coordinate system, to fully account for the position of the tibia relative to the femur while acquiring data.

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DOES SUBCHONDRAL SCLEROSIS PROTECT PROGRESSION OF JOINT SPACE NARROWING IN PATIENTS WITH VARUS KNEE OSTEOARTHRITIS?

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Purpose: Abnormal medial loading in varus knee osteoarthritis (OA) results in decreased cartilage thickness, osteophyte ingrowth and subchondral bone sclerosis in the medial femoral and tibial condyles. Some studies have shown that higher bone mineral density (BMD) is protective for knee OA progression. Also, BMD is not necessarily associated with decreased cartilage thickness of knee OA. In addition, the relationship between BMD and knee OA may differ by measurement site of BMD and severity of knee OA. We hypothesized that subchondral bone sclerosis might protect decreased cartilage thickness in knee OA. The aim of this study was to assess the relationship between joint space narrowing and BMD of the knee condyles in patients with varus knee OA.
Methods: This study involved 192 women with varus knee OA at our institution. All patients underwent knee radiograph, dual energy x-ray absorptiometry at lumbar spine, proximal femur and knee condyles. The femorotibial angle (FTA) was defined as the lateral angle between the axis of the femoral and tibial shafts. The joint space narrowing angle (JSNA) was defined as the angle between the tangents to the femoral condyles and the tibial plateau marginal line, and was used as an indicator of medial joint space narrowing. Medial and lateral condyles BMD at the femur and the tibia were measured. BMD of the femoral and medial condyles was used as the indicator of subchondral sclerosis. According to the Kellgren &Lawrence grading system, 11 knees were grade 1, 29 knees were grade 2, 54 knees were grade 3 and 98 knees were grade 4. Also, according to the Ahlbäck grading system, 37 knees were grade 0, 51 knees were grade 1, 51 knees were grade 2, 47 knees were grade 3 and 6 knees were grade 4. Body mass index (BMI) was calculated as an index of obesity. SPSS for windows was used for statistical analysis. Data was expressed as means ± standard deviations and assessed using Pearson's correlation coefficients. Significance was set at $p<0.05$.
Results: Mean age, BMI and FTA were 69.5 ± 8.9 years, 25.6 ± 4.3 kg/m^2 and 182.2 ± 5.1°, respectively. There was a weak association between lumbar spine BMD and JSNA ($r = 0.176$, $p = 0.015$), but there was no association between femoral neck BMD and JSNA. Medial femoral and tibial condyles BMD were correlated with JSNA ($r = 0.550$, $p < 0.001$ and $r = 0.571$, $p < 0.001$, respectively). Also, lateral femoral and tibial condyles BMD were not correlated with JSNA.
Conclusions: Previous paper reported that high BMD at baseline and BMD gain decreased the risk of progression of radiographic knee OA. The protective effect might be associated with reducing the risk of joint space narrowing. Our cross-sectional results showed that the increase of subchondral bone sclerosis in the medial femoral and tibial condyles might protect the decrease of cartilage thickness in patients with varus knee OA.

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DELAY IN OSTEOARTHRITIC CHANGE IN A LOX-1 KNOCKOUT MOUSE MODEL OF OSTEOARTHRITIS

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Purpose: LOX-1, a vascular endothelial cell receptor for oxidized low-density lipoprotein (ox-LDL), is reportedly involved in the progression of knee osteoarthritis (OA); however, the underlying mechanism has not been elucidated. In the present study, we established a mouse model of OA through destabilization of the medial meniscus (DMM) to investigate the role of LOX-1 in the pathogenesis of OA progression.
Methods: In this study, we used 9-week-old LOX-1+/+ and LOX-1-/- mice. The mice underwent DMM at the right knee (DMM side) as well as a skin incision along with placement of a joint capsule on the contralateral knee (sham-operation side). Samples were obtained from both the knee joints at 4 and 8 weeks after surgery. Safranin staining was performed for each section to assess the osteoarthritic change. Cartilage degeneration, osteophyte formation were assessed by using a scoring system . Moreover, the expression levels of LOX-1 and ox-LDL were assessed in each sample by immunostaining.
Results: On the DMM side, osteophyte formation and cartilage degeneration were significantly suppressed in LOX-1-/- mice at 8 weeks after surgery. However, no significant difference in osteophyte formation or cartilage degeneration was noted on the sham-operation side at both 4 and 8 weeks; OA progression was only slightly observed among these mice . Expression was observed cartilage cells, the formation of osteophytes section . Moreover, the expression levels of LOX-1 and ox-LDL in LOX-1+/+ mice were found to be increased from 4 weeks to 8 weeks after surgery.
Conclusions: Thus, we believe that LOX-1 may play an important role in the pathogenesis of OA progression. Moreover, we suggest that LOX-1 could be used as a potential target for the prevention and treatment of OA progression.

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CARTILAGE MINERALIZATION AND CELL DEATH WITHIN EXPERIMENTAL OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is characterized by the progressive loss of articular cartilage (AC), accompanied by altered subchondral bone remodeling. In addition calcium deposits have been related to AC degeneration and have been observed in late stages of OA. However, the role of those deposits, whether they induce OA pathogenesis or they appear as a consequence of such process, is still unknown. Therefore the main aim of our research was to determine mineralization markers and cell death in AC.
Methods: Knee articular cartilage samples from OA and normal tissue from patients were studied. Macroscopic and microscopic observations, alkaline phosphatase staining for light and electron microscopy (bright and dark fields), TUNEL technique, electron diffraction (EDS), and x-ray microanalyses were performed.In addition we studied the kinetics of expression and tissue localization of osteopontin (OPN), a mineralization biomarker, and calcium deposits in samples from (normal, sham) and osteoarthritic cartilage studied in a rat model. Immunohistochemical and western blot assays for OPN, as well as Alizarin red staining for calcium deposits performed in the superficial, middle, and deep zones of ACwere analyzed.
Results: Chondrocytes from patients displayed a morphology of apoptosis and showed abundant alkaline phosphatase (ALP)-rich matrix vesicles (MV) budding from the plasma membrane with hydroxyapatite microcrystals on their surface. Farther from the cells hydroxyapatite crystals were detected on the MV surface and increased as they approached the subchondral bone. The concentration of Ca and P and their ratio increased inside the ALP-rich MV in relation to the proximity to subchondral bone. In the subchondral bone the ratio Ca,/P varied from 3.936 to 0.974. In normal tissue the ratio was very homogeneous (maximum 1.973, minimum 1.781).
Conclusions: In situ, apoptotic chondrocytes correlate with factor known to be involved in the calcification of the extracellular matrix. This suggests that apoptosis is involved in the abnormal calcification of OA cartilage, and consequently in the altered remodeling of the subchondral bone. In addition, the expression and localization of OPN and calcium deposits during OA pathogenesis suggests that the pathological AC mineralization starts in the superficial zone during OA pathogenesis, which correlates with an increased expression of apoptotic cell death markers found mainly in chondrocytes from SZ in early stages of OA.

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IDENTIFYING THE EARLIEST HISTOPATHOLOGICAL CHANGES IN KNEE OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is a major cause of pain and disability in the elderly population. Defining the earliest structural, cellular and molecular changes specific to disease onset is challenging. The disease is usually diagnosed when pain first develops which is often after initial joint damage has occurred. Consequently this limits investigation into the earliest changes of preclinical knee OA. Furthermore, radiographs and magnetic resonance imaging (MRI) are insensitive to detect cellular or molecular pathological changes in people with newly diagnosed knee OA. We aimed to address these issues by histologically screening knee tissue collected from post-mortem (PM) cases. We hypothesised that histopathological features commonly detected indicate early osteoarthritic changes, whereas features that are less common indicate changes occurring later in the disease process.
Methods: Joint tissues were collected from 183 consecutive PM donations. Osteoarthritic changes in the medial tibial plateaux, synovia and menisci were histologically graded. The extent and severity of articular cartilage loss in the medial and lateral femoral condyles and tibial plateaux were assessed macroscopically. Each articular surface score was summated to give a total tibiofemoral chondropathy score (0-400). Osteophytes were directly visualised on the dissected knee.
Results: PM cases (median age 65 years, 60% male) displayed a wide range of macroscopic chondropathy scores (median 91, full range 9 to 362). Mild fissures to the articular cartilage surface were a feature of over 90% of cases, at least mild proteoglycan loss and meniscal pathology were found in 85% and 80% of cases, respectively. Alterations in chondrocyte morphology and breaching of the tidemark by vessels from the subchondral bone were evident in just over half of the cases (58% and 55%, respectively). Just over a quarter of the cases had osteophytes (29%). The majority of cases had a normal synovium. Mild and moderate synovitis was a feature of 13% and 2% of cases, respectively. Subchondral bone marrow replacement by fibrovascular tissue was observed in 7% of PM cases. Histopathological scores for cartilage surface integrity, chondrocyte morphology, tidemark breaching, menisci degradation and synovitis were significantly correlated with macroscopic chondropathy score ($P < 0.05$).
Conclusions: Mild clefts to the articular surface, mild proteoglycan loss and meniscal changes were most commonly observed in the cases, suggesting these may be either normal or early features of the disease process. Tidemark breaching was found in half of the cases, suggesting this may also be a normal or relatively early feature. Subchondral bone marrow changes and synovitis were observed less frequently, suggesting they occur later in the disease process. Identifying the earliest pathological changes in OA pathogenesis is crucial for the development of targeted treatments to slow down, or even reverse the disease process.

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SERUM SCLEROSTIN IS HIGHER IN MEN WITH SEVERE OSTEOPHYTES AT THE SPINE - THE MINOS STUDY

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Purpose: Sclerostin is a negative regulator of bone formation that might be involved in osteoarthritis (OA) pathophysiology. Therefore, our aim was to analyze the association between spine OA severity and serum sclerostin concentration in older men.
Methods: In this cross-sectional analysis of 694 men aged 50-85 years, spine osteoarthritis was assessed at 6 intervertebral spaces using Lane's score (Lane, J Rheumatol, 1993). Total score of osteophytes was calculated as the sum of osteophyte scores for each intervertebral level. Total scores of disc space narrowing and of subchondral sclerosis were calculated similarly. Sclerostin level was measured in fasting serum using ELISA assay (TECOsclerostin EIA kit, TECOmedical). Bone mineral density (BMD) at the total hip was measured by dual energy X-ray absorptiometry using the Discovery A HOLOGIC device.
Results: After adjustment for age, weight, serum 17β-estradiol concentration and glomerular filtration rate, serum sclerostin level increased across the quartiles of total osteophyte score (p for trend <0.001). Sclerostin level was 15% (0.42SD, $p<0.001$) higher in the highest quartile of total osteophyte score (>12) compared with the lowest quartile (<6). After similar adjustments, the analysis was performed in four groups defined by the most severe grade of osteophytes (no or mild, moderate, severe). Average sclerostin level increased with the increasing osteophyte grade (p for trend <0.001). It was 13% (0.37SD, $p<0.005$) higher in the 471 men who had at least one intervertebral level with severe osteophytes compared with the 109 men who had no or mild osteophytes. The associations between osteophyte severity (quartiles, grades) and sclerostin level remained significant ($p<0.05$ to <0.01) after additional adjustment for total hip BMD, disc space narrowing score and subchondral sclerosis score. After adjustment for confounders, serum sclerostin increased across the quartiles of total disc space narrowing score (p for trend <0.001). It was 12% (0.38 SD, $p<0.001$) higher in the highest quartile vs the lowest quartile. After similar adjustment, the analysis was performed in four groups defined by the most severe grade of disc space narrowing. Average serum sclerostin increased with the increasing grade of disc space narrowing (p for trend <0.005). However, all the associations between disc space narrowing (quartiles, grades) and sclerostin levels lost significance ($p>0.23$) after additional adjustment for total hip BMD, osteophyte severity and subchondral sclerosis score.
There was a non-significant trend to higher sclerostin levels in men with subchondral sclerosis at >1 intervertebral levels in comparison with other men (5%, 0.18SD, $p = 0.053$). This trend became non-significant after additional adjustment for total hip BMD, osteophyte severity and disc space narrowing score ($p = 0.88$).
Conclusions: In older men severe osteophytes - but not severe disc space narrowing or subchondral sclerosis - were independently associated with higher sclerostin concentration.

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DEVELOPMENT OF OSTEOARTHRITIS-LIKE CHANGES IN TRANSGENIC ENDOTHELIN-1 OVEREXPRESSED MICE

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Purpose: Vascular pathology has been implicated in the pathogenesis of OA. Endothelin-1 (ET-1), a potent vasoconstrictor, is known to promote matrix metalloproteinase 13 (MMP13) by chondrocytes in vitro. Use of Endothelin type A receptor antagonist could attenuate the severity of OA in a rat model with anterior cruciate ligament transection. Taken together, we hypothesized that the overexpression of endothelial ET-1 could lead to the development of OA. The objective of this study was to evaluate the phenotypes of articular cartilage and subchondral bone in a transgenic mice, in which ET-1 was purposely over-expressed in endothelium using tie-1 promoter.
Methods: Male heterozygous TET-1 mice were generated by microinjection of the ET-1 construct, which contained the mouse ET-1 cDNA with SV40 polyA driven by the Tie-1 promoter. Recently, we established a transgenic mouse model over-expressing ET-1 (TET-1 mice) in endothelial cells, which developed systemic hypertension with altered vascular reactivity since 8 weeks after birth. Tibiae of male, heterozygous TET-1 mice ($n = 5$) and their non-transgenic littermates ($n = 4$) of 35-weeks-old were obtained. PCR were used to confirm their genotypes. Micro-CT scan on tibiae were performed before tissue processed to wax blocks. Tibiae in wax blocks were sectioned and histology was studied on 5μm-thick wax sections.
Results: Micro-CT data showed a decrease of trabecular bone density (bone volume/tissue volume, BV/TV, 10.9±0.4%) in TET-1 mice primary spongiosa when compared to the age- and gender-matched littermates (12.8±0.5%, $p<0.05$). It was revealed histologically that articular chondrocytes underwent hypertrophic changes together with thickening of calcified cartilage in TET-1 mice as compared to their littermate.
Conclusions: TET-1 mice presented OA-like changes, It strongly suggested that ET-1 plays a pivotal role in the development of OA. Yet individual variations were observed probably due to the heterogenic nature of the genotypes. More samples and the use of homozygous TET-1 mice should be included to confirm these findings.

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IDENTIFICATION OF CONCENTRIC LAMELLAE IN THE ARTICULAR CALCIFIED CARTILAGE OF OSTEOARTHRITIC MICE

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Purpose: The structure, ultrastructure and function of hyaline articular cartilage (HAC) and subchondral bone (SCB) have been the subject of much investigation and their potential involvement in the pathogenesis of osteoarthritis (OA) has been widely recognised. However much less