative bone formation in response to marrow alterations, might explain why sclerosis does not increase significantly with age in certain regions of the spine.[20]

However, the study did not find a significant relationship between osteophyte formation and spinal level, which contrasts with some earlier studies that observed a more pronounced effect of spinal level on degeneration.[21] This discrepancy may be attributed to differences in population characteristics, such as ethnicity or lifestyle, as well as the methodology employed. Despite this, the study's findings align with other research on the role of age and spinal level in influencing degenerative changes, particularly at the lower lumbar levels, which bear greater mechanical load and stress.[15-19]

Macroscopic examination of the CEP revealed structural abnormalities such as fissures, focal defects, and occasional protrusions into the adjacent vertebrae. The IVD exhibited similar morphological changes, with significant damage observed in the nucleus pulposus and annulus fibrosus. All the grades of degeneration, (EP, IVD & overall) correlated significantly with both age and

spinal level. A substantial positive correlation between the macroscopic degeneration scores of endplates and IVDs was observed. This finding implies a shared pathophysiology i.e. degenerative changes in one component can influence and exacerbate changes in the other.[22] These structural changes are often linked to mechanical loading, with microtrauma and prolonged strain contributing to the gradual degeneration of these tissues. The observed fissures and defects in the IVD are indicative of tissue breakdown due to chronic mechanical stress, which disrupts the extracellular matrix and compromises the structural integrity of the spine.[22,23]

Microscopic evaluation further revealed that degenerative changes in the CEP and IVD were characterized by disorganization of the matrix, altered cellular distribution, and the presence of hypercellularity and areas of hypo-cellularity. Hypercellularity suggests a reparative response to tissue damage, whereas hypo-cellularity may reflect compromised cell viability or a failure to adequately restore tissue structure. These changes are consistent with the body's attempt to repair damaged tissues, yet they also indicate that the degeneration process is not entirely reversible. Additionally, areas of chondrocyte clustering, the presence of amorphous material or granulation in the intervertebral disc point to ongoing tissue remodeling in response to degeneration.[24] The interplay between cellular changes in the CEP and IVD suggests a shared pathophysiology, where degeneration in one component exacerbates changes in the other.[22] This interconnected process highlights the need for a holistic approach to understanding spinal degeneration, as changes in one region of the motion segment can influence and amplify degenerative processes in adjacent structures.[22,23]

When different assessment modes were correlated, significant synergies emerged, offering insight into their potential interrelationships and contributing to a comprehensive understanding of degenerative processes. Consistent positive correlations were observed between scores of all the assessment methods including radiological, macroscopic, and microscopic modes, indicating their mutual coherence in evaluating degenerative changes. The strongest correlation was observed between radiological grades with macroscopic grades and macroscopic grades with microscopic grades. Individual sclerosis and osteophyte scores exhibited strong positive correlations with all the scores (macroscopic and microscopic scores) highlighting the consistency in their assessments. The correlation between radiological, macroscopic and microscopic findings has already been established in previous studies not in just overall scores but among the individual scores for the endplate and IVD too.[20,22] But most of these studies are done on animal models in the Pakistani population who have different demographic data and genetic make-up.

CONCLUSION

These positive correlations among all the assessment modes suggested a degree of harmony between these assessment scales but certain microstructural degenerative changes might not be well-captured by radiological imaging alone accentuating the importance of incorporating a multi-modal approach for a comprehensive assessment of spinal degeneration. The findings of this study provided a depth level of understanding and compatibility of the employed techniques.

LIMITATIONS

The availability of only adult male cadavers (due to cultural constraints) was the main limitation encountered in this study which may limit the generalizability of the findings to female population who have different anatomy, lifestyle, and healthcare factors.

SUGGESTIONS / RECOMMENDATIONS

Future research should aim to encompass both genders and different age groups along with the role of external factors like physical activity, diet, and comorbid conditions. This will help in understanding how spinal degeneration manifests across various demographics, taking into account differences in anatomy, lifestyle, and healthcare factors. Incorporating these considerations into future research endeavors will enhance our understanding of spinal degeneration and its implications for clinical practice, ultimately improving healthcare strategies and outcomes for diverse populations.

STRENGTH OF THE STUDY

The study draws its strength from its rigorous quantitative analysis, and comprehensive assessment methods. It contributes to our understanding of spinal degeneration, particularly in the context of the Pakistani population, and emphasizes the importance of considering multi-modal approaches in spinal research. Despite being the first study in the Pakistani population, the outcomes closely correspond with consistent trends observed in other populations; this strengthens the validity of the findings.

CONFLICT OF INTEREST / DISCLOSURE

None.

ACKNOWLEDGEMENTS

I sincerely thank Prof. Dr. Uruj Zehra, Head of Anatomy Department, UHS Lahore for her invaluable guidance,

feedback, and support, which were instrumental in the successful completion of this study.

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