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RESEARCH ARTICLE

Comprehensive Histochemical Evaluation of Age-Related Intervertebral Disc Degeneration

İntervertebral Diskte Yaşlanmaya Bağlı Dejeneratif Değişikliklerin Ayrıntılı Histokimyasal Analizi

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ABSTRACT

Objective: The intervertebral disc (IVD) is a fibrocartilaginous structure that plays a critical role in transmitting spinal loads and maintaining mobility. Age-related degeneration of the IVD leads to the disruption of structural integrity, reduction in mechanical function, and the development of clinical symptoms such as back and neck pain. During the degenerative process, matrix composition, cellular organization, and mechanical resilience are significantly affected. The aim of this study was to investigate the histological and histochemical changes occurring in IVDs across different age groups using various staining techniques and to evaluate their relationship with the degeneration process.

Materials and Methods: This study included 30 patients aged between 40 and 70 years who underwent surgical procedures for disc herniation during which IVD specimens were obtained. Patients were divided into four age groups: Age40s (40–49 years, n=9), Age50s (50–59 years, n=7), Age60s (60–69 years, n=8), and Age70s (70–79 years, n=6). Surgical specimens were fixed in 10% formalin, processed routinely, and embedded in paraffin. Sections of 5 µm in thickness were cut from the paraffin blocks and stained with hematoxylin and eosin, toluidine blue, Masson's trichrome, Congo red, and the Armed Forces Institute of Pathology method. Histological changes were examined under a light microscope and semi-quantitatively scored.

Results: With advancing age, IVD tissues exhibited a marked decrease in proteoglycan content, an increase in collagen fiber density, accumulation of lipofuscin granules, and the presence of amyloid deposits. In the older age groups, the matrix was observed to become denser and more fibrotic, and to show disruption of the lamellar organization.

Conclusion: Histochemical staining techniques are effective in the detailed identification of cellular and extracellular matrix changes occurring during IVD aging and degeneration. These methods contribute to the characterization of age-related structural alterations and provide valuable information for comparative histopathological assessments of the degenerative process.

Keywords: Aging, degenerative disc disease, histochemistry, intervertebral disc

ÖZET

Amaç: Omurlar arası disk (intervertebral disk, IVD), omurga yüklerinin iletilmesi ve hareket kabiliyetinin sağlanmasında kritik rol oynayan fibro-kıkırdak yapıda bir oluşumdur. Yaşlanmaya bağlı olarak gelişen IVD dejenerasyonu, yapısal bütünlüğün bozulmasına, mekanik fonksiyonların azalmasına ve klinik olarak bel ve boyun ağrısı gibi semptomlara yol açmaktadır. Dejeneratif süreçte matriks kompozisyonu, hücresel organizasyon ve mekanik dayanıklılık belirgin şekilde etkilenmektedir. Bu çalışmanın amacı, farklı yaş gruplarında IVD'de meydana gelen histolojik ve histokimyasal değişiklikleri çeşitli boyama teknikleri ile ayrıntılı olarak ortaya koymak ve bu değişikliklerin dejenerasyon süreciyle ilişkisini değerlendirmektir.

Gereç ve Yöntemler: Çalışmaya, 40–70 yaş aralığında olup disk herniasyonu tanısıyla cerrahi müdahale sırasında IVD materyali elde edilen toplam 30 hasta dahil edilmiştir. Hastalar, yaş aralıklarına göre dört gruba ayrılmıştır: 40–49 yaş aralığını kapsayan Yaş40 grubu (n=9), 50–59 yaş aralığını kapsayan Yaş50 grubu (n=7), 60–69 yaş aralığını kapsayan Yaş60 grubu (n=8) ve 70–79 yaş aralığını kapsayan Yaş70 grubu (n=6). Elde edilen cerrahi materyaller %10'luk formalin ile fikse edilmiş, rutin takip işlemlerinden geçirilerek parafine gömülmüştür. Parafin bloklardan 5 µm kalınlığında kesitler alınmış ve Hematoksilen–Eozin (H&E), Toluidin Mavisi, Masson Trikrom, Kongo Kırmızısı ve Armed Forces Institute of Pathology (AFIP) yöntemleri ile boyanmıştır. Histolojik değişiklikler ışık mikroskobu altında değerlendirilmiş ve yarı kantitatif yöntemle skorlanmıştır.

Bulgular: Yaş ilerledikçe IVD dokusunda proteoglikan içeriğinde belirgin azalma, kollajen lif yoğunluğunda artış, lipofuksin granüllerinin birikimi ve amiloid depozitlerinin oluşumu gözlenmiştir. İleri yaş gruplarında matriks yapısının yoğunlaştığı, fibrotik karakterin belirginleştiği ve lameller organizasyonun düzenliliğini kaybettiği saptanmıştır.

Sonuç: Histokimyasal boyama teknikleri, IVD yaşlanması ve dejenerasyonu sürecinde meydana gelen hücresel ve ekstrasellüler matriks değişikliklerinin ayrıntılı olarak belirlenmesinde etkili yöntemlerdir. Bu yöntemler, yaşa bağlı yapısal değişikliklerin karakterizasyonuna katkı sağlamakta ve dejeneratif sürecin histopatolojik değerlendirilmesinde karşılaştırmalı çalışmalar için değerli bir temel oluşturmaktadır.

Anahtar Kelimeler: Yaşlanma, dejeneratif disk hastalığı, histokimya, omurlar arası disk

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INTRODUCTION

The intervertebral disc (IVD) is a fibrocartilaginous structure that transmits and distributes spinal loads between the vertebral bodies while also allowing mobility (1). It is structurally organized with a centrally situated nucleus pulposus (NP), characterized by high water content and abundant proteoglycans, encased by the annulus fibrosus (AF), which comprises concentrically layered lamellae enriched with collagen. Thin cartilaginous endplates (CEPs) located on the superior and inferior surfaces of the disc regulate the nutrition and metabolic exchange of the IVD (2). The outer annulus comprises highly organized collagen lamellae, where type I collagen fibers are aligned in parallel with longitudinal fibroblasts (3, 4). The inner annulus, in contrast, has a more cartilage-like structure, containing chondrocyte-like cells and higher levels of type II collagen and proteoglycans (5). The centrally located NP consists of a highly hydrated, gelatinous matrix rich in proteoglycans, mainly synthesized by large notochordal cells. Together, the AF, NP, and CEPs constitute the essential components of the functional spinal motion segment, enabling the IVD to provide shock absorption during load bearing and resist tensile and torsional forces (6).

Age-related degenerative joint disorders are among the most common chronic conditions, exerting a profound impact on public health and generating substantial social and economic challenges (7). In the elderly, IVD degeneration and osteoarthritis are the primary causes of persistent joint-related pain and functional impairment (8). Impaired mobility, in turn, is widely recognized as a strong predictor of functional decline, loss of autonomy, and mortality in later life (9). Accordingly, safeguarding joint integrity, and particularly that of IVDs, plays a pivotal role in sustaining mobility with advancing age. IVDs tend to manifest age-related degenerative changes earlier than many other connective tissues (10). As the NP ages, its proteoglycan content and hydration progressively diminish, resulting in a gradual shift toward a more fibrotic composition. These alterations promote the development of fissures, reduce NP volume and intradiscal pressure, and ultimately lead to a measurable loss of disc height. Over time, oxidative modification of matrix proteins produces further structural changes, transforming the translucent and gelatinous NP into a yellow, fibrotic tissue. This transition is compounded by the accumulation of lipofuscin, a brown "aging pigment" formed through the slow peroxidation of lipids (11).

Amyloidosis is a systemic disorder characterized by the extracellular deposition of misfolded protein aggregates in various tissues and organs. Depending on the site of deposition, these amyloid accumulations can disrupt normal function through mechanical compression or by triggering degenerative changes (12). IVDs are frequently reported as sites of localized articular amyloid deposition and, in some cases, as a location affected by systemic forms. While the mechanisms underlying this phenomenon are not fully understood, it has been suggested that intrinsic properties of the IVD extracellular matrix may favor amyloid aggregation. Small, localized deposits are not uncommon within IVDs and have been documented in both pathological and age-related contexts (13).

The present study aimed to investigate the histological alterations occurring in disc cells throughout the processes of IVD aging and degeneration, and to characterize these changes with the application of various histochemical staining techniques. Specifically, assessments were performed to evaluate cell type, cellular density, proliferative activity, accumulation of lipofuscin granules as an indicator of cellular senescence, and deposition of amyloid plaques.

MATERIALS AND METHODS

Patient Selection and Sample Collection

This study included 30 patients aged 40–70 years who underwent surgical removal of IVD material due to disc herniation in the Department of Neurosurgery of Medova Hospital. Written informed consent was provided by all participants. Residual disc material obtained during surgery was used for the study. To ensure the consistency and relevance of the findings, samples from young adult patients under 40 years of age were excluded. Therefore, analyses were conducted on samples from patients aged 40 and above, representing sufficiently aged discs. This approach allowed for the assessment of histological changes associated with both age-related degeneration and reduced physical activity, providing a clearer understanding of pathological features in a more representative patient population. Ethical approval for the study was granted by the Non-Drug and Non-Medical Device Research Ethics Committee of Necmettin Erbakan University on June 13, 2025 (Approval No: 2025/5818).

Inclusion criteria were as follows:

- Radiological confirmation of disc herniation by MRI or CT
- Availability of surgically obtained disc material
- Complete recording of the patient's clinical and demographic data (age, sex, symptom duration, level of herniation, etc.)

Exclusion criteria were as follows:

- Presence of pathologies other than disc herniation, such as infection, tumor, or trauma
- Insufficient or damaged tissue material

Clinical data of the patients were obtained from hospital records and surgical notes.

Patients were divided into four age groups for analysis: Age40s (40–49 years, n=9), Age50s (50–59 years, n=7), Age60s (60–69 years, n=8), and Age70s (70–79 years, n=6).

Histological Analyses

IVD tissues were fixed in 10% neutral buffered formalin. Following fixation, samples were dehydrated through a graded ethanol series (70%, 80%, 90%, 96%, and absolute alcohol; 1 h each). Dehydrated specimens were cleared in xylene and subsequently infiltrated with molten paraffin in a laboratory oven for 4–5 h. Tissues were then embedded in paraffin to obtain paraffin blocks. Serial sections of 5 µm in thickness were cut using a rotary microtome. For deparaffinization, the sections were immersed in xylene (3 × 20 min) and rehydrated through descending grades of ethanol (100%, 90%, 80%, 70%,

and 50%). The slides were then stained with hematoxylin and eosin (H&E), toluidine blue, Masson's trichrome, Congo red, and the Armed Forces Institute of Pathology (AFIP) lipofuscin staining method. After staining, sections were mounted with Entellan® mounting medium. All stained sections were examined using a Zeiss Primo Star light microscope, and digital images were captured and analyzed with the Zeiss AxioCam ERc 5s imaging system. Histological alterations in the IVD tissues were evaluated for each age group according to predefined criteria (Table 1) (14). Scoring was performed independently by two blinded observers.

Hematoxylin–Eosin Staining

H&E staining was employed to score and evaluate the proliferation of hypertrophic chondrocytes, as well as granular alterations characterized by eosinophilic granular material within the fibrocartilaginous matrix, under light microscopy (15).

Masson's Trichrome Staining

Masson's trichrome staining (BesLab, Lot: 072022.036) was performed to demonstrate vascularization and the structural organization of collagen fibrils within the IVD tissue (16).

AFIP Method for Lipofuscin

Deparaffinized sections were passed through distilled water and stained with Kinyoun carbol fuchsin (J-608-1) for 30 min, then rinsed with tap water. Sections were dipped in acid alcohol (J-608-3) until pale pink, rinsed under tap water for 5 min, and washed with distilled water. Counterstaining was performed with picric acid (J-608-2) until sections appeared yellow. Sections were then dehydrated in 95% and absolute alcohol, cleared with xylene, and cover-slipped with Entellan®. On each slide, lipofuscin granules were observed as red-orange structures in yellow-stained areas and were scored accordingly (17,18).

Congo Red Staining

Congo red staining was used to detect amyloid protein in IVD tissue (19). Deparaffinized sections were stained according to the instructions for the Pato Lab Congo Red Staining Kit (PLKit10-150).

Toluidine Blue Staining

A stock solution was formulated by dissolving 0.1 g of toluidine blue powder (Sigma-Aldrich, T3260) in 100 mL of distilled water, which was subsequently diluted at a ratio of 1:2.

Table 1. Comparative scoring of age-related morphological alterations in intervertebral discs by different histological stains

	Score	Scoring criteria	
		Hematoxylin and Eosin (H&E)	
Hypertrophic chondrocyte proliferation	0	No hypertrophic chondrocytes	Fig.1-b
	1	Few hypertrophic chondrocytes (minimal foci, not diffuse)	
	2	Moderate hypertrophic chondrocytes (several foci of clustering)	
Eosinophilic granular structure	0	No eosinophilic granular structures	Fig.1-c
	1	Few eosinophilic granular structures (isolated foci)	
	2	Prominent eosinophilic granular structures (diffuse or multiple dense foci)	
		Masson's Trichrome (MT)	
Vascularization	0	No vascularization	Fig.2-b
	1	Mild vascularization	
	2	Marked vascularization	
Structure of collagen fibrils	0	Collagen fibrils well-organized, no structural disruption	Fig.2-c
	1	Mild irregularity (partial misalignment or waviness in some fibrils)	
	2	Marked irregularity (diffuse misalignment, fragmentation, or dense fibrotic areas)	
		Armed Forces Institute of Pathology Staining Protocol (AFIP)	
Presence of lipofuscin granules	0	No lipofuscin granules	Fig.3-b
	1	Few lipofuscin granules (isolated or small foci)	
	2	Marked lipofuscin granules (diffuse or multiple dense foci)	
		Congo Red (CR)	
Amyloid deposits	0	No amyloid deposits	Fig.4-b
	1	Mild amyloid deposits (few or isolated foci)	
	2	Marked amyloid deposits (diffuse or multiple dense foci)	
		Toluidine Blue (TB)	
Intense metachromatic staining	0	No or minimal metachromatic staining	Fig.5-b
	1	Mild metachromatic staining (few small areas)	
	2	Intense and widespread metachromatic staining (large, prominent areas)	

Sections were stained with this solution for 40 s. Toluidine blue interacts with acidic mucopolysaccharides (e.g., chondroitin sulfate, keratan sulfate), producing metachromasia. The stain was used to visualize cartilage-like structures, proteoglycan content, and mucopolysaccharides (14).

Statistical Analysis

All data were expressed as mean ± standard deviation. The normality of the data distribution was assessed using the Shapiro–Wilk normality test. Statistical comparisons among groups were performed using one-way ANOVA, followed by the Tukey post hoc test. Values of $p < 0.05$ were considered statistically significant. Statistical analyses were performed using GraphPad Prism 5.0 Demo and Microsoft Office 365 software.

RESULTS

Hematoxylin and Eosin Staining Findings

Examination of H&E-stained sections from all age groups revealed that those obtained from the Age40s group exhibited a more organized lamellar structure, with uniformly shaped chondrocytes and fewer eosinophilic regions. With advancing age, disruptions in lamellar organization and the development of fissures were observed, along with more prominent chondrocyte clusters and an increase in eosinophilic regions. Based on these changes, hypertrophic chondrocyte proliferation scores were significantly higher in the Age60s and Age70s groups compared to the Age40s group, whereas eosinophilic granular structure scores were significantly higher in the Age60s group compared to the Age40s group (Figure 1).

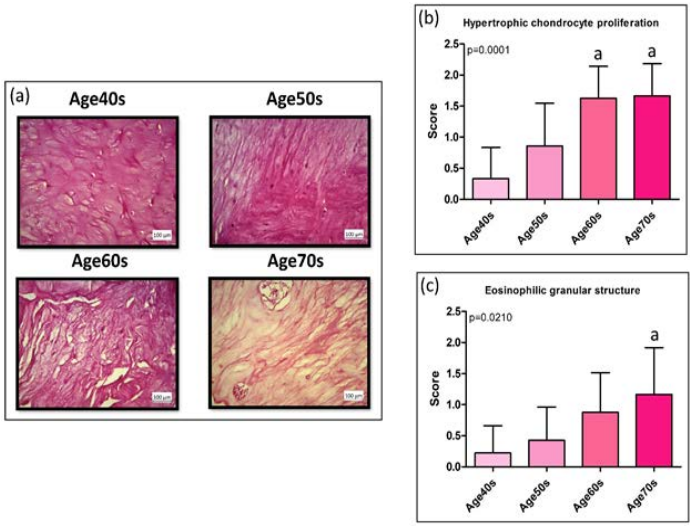

Figure 1. (a) Histological images of intervertebral disc tissue stained with hematoxylin and eosin, (b) histological scoring of hypertrophic chondrocyte proliferation, (c) histological scoring of eosinophilic granular structures. The letter "a" indicates a statistically significant difference ($p < 0.05$) compared to the Age40s group.

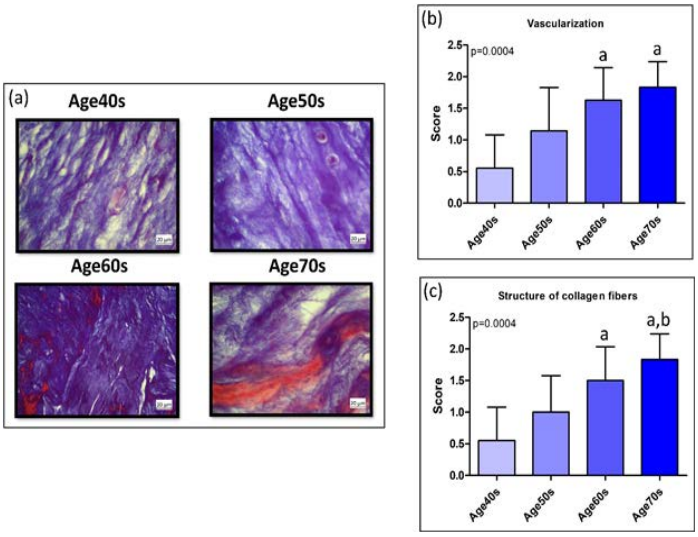

Figure 2. (a) Histological images of intervertebral disc tissue stained with Masson's trichrome, (b) histological scoring of vascularization, (c) histological scoring of collagen fibril structure. The letters "a" and "b" indicate statistically significant differences ($p < 0.05$) compared to the Age40s and Age50s groups, respectively.

Masson's Trichrome Staining Findings

Examination of Masson's trichrome-stained sections from each age group revealed that the blue tone indicating collagen was lighter in sections from the Age40s group compared to the other groups. In particular, the Age60s and Age70s groups showed a darker blue tone, reflecting higher collagen contents. This finding suggests an age-related increase in fibrosis. Statistical evaluation of the scoring based on collagen staining intensity demonstrated a significant increase in vascularization in the Age60s and Age70s groups compared to the Age40s group. Moreover, structural alterations in collagen fibers were more pronounced in the Age60s and Age70s groups than in the Age40s group. The score indicating collagen fiber alterations in the Age70s group was also statistically significantly different from that of the Age50s group (Figure 2).

AFIP Method for Lipofuscin Findings

Examination of IVD tissue samples stained with the AFIP method to detect the presence of lipofuscin granules showed red-orange lipofuscin granules within yellow-stained background structures. The presence of these granules was decreased in the Age40s and Age50s groups compared to the Age60s and Age70s groups. Based on the scoring data, a significant difference existed between the Age70s and Age40s groups (Figure 3).

Congo Red Staining Findings

In cases of Congo red-positive staining, amyloid plaques in the disc tissue appeared red. Examination of stained preparations from all age groups revealed very faint coloration

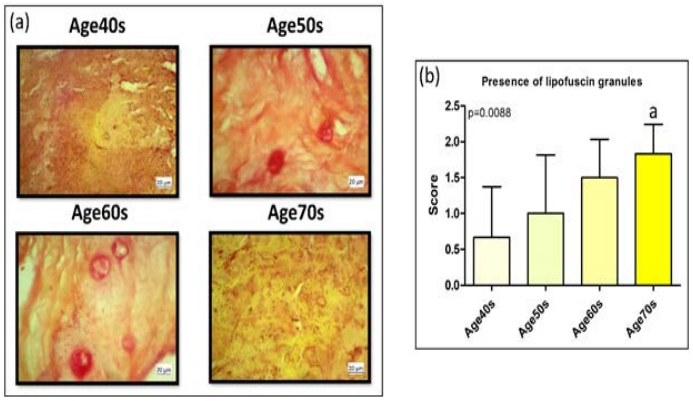

Figure 3. (a) Histological images of intervertebral disc tissue stained using the AFIP method, (b) histological scoring of the presence of lipofuscin granules. The letter "a" indicates a statistically significant difference ($p < 0.05$) compared to the Age40s group.

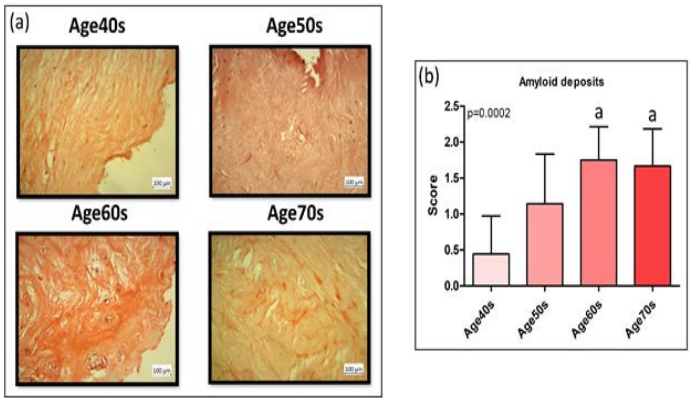

Figure 4. (a) Histological images of intervertebral disc tissue stained with Congo red, (b) histological scoring of amyloid deposits. The letter "a" indicates a statistically significant difference ($p < 0.05$) compared to the Age40s group.

with minimal red areas in the Age40s group, indicating little to no amyloid deposition. In contrast, in the older groups, and particularly in the Age60s and Age70s groups, more distinct red-orange areas were observed, consistent with Congo red-positive regions suggestive of amyloid accumulation. Statistical analysis identified a significant difference between the Age60s and Age70s groups compared to the Age40s group (Figure 4).

Toluidine Blue Staining Findings

Because toluidine blue is a metachromatic dye, it clearly reveals age-related changes in proteoglycan content and matrix structure in IVDs. Examination of the sections showed that the Age40s and Age50s groups exhibited relatively more intense metachromatic (blue-purple) staining due to their higher proteoglycan content. In contrast, the Age60s and Age70s groups had reduced metachromasia and lighter staining. Statistical analysis of the staining intensity scores indicated significant differences between the Age60s and Age70s groups compared to the Age40s group (Figure 5).

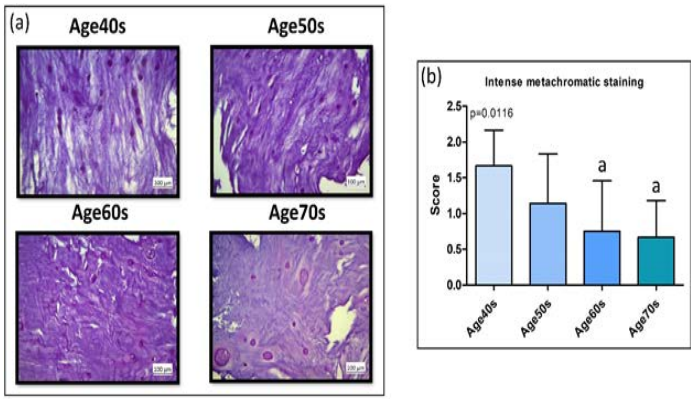

Figure 5. (a) Histological images of intervertebral disc tissue stained with toluidine blue, (b) histological scoring of intense metachromatic staining. The letter "a" indicates a statistically significant difference ($p < 0.05$) compared to the Age40s group.

DISCUSSION

The histopathological examination of IVD tissues continues to serve as a fundamental reference method in the evaluation of degenerative processes. However, more detailed characterization and interpretation of IVD pathologies could contribute to a better understanding of the mechanisms underlying the clinical complications of current treatments. Moreover, reaching a consensus on the simultaneous changes occurring in realms such as cellular function, matrix composition, and biomechanical behavior would allow histological findings to be interpreted more comprehensively from both mechanistic and clinical perspectives. In this context, the structural changes that occur in IVDs throughout life ultimately lead to tissue degeneration and the need for medical intervention. Although the patients included in this study were within different age ranges, they had all been diagnosed with disc herniation as a result of degenerative changes observed in their disc structures.

Numerous studies have reported a marked increase in the proliferative activity of chondrocytic cells during disc aging and degeneration. Miyamoto et al. (20) documented heterogeneous levels of cartilage tissue proliferation in cervical discs collected from murine models simulating cervical spondylosis. Similarly, Johnson et al. (21) observed that in degenerative human discs, proliferative activity was predominantly concentrated in regions containing dense cell clusters. Consistent with these findings, Zhao et al. (22) demonstrated that chondrocytic cells in herniated human cervical discs, which stained positively

for proliferating cell nuclear antigen, were typically localized in clusters adjacent to tissue fissures. Furthermore, both the number and size of these clusters near cracks and fissures were shown to increase with age in human lumbar discs. Collectively, these observations suggest that enhanced proliferation of chondrocytic cells constitutes a common histological marker of disc degeneration and may serve as a morphological indicator of disease progression. The findings of our H&E-stained sections were consistent with previously reported observations regarding chondrocytic cell proliferation.

The eosinophilic granular staining observed in our study is consistent with lipofuscin accumulation, which is described in the literature as one of the histological markers of disc aging and degeneration. Lipofuscin is an age-related pigment that accumulates in lysosomes as a byproduct of cellular metabolism. Saluja et al. (23) and Gower and Pedrini (24) reported that lipofuscin granules are frequently observed in both the AF and NP, particularly in advanced degenerative discs. Veroutis et al. (25) developed the GL13 staining method, which confirmed the association of lipofuscin accumulation with senescence in human disc tissues and demonstrated a significant increase in pigment-positive cells in herniated discs. These findings suggest that the eosinophilic granular staining observed in our study is not merely a morphological alteration but also an indicator of the biological process of cellular senescence, associated with aging and degeneration in disc cells.

Advanced degenerative changes have been particularly associated with alterations in collagen type and organization. Meisel et al. (26) demonstrated that degenerative discs exhibit increased type I collagen accumulation, a reduction in fibril diameter, and the formation of a denser, more fibrotic matrix. Additionally, age-related collagen deterioration is known to be linked not only to mechanical stress but also to molecular-level post-translational modifications. Gruber et al. (27) noted that age-related IVD changes, including the compaction and structural irregularity of collagen fibrils, are closely associated with cellular senescence, oxidative stress, and the accumulation of advanced glycation end products. Consistent with these findings, the present study demonstrated increased collagen fiber deposition in aged discs, as confirmed by Masson's trichrome staining. These alterations reflect the pronounced fibrotic remodeling that typifies advanced disc aging.

Although there is consensus in the literature that blood vessels are limited to the outer AF in normal adult IVDs, there is still debate as to whether vascular ingrowth occurs into the inner portions of the discs during degeneration. Corroborating this hypothesis, Freemont et al. (28) and Johnson et al. (29) identified vascularized areas within IVDs at sites where the lamellar organization of the AF was compromised. Consistent with our observations, multiple studies have documented the occurrence of vascular penetration into the inner annular layers in discs exhibiting advanced age-related or degenerative changes (30, 31). Stefanakis et al. (32) proposed that annular fissures or tears arising during disc degeneration promote vascular ingrowth, primarily as a consequence of localized proteoglycan depletion.

Lipofuscin accumulation in IVD degeneration is considered an important histological indicator of cellular senescence and oxidative stress in the tissue. Urban and Roberts (33) attributed lipofuscin accumulation in these tissues to the avascular nature of the IVD, resulting in a lack of nutrients and growth factors. On the other hand, Dimozi et al. (34) proposed that the intense stress to which IVD cells are exposed may trigger premature senescence. Zhao et al. (35) further demonstrated that excessive mechanical forces on IVD cells, particularly tensile forces applied to the AF, can suppress autophagy and induce premature aging in vitro in AF cells of the IVD. In line with these studies, we also found increased lipofuscin granules in the IVDs of elderly individuals using the AFIP method.

Amyloid deposits within or around joints have been reported in patients with severe arthropathy associated with systemic amyloidosis. Electron microscopy studies have revealed the accumulation of fibrils with ultrastructural features characteristic of amyloid in intervertebral discs. Consistent with this, Wullbrand et al. (36) demonstrated that amyloid deposition in IVDs, including both surgically removed tissues and autopsy samples, increases with age. Similarly, Madhani et al. (37) reported that individuals undergoing decompression surgery for cervical or lumbar spinal stenosis who had amyloid deposits in the ligamentum flavum and disc tissue were significantly older than those without such deposits. Consistent with these findings, our study also demonstrated a greater presence of amyloid deposits in IVDs from elderly individuals stained with Congo red.

One of the most prominent biochemical changes observed in the degenerative process is the reduction in proteoglycan content. Proteoglycans, the main components of the IVD extracellular matrix, maintain disc hydration and matrix organization. In particular, aggrecan, the most abundant proteoglycan in the IVD, decreases during degeneration, negatively affecting the mechanical strength and functional integrity of the tissue. Urban and Roberts (33) noted that the loss of proteoglycans reduces the osmotic pressure and water retention capacity of the NP. Antoniou et al. (38) demonstrated that aggrecan synthesis decreases and proteoglycans undergo degradation with age. Similarly, Boos et al. (39) histologically confirmed that proteoglycan content is significantly reduced in older age groups, contributing to the loss of disc elasticity. In line with these findings, our study demonstrated weaker metachromatic staining in toluidine blue-stained sections of the older groups due to decreased proteoglycan content.

Study Limitations

Due to the retrospective design of this study, the effects of additional factors that may influence IVD degeneration, such as diabetes mellitus, obesity, smoking, mechanical stress, scoliosis, postural abnormalities, and chronic inflammatory processes, could not be fully controlled. Although individuals under 40 years of age were excluded in order to reduce age-related variability, the influence of potential confounding factors could not be entirely eliminated. Such limitations should be taken into account while interpreting our findings.

CONCLUSION

Comparisons of staining methods have the potential to facilitate the selection of appropriate techniques for the histological evaluation of degenerative changes in human IVDs. The findings of this study offer guidance in efforts to achieve a more detailed characterization of structural alterations in different regions of the IVD and contribute to improving comparability across different studies.

DECLARATIONS

Conflict of Interest: *The authors disclosed no conflict of interest during the preparation or publication of this manuscript.*

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