ORIGINAL RESEARCH

Histopathological changes due to osteoarthritis in articular cartilages of the knee

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Abstract

Introduction: The bony ends of a synovial joint are lined by a specialised hyaline cartilage termed the articular cartilage. Articular cartilage can be affected with degenerative diseases such as osteoarthritis. Knee being one of the larger joints built for weight bearing is the commonest site for osteoarthritis. The aim of this study was to describe the macroscopic and microscopic features of articular cartilages of the knee joint in patients diagnosed with primary osteoarthritis.

Methods: Seventy-two articular cartilages removed during total knee replacement surgery were observed for macroscopic damage and graded according to the direct visual assessment score. Thirty specimens randomly selected were processed for histology and stained with Safranin-O-fast green-iron hematoxylin and observed and scored using the Mankins histological and histochemical grading system.

Results: majority of specimens showed severe damage in the medial compartment and much less damage in the lateral compartment. In the tibia the damage was greatest on the medial most edge where the articular cartilage was absent leaving the bone exposed. Anteromedial area was relatively spared. In the femur the middle part of the medial condyle was seen to be more severely damaged. A similar pattern was observed in 19 (63.3%) of the specimens and showed exposure of bone. In the lateral compartment worst damage was observed in the middle of the lateral femoral condyle, with the rest of the lateral compartment being well persevered. This pattern was observed in 26 (86.6%) of the knee specimens.

Conclusion: OA of the medial compartment causes the two cartilage surfaces to grind together, mechanically denuding the cartilage layers, while the lateral compartment which receives much less mechanical stress is well preserved.

Running title: histopathological changes in osteoarthritis

Key words: articular cartilage, osteoarthritis

Introduction

The bony ends of a synovial joint are lined by a specialised hyaline cartilage termed the articular cartilage. The articular cartilage is about 2.5mm thick in a normal adult (1).

However, the thickness of the cartilage changes from bone to bone in accordance with the tensile stresses it has to withstand. On histological examination the articular cartilage shows a transparent matrix with chondrocytes arranged in layers. Four layers are commonly described (Figure 1).

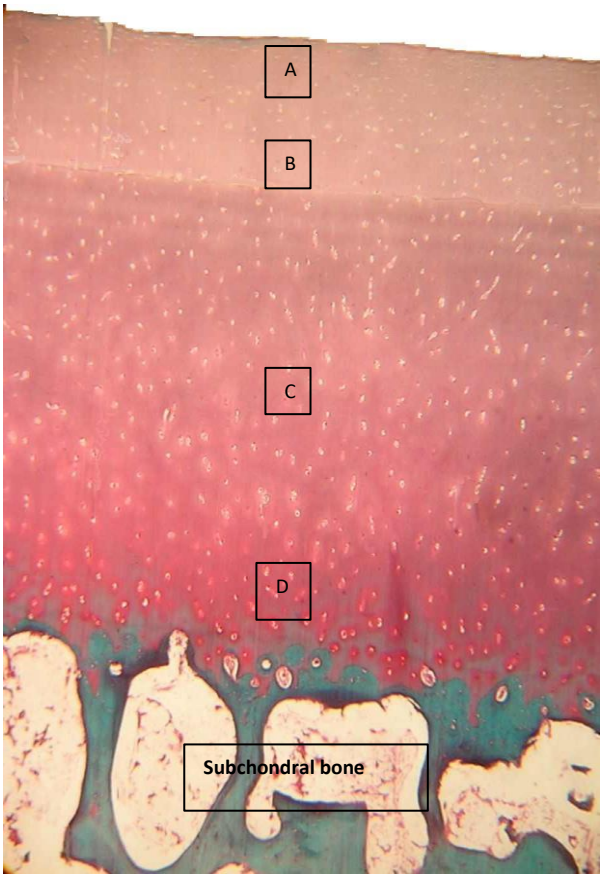


Figure 1: Histological appearance of the articular cartilage of a normal tibia

A. The tangential layer, where the chondrocytes are small and flattened and arranged parallel to the surface.

B. The transitional zone, where the chondrocytes are slightly larger and can be seen singly as well as in isogenous groups.

C. The radial zone being the thickest show large chondrocytes forming radial columns arranged perpendicular to the articulating surface.

D. The calcified cartilage layer rests on the underlying bone. The matrix of this layer consists of calcium salts as the name suggests.

The articular cartilage in the knee joint has to withstand the body weight as well as the stresses of bending and straightening during the many movements of everyday living. Therefore, it is designed specially to withstand these stresses. However, disease processes such as osteoarthritis (OA) can damage this intricate structure. The articular cartilage lacks a perichondrium and has no blood vessels penetrating it. Therefore, the regenerative ability is minimal in this type of cartilage.

OA is defined as a heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage in addition to related changes in the underlying bone at the joint margins (2). The characteristic features include focal areas of damage to the articular cartilage of synovial joints and sclerosis of the underlying subchondral bone.

OA of the knee is the most common form of OA worldwide and is commoner in females. (3) The documented risk factors for knee OA are female gender, increasing age, obesity, ethnicity, genetic factors and local factors affecting the knee, such as ligament laxity, reduction in proprioception with age and knee joint mal alignment (4).

The knee joint formed by the articulation of the tibia and femur, is described as having three

compartments: the medial compartment formed between the medial tibial plateau and the medial femoral condyle, the lateral compartment formed between the lateral tibial plateau and the lateral femoral condyle and the anterior compartment formed between the intercondylar area and the patella. OA is commonly seen in the medial compartment with varus malalignment of the knee.

The OA disease process is thought to start at around the age of 30 years and slowly progress. It may range from mild to moderate knee pain increased with activity to severe deformity and loss of ambulation. Initial treatment methods include management of pain and advice on minimizing the strain on the knee joint. However, severe disease requires surgical intervention with joint replacement.

The first demonstrable change in articular cartilage with OA is the softening of the cartilage on palpation (5). These softened areas develop macroscopic fibrillations which finally split to form fissures. The fissures become deeper and deeper until it reaches the subchondral bone. Complete loss of the cartilage layers result in exposure of the bone surface (6,7).

Microscopically the first change is loss of stainability with special stains such as Safranin O, which is the result of reduced proteoglycans in the cartilage matrix (8,9). Then chondrocyte necrosis occurs. Chondrocyte clusters are observed and the tangential and radial orientation of chondrocytes is lost. The boundary between the deep cartilage layers and the calcified layer is disrupted and blood vessels are seen to cross the tide mark (10). Finally, the architecture of the cartilage is completely disrupted and the bone is denuded and exposed.

The aim of this study was to study the histopathological changes seen in the articular cartilages of patients with osteoarthritis of the knee joint and grade the lesions observed, macroscopically and microscopically.

Methods

Articular cartilages removed from 72 knee joints during total knee replacement in patients diagnosed with primary knee joint osteoarthritis were obtained from the theatre at Teaching Hospital Peradeniya. Femoral condyles and the tibial plateau removed at surgery were retrieved and placed in 10 formal saline. Five articular cartilage specimens from above knee amputations due to trauma were obtained as a control sample.

All collected specimens were examined under normal light to assess the cartilage wear patterns. The widest diameter of each lesion was measured using a calibrated Vernier calliper (120 mm dual scale metal). The two articular surfaces of each joint were accessed using the scoring system described by Noyer et. al. in 1989 (11). This scoring system takes into account the depth, severity and the size of the lesion on the articular cartilage and assigns a stage and a numerical value for each (Figure 2). Table 1 shows the scoring system in detail.

Table 1: Direct visual assessment score

Grade	**Description of lesion**	**Points for 10 - 15mm sized lesion**	**Points for >15mm sized lesion**
1A	No visible lesion	0	0
2A	Lesion with fissures, fragmentation, half thickness	2	4
2B	Lesion with fissures, fragmentation full thickness	3	6
3	Bone exposed	5	10

In this scoring system normal appearing cartilage is classed as grade 1A and does not receive any points. A lesion with fissures and fragmentations of the articular cartilage extending to half its thickness is classed as grade 2A. A grade 2A lesion with the widest diameter between 10-15mm is given 2 points and if the lesion is wider than 15mm it's given a score of 4. Grade 2B is given to a lesion where the articular cartilage has fissures and fragmentation extending to its full thickness. If a grade 2B lesion has a diameter between 10-15mm it is given 3 points and if the diameter exceeds 15mm 6 points are given. A lesion where the articular cartilage is denuded and the bone is exposed is a grade 3 lesion and is given 5 points if the diameter is between 10-15mm and 10 points if it exceeds 15mm.

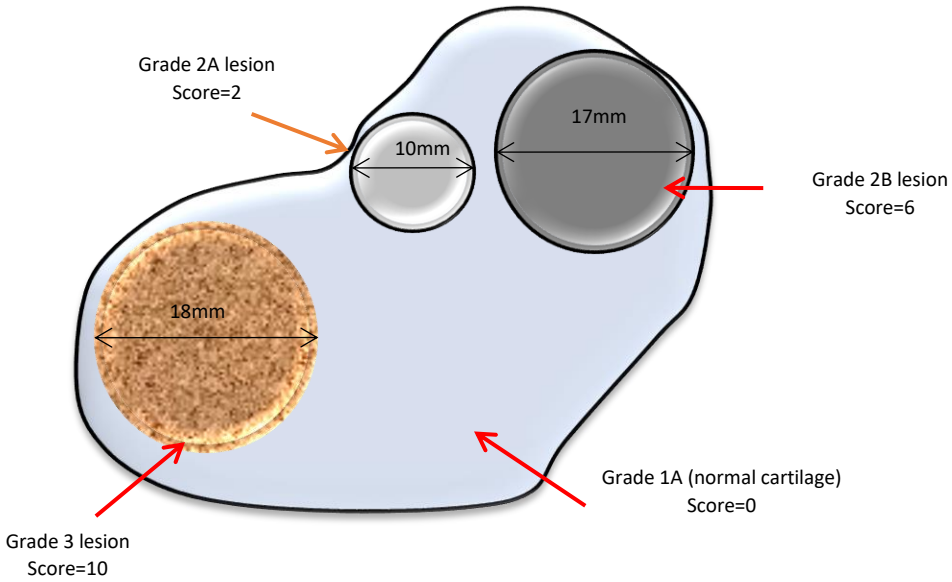


Figure 2: Tibial plateau with the articular cartilage lesions graded and scored

Using these scores, the final macroscopic damage severity was expressed out of a score of 20 for each compartment. A score of 20 for the medial compartment means that the

articular cartilages on the medial tibial plateau and the medial femoral condyles are completely destroyed with exposure of bone. A score of 10 for the compartment means that half the articular cartilage in the relevant compartment is damaged.

Articular cartilage specimens from osteoarthritic knees and the control samples were fixed in 10% formal saline for two days, and processed for histopathological studies. The fixed specimens were cut into 1×1cm size blocks using a hacksaw and a scalpel as specified in figure 4.

All blocks contained bone with the articular cartilages, therefore they were decalcified in 20% EDTA and for three weeks. Decalcified specimens were then processed for normal microscopy and embedded in paraffin wax. Three longitudinal consecutive sections, of 2 µm thickness were cut from each wax block using a microtome (Reichert-Jung BIOCUT 2030), mounted on clean glass slides and stained with Safranin-O-fast green-iron hematoxylin according to the regular procedure.

A total of 42 sections for each knee joint were finally prepared and examined under a normal light microscope. The damage to the articular cartilage was graded according to the Mankins histological and histochemical grading system given in table 2 (12). This grading system assigns separate scores to structure of the cartilage, cell distribution and density, intensity of Safranin-O staining, and the integrity of the tile tidemark. Each of these subcategories is given a separate numerical value. Once the values given for the subcategories are totaled the final score for each area assessed is produced. The highest score thus obtained is 14 and denotes completely destroyed cartilage or absence of cartilage in the area. A score of 0 denotes a histologically normal articular cartilage.

From each of the slides, three non-overlapping fields were examined and the histological score was determined. Slides prepared from the normal cartilage specimens were used as a reference when deciding the grade. The histological score for each visual field was totaled and an average score was obtained for each slide. This score was taken as the score for the area of the articular cartilage sampled. The scores obtained for the areas studied in the two knee compartments namely the medial and the lateral was totaled to arrive at a histological score for each knee.

Table2: Mankin's Histological and histochemical grading system

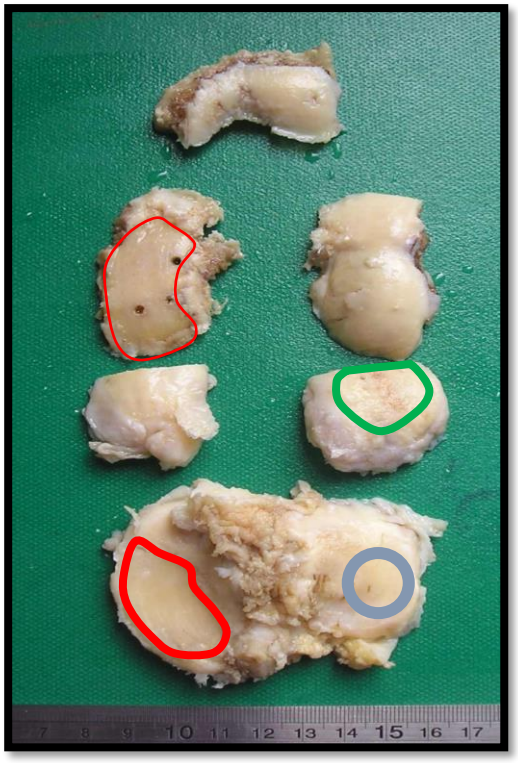
		Grade
I. Structure	a. normal	0
	b. surface irregularities	1
	c. pannus and surface irregularities	2
	d. clefts to transitional zone	3
	e. clefts to radial zone	4

	f. clefts to calcified zone	5
	g. complete disorganizations	6
II. Cells	a. normal	0
	b. diffuse hypercellularity	1
	c. cloning	2
	d. hypocellularity	3
III. Safranin-O staining	a. normal	0
	b. slight reduction	1
	c. moderate reduction	2
	d. severe reduction	3
	e. no dye noted	4
IV. tide mark integrity	a. intact	0
	b. crossed by blood vessels	1
		14

Results

Direct visual assessment of the articular cartilages showed that the medial compartment was more severely damaged than the lateral compartment. The damage score of the medial compartment ranged from 12 (60%) to 20 (100%) with a mean of 12.53 (±5.3). It was observed that 37% of the specimens had lesions with a score of 20 in the medial compartment. This denotes that these specimens had lesions more than 15mm in diameter where bone was exposed on the medial tibial plateau and the medial femoral condyle. Damage to the lateral compartment was comparatively less and ranged from 0 to 10 with a mean of 1.28 (±2.2). Of these 44 knee specimens (61.1 %) had macroscopically normal appearing articular cartilages in the lateral compartment.

The knee joint specimen of a single patient with the lesions outlined and scored is given in figure 3 for reference.



The 2 lesions outlined in red has a diameter more than 15mm and the bone is exposed therefore they are grade 3 lesions and are given 10 points for each.

The lesion outlined in green has a diameter between 10mm-15mm and the lesion extends to the full thickness of the articular cartilage and therefore is a grade 2B lesion and is given a score of 3.

The area outlined in blue has a normal appearing articular cartilage

Figure 3: Articular cartilage specimen with the lesions graded

Microscopic examination confirmed that the medial compartment showed more severe damage than the lateral compartment. The specific Mankins histopathological score for each area in the tibia and femur is given in figure 5 for reference.

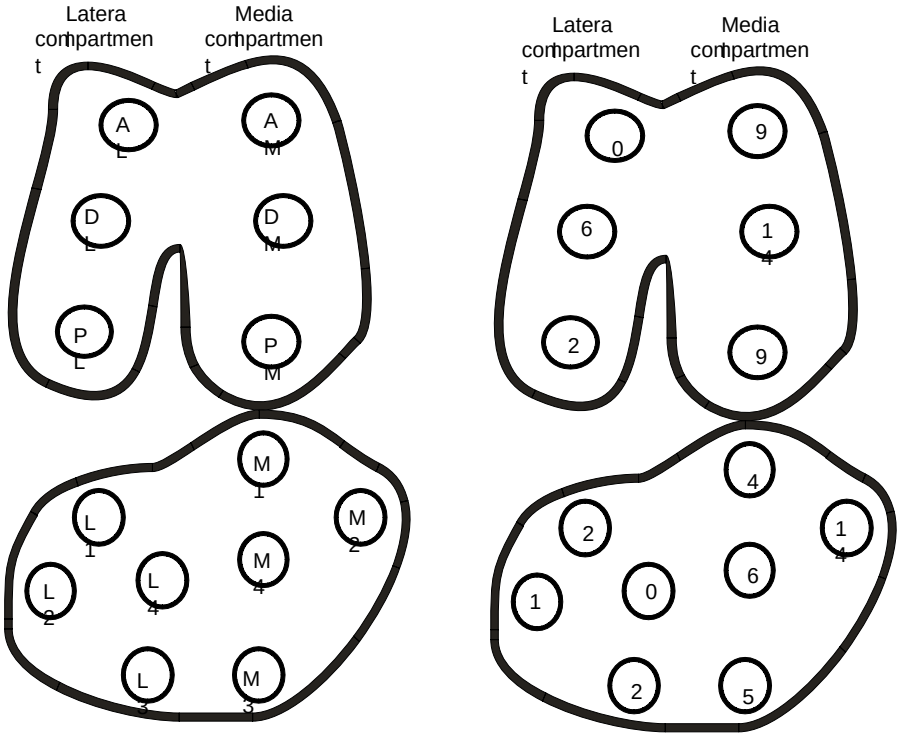


Figure 4: Mankins score for each area in the tibia and femur of the right knee

Cartilages with maximum damage were completely denuded of cartilage and only bone with a thin layer of cartilage matrix and few scattered cells could be seen. Less serve damage showed cartilage with varying thickness and amounts of Safranin O staining with rapid loss of staining as the severity progressed. Macroscopically normal appearing cartilages showed fissuring and fragmentation of the surface layers under microscopy. Chondrocyte orientation was lost and cell clustering and nesting was observed in many specimens.

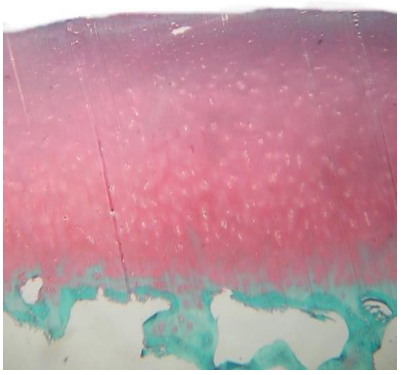
Cartilage in the lateral compartment was well preserved with good staining and well-preserved cell architecture and orientation.

The score in the medial compartment of both the femur and tibia ranged from 4-14. In the tibia the damage was greatest on the medial most edge in the area marked M2 in figure 5. These areas did not show articular cartilage leaving the bone exposed. This pattern was seen in most specimens. The areas marked M1 and M3 were relatively spared. In the femur the middle part of the medial condyle marked DM was seen to be more severely damaged. A similar pattern was observed in 19 (63.3%) of the specimens and showed exposure of bone.

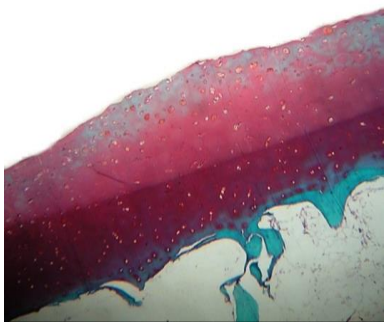
The score in the lateral compartment in both the tibia and femur ranged from 0-6. The worst damage was observed in the middle of the lateral femoral condyle, the area marked DL. The rest of the lateral compartment was well persevered with most of the areas having a score of 0-2, which is considered similar to normal cartilage. This pattern was observed in 26 (86.6%) of the knee specimens.

The overall microscopic score for the medial compartment of each knee specimen was calculated by adding the scores from all the areas sampled in the medial tibial plateau (M1, M2, M3, M4) and the medial femoral condyle (AM, DM, and PM). The overall score ranged from 43-79 with a mean of 55.34±10.59. The score for the lateral compartment was also calculated similarly and it ranged from 6-38 with a mean of 14.2±9.15

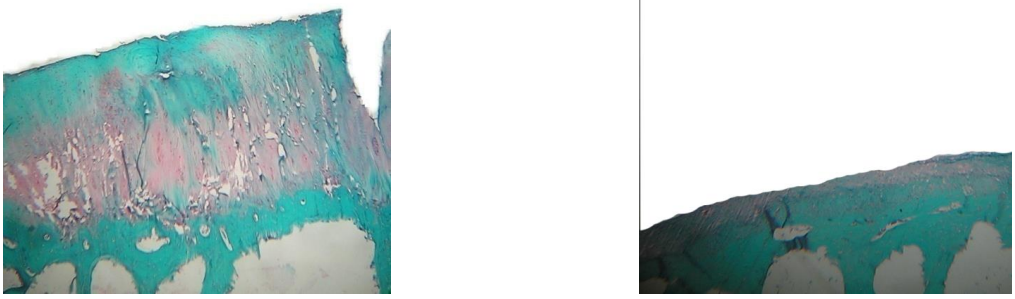
Figure 5 shows the microscopic appearance of the articular cartilage of the tibia in four different stages of damage with the specific Mankins scores.



Score = 0 – denotes normal cartilage. Maximum thickness and staining



Score = 5 – 50% loss of thickness. Fissuring and slight loss of staining



Score = 9 – loss of staining. Complete disruption of chondrocyte architecture.

Score = 14 – complete loss of cartilage thickness

Figure 5: Microscopic appearance of articular cartilage with the specific Mankins scores

Discussion

The direct visual assessment score described by Noyers et al (11) has been designed to stage lesions in arthroscopy. This scoring system has the advantage of considering the depth as well as the size of the lesions and assigning a stage and a numerical score for each observed lesion. It can be stated that this scoring system is comprehensive and provides an excellent idea about the lesion. The description of lesions and the scoring system devised by them has been used for staging lesions by naked eye examination in international studies (13.14).

Mankins histopathological score was designed by Mankin et al in 1971(12) by studying osteoarthritic human hips. It is a widely accepted score for assessment of articular cartilage damage in joints with osteoarthritis. van der Sluijs et al (1992) (15) validated this score and demonstrated an excellent inter and intra observer agreement. Although other scoring systems have been designed in the recent past Mankin score is still widely used in international studies (16,17). It is considered a reliable score and its use in this study can be justified since it offers more opportunities for comparing results with previous studies.

Two studies available in the literature have looked at macroscopic changes in articular cartilage due to OA (18,19). Both these studies have looked at only the tibial plateau, while the current study looks at the tibia as well as the femur. These studies do not offer histopathological evidence. In the current study the medial compartment articular cartilage was seen to be severely damaged. The knees with least damage had 60% of the medial compartment articular cartilage affected and the severely damaged knees had 100% of its medial compartment articular cartilage affected. Histological assessment confirmed these findings.

Histological assessment of the lateral compartment showed the articular cartilage to be normal in more than 80% of the knees examined. The microscopic score was seen to be 0-2 in these knee specimens with the articular cartilage thickness being normal.

The above findings can be due to the excessive medial orientation of the femur on the medial tibial plateau which shifts the whole of the body weight on to medial compartment of the knee. This causes severe stress on the articular cartilage of the medial compartment causing the two cartilage surfaces to grind together, mechanically denuding the cartilage layers. The diseased cartilage has very little potential to regenerate and the severe trauma caused by day-to-day activities makes any attempts at healing unsuccessful. Examination of the lateral compartment in these knees however revealed well preserved articular cartilage. When the weight bearing is shifted to the medial compartment the lateral compartment receives very little mechanical stress on its articular surface. This prevents grinding of the articular surfaces on each other and removes most of the mechanical stress, allowing the cartilage to maintain its normal structure.

The pathophysiology of OA is complex and treatment options are limited (19). In-depth studies in to pathological mechanisms and treatment options are ongoing even though disability and deformity due to this debilitating disease is still common and continue to burden the health sector.

Conflict of interest

None

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