Development of a compartment-specific histopathological scoring system for the cartilage endplate and intervertebral disc in aging C57BL/6J mice.

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INTRODUCTION: Low back pain (LBP) is a leading cause of disability worldwide, strongly linked to degeneration of the cartilaginous endplate (CEP) and intervertebral disc (IVD), which together maintain spinal stability and flexibility. Histologically, spinal aging involves structural alterations in the CEP, nucleus pulposus (NP), and annulus fibrosus (AF). The vertebral body's bony endplate (BEP), together with the CEP, forms the vertebral endplate complex. In C57BL/6J mice, spontaneous disc degeneration becomes evident at around six months of age. Existing histological scoring systems often fail to distinguish the CEP from the IVD, limiting accurate evaluation of tissue-specific degeneration. To overcome this, we reviewed current methods and developed a novel semi-quantitative scoring system that independently assesses CEP and IVD compartments. This compartment-specific approach enhances sensitivity and specificity in detecting early, distinct degenerative changes, providing a refined and reliable histological standard for studying spinal aging and disc pathology in mouse models.

METHODS: All animal experiments were approved by the Institutional Animal Care and Use Committee of Hiroshima University. Male C57BL/6J (B6) mice (n = 8 per group, total n = 64) were obtained from The Jackson Laboratory and sacrificed at 2, 3, 6, 9, 12, 15, 18, and 20 months of age. Seven histological parameters were evaluated. For the annulus fibrosus (AF), assessments included lamellar organization and the presence of tears. For the nucleus pulposus (NP), evaluations covered shape, cellular composition, and matrix quality. The NP–AF interface was analyzed by border clarity and NP area ratio using ImageJ for quantitative comparison. Immunohistochemistry assessed anabolic factors (Type II collagen, aggrecan), NP marker (cytokeratin-19), catabolic enzymes (MMP-13, ADAMTS-5), and the pain marker CGRP. Data were analyzed using GraphPad Prism 10 and expressed as mean ± SD. Statistical differences were determined using one-way ANOVA.

RESULT: Histological evaluation of the IVD and CEP at the L5–L6 level in B6 mice with aging was performed using sagittal sections stained with Saf-O staining. In the IVD By 3 months, histological changes were observed in both regions, occurring spontaneously with aging, as part of physiological maturation or as early degenerative alternations. Type II COLLAGEN immunostaining demonstrated a distinct age-related pattern, with staining intensity increasing up to 6 months of age. CYTOKERATIN-19 staining was strong in the NP at 2 months, but gradually decreased thereafter. From 12 months onward, staining intensity declined in both AF regions. MMP-13 and ADAMTS-5, In the NP, staining was weak at 2 and 6 months but became stronger from 9 to 18 months, indicate that catabolic activity contributes to matrix degradation and structural decline during disc aging. The CGRP-positive cells increased markedly between 6 and 15 months of age. Histological changes in the CEP during aging were classified into three phases: pre-maturation (= growth phase), maturation, and post-maturation (= degenerative phase). The growth phase, defined as the developmental phase preceding maturity, was characterized by abundant chondrocytes within the CEP. The subsequent maturation phase was marked by the appearance of distinct endochondral ossification-like changes. At 3 months of age, bone marrow appeared more prominent on the cranial side, and by 6 months it had extended comprehensively from the anterior to the posterior regions. Significant histological changes in the CEP were observed between 2 and 6 months of age ($p \leq 0.001$). The degenerative phase was characterized by a gradual reduction in bone marrow volume within the CEP, progressively replaced by sclerotic-like tissue. Furthermore, the evaluation revealed a significant increase from 12 months ($p \leq 0.05$). In the AF, The scoring system showed statistically significant changes as early as 2 months compared to 3 months of age ($p \leq 0.0001$). In contrast, the age range from 3 to 6 months of age, considered the peak maturation period ($p \leq 0.001$). In the degenerative phase following 6 months of age, the outer AF exhibited extensive tearing and loss of fibrous integrity, while the inner AF exhibited an accumulation of chondrocyte-like cells. In the NP, NP cells were markedly reduced, and the gelatinous matrix was severely disrupted. In advanced phase of degeneration, the NP was almost entirely resorbed and replaced by chondrocyte-like cells. A comparison between the control group (6 months) and the 9 month group revealed a significant difference ($p \leq 0.01$), and the severity of changes continued to increase up to 20 months. Notably, a statistically significant difference was observed at 18 and 20 months of age ($p \leq 0.0001$)

DISCUSSION: In this study, we established a novel histopathological scoring system designed to comprehensively assess age-related changes in both the IVD and the CEP compartments of C57BL6 mice. This compartment-specific approach enables a more precise and anatomically integrated evaluation of degenerative processes during maturation and aging. Our histological and immunohistochemical data revealed three distinct phases of CEP development: pre-maturation, maturation, and post-maturation (degeneration). During the maturation phase (2–6 months), the CEP underwent endochondral ossification and bone-marrow expansion, culminating in structural stabilization by 6 months. Thereafter, the degenerative phase was characterized by a progressive replacement of bone marrow with sclerotic-like tissue. These degenerative changes in the CEP, such as sclerotic alterations beginning at 9 months of age, are consistent with previous studies that describe similar patterns of ectopic bone in aging models. However, degeneration in the AF and NP became evident around 9 months, preceding the prominent sclerotic changes in the CEP. Thus, the CEP and the IVD (AF and NP) implying partially independent mechanisms driving disc aging. On the other hand, the AF and NP exhibited sequential and distinct degenerative patterns. Lamellar disorganization and fissures developed in the AF, while the NP showed condensation of the gelatinous matrix and a decline in notochordal-like cells. The coordinated yet compartmentalized nature of these changes indicates that IVD aging is not a uniform process but rather a mosaic of region-specific pathologies.

SIGNIFICANCE/CLINICAL RELEVANCE: This study establishes a simple and reproducible, compartment-specific histopathological scoring system for the mouse IVD and CEP.

REFERENCES:[1]Zhang+. *Osteo and Cartilage*. (2021), [2]Kirnaz+. *Int J Spine Surg*. (2021), [3]Crump+, *JOR Spine*. (2023), [4]Kirnaz+. *Int J Spine Surg*. (2021), [5]Ohnisi+. *JOR*. (2018), [6]Wang+, *Osteo Cart*. (2016).

Figures legend: Representative pictures of sagittal sections of the Intervertebral disc (IVD) **(1)** Saf-O, **(2)** Type II Col and **(3)** MMP13. **(4&5)** IVD Histology score

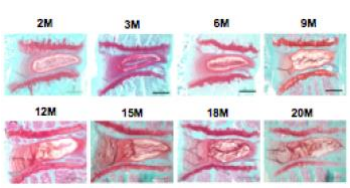

Figure 1.

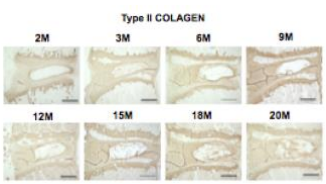

Figure 2.

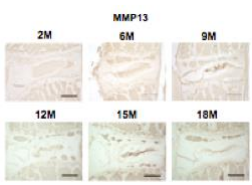

Figure 3.

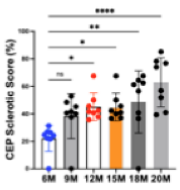

Figure 4.

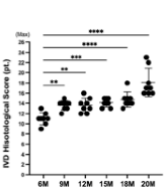

Figure 5.