J. Vet. Malaysia (2025) 37 (2): 4-8

Short Communication

HISTOLOGICAL AND HISTOMORPHOMETRICAL EVALUATION OF TIBIAL PLATEAU IN CATS WITH OSTEOARTHRITIS

S.A. DUHAIRI[1], N.S. SUKRY[1], N.I.I. ZABIDDIN[1], M.F. GHAZALI[2,3] AND S.M.Z. ARIFFIN[1*]

[1]Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), 43400 UPM, Serdang, Selangor, Malaysia.
[2]Faculty of Veterinary Medicine, Universiti Sultan Zainal Abidin, 22200 Besut, Terengganu, Malaysia.
[3]Halal Research Center, Univesiti Sultan Zainal Abidin, 21300 Kuala Nerus, Terengganu, Malaysia.

SUMMARY

Feline osteoarthritis (OA) is a joint disease that causes cartilage degeneration and bone changes, impairing load transmission and joint function in ageing cats. This study aimed to assess histologic and histomorphometric changes in the tibial plateau of cats with naturally occurring stifle OA and to determine the relationship between cartilage damage and bone changes. Osteochondral tissues from 12 OA and 10 normal cats were examined with H&E and Safranin O staining. Histology showed higher total articular damage scores in OA cats (p<0.05), with cartilage changes ranging from fibrillation to complete loss. Bone histomorphometry revealed increased trabecular bone volume fraction (BV/TV) and subchondral bone plate thickness (Subcho. BP.Th) in OA cats, though trabecular thickness did not differ (p>0.05). However, damage to the articular cartilage showed no relationship with changes in the underlying subchondral bone. These findings highlight the importance of evaluating both cartilage and subchondral bone in OA.

Keywords: osteoarthritis, tibial plateau, histology, histomorphometry, cat

INTRODUCTION

Feline osteoarthritis (OA) is a slowly progressing joint condition in cats over the age of 9 years (Kimura et al., 2020). It is marked by the gradual degeneration of articular cartilage and the underlying subchondral bone in synovial joints. Most feline OA cases appear primary with no apparent underlying cause. However, cases of cranial cruciate ligament rupture have been reported as a factor leading to secondary stifle OA in cats (Boge et al., 2020). The joint structures work together to support function and bear weight, with the tibial plateau microstructure adapted to withstand mechanical stress. Histological characteristics of OA in cats have been documented (Leijon et al., 2017). Cartilage degeneration that ranges from surface fibrillation to complete ulceration is common (Bennett et al., 2012). Additionally, in affected areas, thickening of the subchondral bone plate and trabeculae was also observed beneath the damaged articular cartilage (Ariffin, 2015; Hu et al., 2021). However, no systematic scoring of these microscopic lesions has been conducted. Research in human and murine models has demonstrated that subchondral bone remodelling plays a crucial role in OA progression and the response to cartilage damage (Aho et al., 2017; Donell, 2019). However, the cartilage damage and subchondral bone remodelling relationship in the tibial plateau of OA-affected joints in cats remains unclear. A comprehensive histological evaluation in cats would enhance the understanding of OA natural course and pathology.

This study aims to assess histological and histomorphometrical changes in the tibial plateau of cats with OA and investigate the relationship between cartilage defects and subchondral bone alterations. The study hypothesises that cartilage damage is significantly associated with the underlying subchondral bone changes.

MATERIALS AND METHODS

The study utilised osteochondral tissue samples from 22 cat cadavers (12 OA and 10 normal) that had been euthanised for reasons unrelated to this research and opportunistically collected from state animal control facilities. The stifle joints were classified as OA or normal according to the criteria previously described by Ariffin (2015). OA samples included ten females and two males, with an age range of 4-15 years and a mean of 7.1 years. The normal group consisted of six females and four males with an age range of 1-5 years and a mean of 3 years. The osteochondral tissues from the tibial plateau were fixed in 10% buffered neutral formalin for at least three days, followed by decalcification in 10% formic acid for three days. The osteochondral tissues were routinely processed, embedded in paraffin and sectioned at 5 µm. Haematoxylin and eosin (H&E) staining was performed to assess general tissue morphology, while Safranin O staining was used to evaluate proteoglycan content within the cartilage matrix. Histological assessment was conducted using a modified method (Table 1) (Aho et al., 2017; Voss et al., 2017). This study individually evaluated articular cartilage changes, including structural integrity, chondrocyte distribution, tidemark integrity, and proteoglycan content (assessed using Safranin O staining). Each parameter was scored, and the individual scores were then summed to obtain a

*Corresponding author: Siti Mariam Zainal Ariffin (S.M.Z. Ariffin);
Email: sitimariam_za@upm.edu.my

Editorial history:
Paper received: 30 October 2024
Accepted for publication: 3 August 2025
Issue Online: 1 September 2025

Table 1. Histological scoring of articular cartilage in OA.

	Histologic features	Score	Description
Articular cartilage	Cartilage structure	0	Smooth articular surface with all layers intact
		1	Fibrillation with fissures in the superficial layer
		2	Fissures to the middle layer and/or erosion of the superficial layer
		3	Fissures that extend to the deep layer and/or erosion through the middle layer
		4	Full thickness loss of cartilage
	Chondrocyte	0	Normal
		1	Loss of chondrocytic cellularity at the superficial layer
		2	Chondrocyte clusters mainly in the middle and deep layers
		3	Total loss of chondrocytes
	Tidemark integrity	0	Intact and distinct
		1	Increase the number of tidemarks.
		2	Loss of the tidemark, which is crossed by blood vessels
	Safranin O staining intensity	0	Normal (staining except for surface zone)
		1	Slight reduction (superficial zone)
		2	Moderate reduction (extending down to mid zone)
		3	Severe reduction (entire cartilage thickness)
		4	No dye noted
Global score		0	No abnormality (Total histologic score 0)
		1	Mild (Total histologic score 1-5)
		2	Moderate (Total histologic score 6-10)
		3	Severe (Total histologic score 10-13)

total OA score for each sample. Based on this total, a global OA severity grade was assigned to classify the overall extent of joint degeneration. Two observers independently assessed each sample on two separate occasions. For histomorphometry, images captured under X40 magnification included the articular cartilage and subchondral bone layers. Bone histomorphometric measures included trabecular bone volume fraction (BV/TV, %), thickness of the subchondral bone plate (Subcho.BP.Th, mm) and thickness of trabecular bone (Tb.Th, mm). Measurements were done according to the method described by Nagira et al. (2020). All H&E and Safranin O sections were examined under a light microscope, and histological images were exported into ImageJ version 10 software for analysis. The normality of the data was assessed using the Shapiro-Wilk test. Differences in histologic scores and histomorphometric data between OA and normal cats were analysed using either an independent t-test or the Mann-Whitney U test, with a statistical significance set at 0.05. Pearson's correlation coefficient was used to evaluate the relationship between the total histologic score of articular cartilage and histomorphometric bone parameters.

RESULTS

The histological evaluation demonstrated that the total articular damage score in cats with OA was significantly greater than in cats without OA ($p<0.0001$) (Figure 1.A). The total possible histologic score was 13, with the highest recorded score being 13 and the lowest being 6. The majority of global scores were 2 (moderate), while only two global scores of 3 (severe) were recorded. There were no microscopic changes observed in normal samples. The alterations in cartilage varied from fibrillation to total cartilage loss. Blood vessel invasion at the tidemark, chondrocyte aggregation near severely degenerated cartilage, mainly in the middle zones and loss of Safranin O staining were observed in the cartilage (Figure 2). The histomorphometrical analysis of the tibial plateau revealed significant differences in the bone volume fraction (Figure 1.B) and subchondral bone thickness (Figure 1.C) in OA compared to the normal samples. In contrast, the trabecular bone thickness in cats with OA did not differ significantly from that in cats without OA (Figure 1.D). A slight positive relationship was observed between parameters of bone histomorphometry and the total cartilage damage score (BV/TV, rs=0.25, $p>0.05$; Subcho. BP.Th, rs=0.06, $p>0.05$; Tb. Th, rs=0.09, $p>0.05$). However, the relationships were not statistically significant.

DISCUSSION

The study evaluates the histologic and histomorphometric changes in the tibial plateau of cats with naturally occurring stifle OA and their relationship with bone changes. Histological assessment of the articular cartilage can offer important insights into the damage experienced by affected animals. The articular cartilage examined in this study exhibited a range of changes, including fibrillation and complete loss of cartilage.

Additionally, chondrocyte aggregation, blood vessel crossing the tidemark, multiple tidemarks and loss of Safranin O staining were also observed. These findings align with previous studies (Ariffin, 2015; Leijon et al., 2017).

Fibrillation is characterised by the formation of vertical cleft cartilage, giving it a roughened texture. It is often seen in the early stages of cartilage degeneration and may indicate the onset of further damage (Aho et al., 2017). Fibrillation is often seen in weight-bearing areas and may be a result of repetitive mechanical stress or ageing. In advanced stages of OA, the total loss of cartilage is often seen, leading to increased friction, pain, and impaired joint function. Chondrocyte aggregation is a common feature of feline OA. The exact mechanisms of chondrocyte aggregation are not fully understood. Chondrocyte proliferation is considered an important mechanism for repairing and counteracting cartilage degeneration. By producing more matrix proteins, proliferating chondrocytes contribute to the regeneration and maintenance of cartilage structure and function (van Der Kraan and van Den Berg, 2012).

Crossing blood vessels over the tidemark is another sign observed in articular cartilage. The tidemark functions as a demarcation that separates uncalcified and calcified cartilage. This region is typically lacking in blood vessels. However, in samples where cartilage degradation was severe, blood vessels were seen to penetrate beyond the tidemark. Furthermore, multiple tidemarks were also seen. As reported in previous studies, the number of tidemarks observed on the affected joints correlates with the severity of the disease (Roudier et al., 2013).

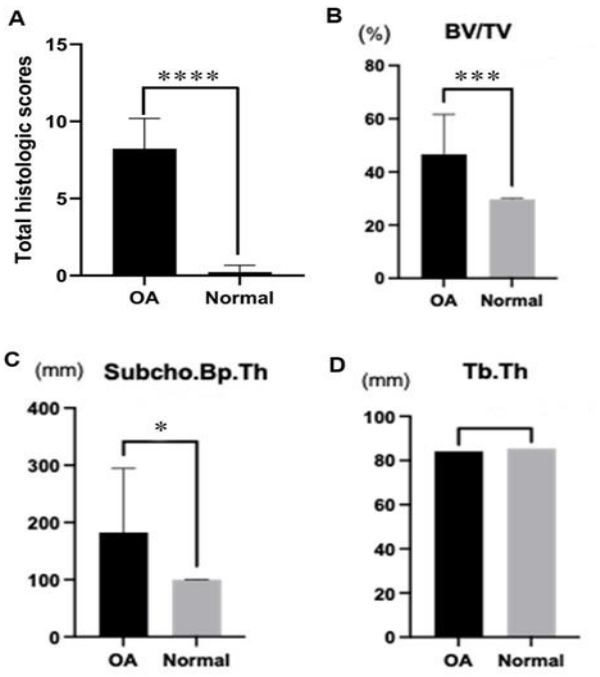

Figure 1. (A) Comparative analysis of total histological scores in articular cartilage samples, highlighting significantly higher scores in the OA group relative to the normal group. (B) In cats with OA, the bone volume fraction (BV/TV) is elevated than the normal group. (C) Increase in subchondral bone plate thickness (Subcho.Bp.Th) in OA-affected cats compared to unaffected cats. (D) No significant difference in trabecular thickness (Tb.Th) is observed between OA and the normal group.

A- C: data are presented as mean±SD, D: data are expressed as median. (represents p<0.05; *** represents p<0.001; **** represents p<0.0001; ns: not significant).*

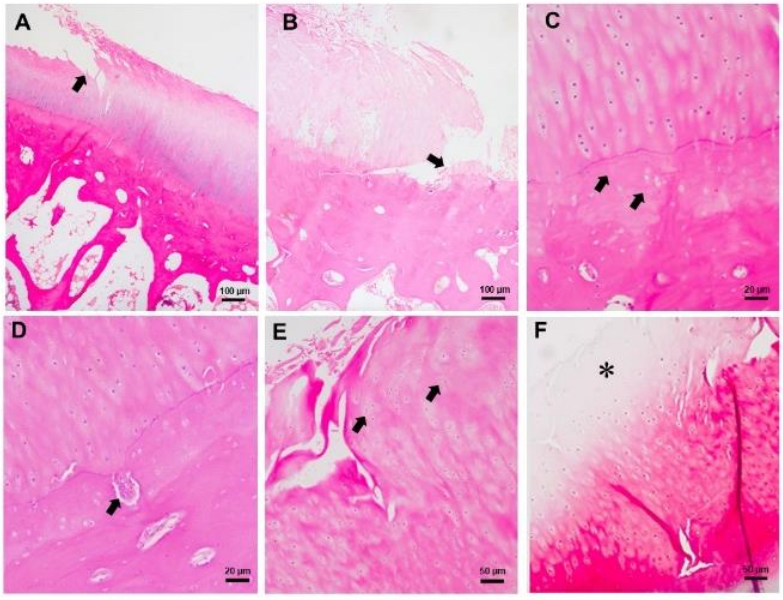

Figure 2. Tibial plateau cartilage affected with OA stained with H&E (A-E) and Safranin O (F). A: Fissuring in the articular cartilage reaching the deep layer, with erosion progressing through the middle layer (black arrow). B: Complete loss of cartilage thickness is observed (black arrow). C: Multiple tidemarks are visible within the calcified cartilage (black arrows). D: Blood vessels crossing the tidemark (black arrow). E: Chondrocyte aggregation is observed in the middle layer (black arrows). F: Loss of Safranin O staining is observed (asterisk).

A, B: X40 magnification; C & D: X400; E-F: X100 magnification.

tidemark. Furthermore, multiple tidemarks were also seen. As reported in previous studies, the number of tidemarks observed on the affected joints correlates with the severity of the disease (Roudier et al., 2013).

Loss of Safranin O staining serves as a key indicator of cartilage degradation. Safranin O selectively binds to proteoglycans, which are important components of the cartilage matrix. In healthy cartilage, the presence of intact proteoglycans results in strong red staining (Di Francesco et al., 2021). Degradation of proteoglycans reduces dye retention, leading to diminished staining intensity as seen in Figure 2 (F). This reduction reflects advanced cartilage degeneration and is associated with impaired structural integrity and function (Euppayo et al., 2016).

In this study, the histomorphometrical analysis of the tibial plateau revealed significant differences in BV/TV and Subcho.BP.Th between OA and normal samples. Contrary to this, trabecular bone thickness did not differ significantly between cats with and without OA. Bone volume fraction (BV/TV) represents the proportion of bone volume to total tissue volume and serves as a key indicator of bone mass changes. In OA, an increase in BV/TV reflects modifications in trabecular bone structure due to subchondral bone remodelling in response to joint degeneration and altered mechanical loading (Han et al., 2022).

Bone remodelling is a normal physiological process that maintains bone health and function. However, in OA, bone remodelling in the subchondral bone is altered by an increased bone turnover to adjust and adapt to mechanical load changes (Zamli et al., 2015). As the bone turnover is high, the balance between resorption and formation of new bone may be disrupted. The new bone tissue formed may not have sufficient time to mineralise, thus becoming more porous and reducing stiffness fully (Ariffin, 2015). In response to reduced mineralisation, the bone enhanced its volume through the thickening of the subchondral bone plate, which is consistent with the findings in this study. This thickening can enhance the stiffness of the subchondral bone, which acts as a boundary between the articular cartilage and the trabecular bone below. No significant difference in the trabecular bone thickness between OA and normal samples was seen in this study, as the subchondral bone plate remodelled more rapidly than the trabecular bone. The changes might only happen in the late stage of OA (Huang et al., 2021).

Although the relationship between cartilage damage and changes in the underlying subchondral bone was hypothesised, our findings did not support this relationship at a statistically significant level. These findings indicate that the relationship between articular cartilage deterioration and changes in the subchondral bone in feline OA can vary. The changes do not always occur in parallel, and the presence and extent of each may differ among individual cats and joints. For example, some cats may have extensive cartilage damage without significant subchondral bone changes, while others may exhibit pronounced bone changes. This study contradicts the findings of Aho et al. (2017), who reported a strong relationship between increased bone remodelling and cartilage damage. Therefore, the histological assessment of feline OA should take the subchondral bone into account, and there is a need for a universal grading scale that integrates both cartilage and bone changes for comprehensive analysis.

CONCLUSION

In conclusion, the OA tibial plateau showed histological changes characterised by loss of cartilage structure, decreased cellularity, loss of tidemark integrity, and low safranin O intensity. Furthermore, bone changes such as increased bone volume fraction and subchondral bone plate thickening were observed. However, the articular cartilage damage did not correlate with changes in the underlying subchondral bone. For future studies, longitudinal research with larger sample sizes is recommended to better understand OA progression, establish cause-and-effect relationships and observe changes in various contributing factors.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the staff of the Histopathology Laboratory, Faculty of Veterinary Medicine, UPM, for their help in completing this research project.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING

This study was funded by the Faculty of Veterinary Medicine, Universiti Putra Malaysia, through the Final Year Project Research Grant, Academic Session 2023/2024.

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