The validity of in vitro ultrasonographic grading of osteoarthritic femoral condylar cartilage – a comparison with histologic grading


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Summary

Objective: To establish an ultrasonographic grading for semi-quantitative evaluation of the femoral condylar cartilage of knee osteoarthritis (OA), in vitro, and compare the ultrasonographic grading with the histologic grading.

Design: Thirty-four patients going to receive total knee arthroplasty because of OA of the knee were recruited. Specimens of the distal medial and lateral femoral condyles were taken during the operation. The anterior and middle areas of the articular cartilage in each specimen were graded by in vitro ultrasonography (US) examination and histologic evaluation. The correlation between the US and histologic findings was analyzed.

Results: Sixty-seven specimens were collected. Both US and histologic changes were graded from I to IV. The correlation between the grading of US and histology was good in both anterior and middle areas (Rho = 0.78, 0.89, P < 0.001). The correlation between US grading over anterior area and histology grading over middle area was moderate (Rho = 0.70, P < 0.001).

Conclusions: The moderate correlation between in vitro US and histology might permit quantitative in vivo assessment of cartilage.

Key words: Osteoarthritis, Articular cartilage, Ultrasonography, Histology.

Introduction

Osteoarthritis (OA) is a severe musculoskeletal disease, commonly diagnosed in elderly people. OA of the knee is particularly prevalent and people suffer due to pain, stiffness, and muscle weakness. If conservative symptomatic treatment cannot relieve the pain, patients have to receive surgical intervention such as total joint arthroplasty. However, surgical implants are not a satisfactory procedure because implants have a limited life span and limited tolerance for heavy loading and vigorous activity. OA is a progressive degradation of the articular cartilage. Relative to the collagen in the matrix, the earliest change in the OA cartilage is diminution of mucopolysaccharide chondroitin sulphate. This results in depletion of the ground substance and unmasking of the collagen. The injury of the collagen network is thought to represent the “point of no return” in the process of OA progression. There are several methods suggested for treating early knee OA, such as intra-articular injection of hyaluronic acid and taking glucosamine, etc. Therefore, early detection of the pathologic changes in cartilage is essential in order to treat the disease successfully.

Current modalities for knee OA diagnosis include non-invasive techniques, such as plain film, computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography (US), and invasive techniques, such as arthrography and arthroscopy. Plain film demonstrates osseous abnormalities but is insensitive for detecting lesions of soft tissue such as articular cartilage. The CT images can be obtained in the transverse plane, which is perpendicular to the direction of weight bearing and in which cartilage over the weight bearing area cannot be visualized. Although the image reconstruction in the sagittal and coronal planes is available, the resultant image quality is inferior to those obtained by a direct imaging technique. Arthrography and arthroscopy are not ideal screening tests.
because they need either a fluoroscopy suite or an operation room and both of them are invasive procedures\textsuperscript{12}. Besides, arthroscopy reveals only macroscopic changes over the surface of degenerated cartilage. MRI is a powerful tool for thorough evaluation of the articular cartilage. However, there are several technical and economic limitations for routine use in assessing osteoarthritic cartilage\textsuperscript{11,13}. Nuclear medicine study has also been used for early detection of OA, but the patient is exposed to radiation during the examination\textsuperscript{14}.

US is a quick, inexpensive and non-invasive method for articular cartilage assessment\textsuperscript{15}. It plays an important role in imaging the musculoskeletal system to detect the minimal change of soft tissue\textsuperscript{16,17}. Several studies had used US to assess the thickness and surface characteristics of articular cartilage\textsuperscript{9,18-20}. Although US is a skill-dependent method, Disler et al.\textsuperscript{21} showed that US was accurate and reliable for detection and grading of knee articular cartilage defects. W. Joseph McCune only graded the clarity and sharpness of the femoral condylar cartilage individually and made a correlation with gross operative findings\textsuperscript{22}. It still lacked a US grading of OA cartilage using all the US characteristics including homogeneity of cartilage, sharpness of superficial and deep margin and narrowing of cartilage band. Further its correlation with histologic change is also lacking. The aim of this study was to establish a semi-quantitative method to evaluate the femoral condylar cartilage by in vitro US grading, and compare with its histologic grading.

Material and method

SUBJECTS

Thirty-four patients who had a diagnosis of knee OA and were going to receive total knee arthroplasty were recruited. All of them were classified equal to or greater than Ahlba¨ck stage III\textsuperscript{23} and most of the lateral compartments of the femoral condyle were less severe than medial compartments.

SPECIMEN

During the knee arthroplasty operations, distal femoral condyles were excised with an oscillating saw. The distal medial and lateral condyle specimens were chosen and rinsed in saline.

The degeneration of the cartilage is usually inhomogeneous. It is difficult to select an area to represent the whole changes. We selected the areas for examination just according to the location. Two transverse areas on each specimen, including anterior and middle areas, were chosen (Fig. 1). The middle area of the specimen was located over the middle part of the specimen and represented the weight bearing portion of the femoral condyle. The anterior area of the specimen was located over the middle of the anterior part of the specimen and represented the sub-weight bearing portion. Both these areas were marked with gentian violet and evaluated by US and histologic examinations.

HISTOLOGIC EXAMINATION

After US examination, tissue specimens were rinsed and fixed with 4% paraformaldehyde for 24 h and decalcified in a decalifier for a few days. Then the specimens were processed for embedding in paraffin wax. Standardized 4 µm sections of the specimen were prepared from anterior and middle areas. The sections were stained with haematoxylin and eosin (HE). The characteristics of histologic changes in osteoarthritic cartilage under light microscope are described in Table 4\textsuperscript{4,25}. Two pathologists, who were blinded to US results, evaluated the histologic changes in the articular cartilage under light microscope. They coded a histologic grading according to these histologic characteristics\textsuperscript{5} (Fig. 3).
Spearman’s correlation was performed to determine the correlation between *in vitro* US grading and histologic grading of articular cartilage. A significant correlation was defined as $P$ value less than 0.05. A Rho score of less than 0.40 indicated poor agreement; that of 0.40–0.75, moderate agreement; and that of 0.75–1.00, good agreement.

**Results**

Thirty-four patients were recruited in this study. Each arthritic knee offered two specimens. One specimen was excluded due to poor decalcification. Sixty-seven specimens of femoral condylar cartilage were used for analysis.

The distribution of US grading and histologic grading over various locations is listed in Table II. The cartilage degeneration was less severe in lateral femoral condyles than in medial parts (Table II). The grades of overall specimens, including medial and lateral, ranged from grades 1 to 4.

The distribution of US and histologic grading over the anterior area is shown in Table III, and the distribution of US and histologic grading over the middle area is shown in Table IV.

**Discussion**

The US feature of normal articular cartilage is a homogeneous anechoic band with a sharp superficial cartilage profile (superficial margin) and subchondral bone profile (deep margin) which is due to the water content of cartilage being high and the cartilage surface being flat and smooth. The earlier US features of OA are loss of clarity of the cartilage.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Finding on light microscope</th>
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<tbody>
<tr>
<td>Flaking</td>
<td>Indentation of cartilage capsule</td>
</tr>
<tr>
<td>Fibrillation</td>
<td>Superficial breading of cartilage</td>
</tr>
<tr>
<td>Chondrocyte enlargement</td>
<td>Chondrocyte cytoplasm filled with hyaline-like substance in Zones II–IV</td>
</tr>
<tr>
<td>Hyalinization</td>
<td>Chondrocyte loss in Zones II–III</td>
</tr>
<tr>
<td>Pitting</td>
<td>Irregular surface of cartilage</td>
</tr>
<tr>
<td>Cartilage loss</td>
<td>Decreased thickness of cartilage</td>
</tr>
</tbody>
</table>

*Zone I: superficial zone; Zone II: mid zone; Zone III: deep zone; Zone IV: calcified cartilage.*
band and loss of sharpness of the margin. The loss of sharpness of the interface is due to scattering of sound by a rough surface. The increased echogenicity may represent structural alteration such as fibrillation of cartilage and cleft formation. In the cases with more severe OA, the thickness of cartilage band becomes narrowed and may have disappeared. However, the loss of sharpness of the interface made placement of markers for thickness measurement difficult.

Disler et al. evaluated porcine and human knee specimens. Defects on the porcine knee cartilage were created for surgical grades. The human knee specimens were retrieved from patients who underwent total knee arthroplasty. Both these kinds of specimens were graded using surface observation (surgical grades) and US examination (US grades). Interobserver agreement of US grade was found to be excellent. The concordance of US and surgical grades was also excellent. The study showed that US was reliable for the detection of defects regardless of them being artificial or pathologic. However, the US grades used in this study were only determined by the depth of defects in morphological change.

McCune et al. used the US features of femoral condylar cartilage, including thickness, clarity and sharpness to evaluate the OA change. They compared the individual US features with operative gross findings. They found the clarity and sharpness of the cartilage images correlated significantly with gross findings of the specimen. They also found that evaluation of US images obtained in the transverse plane showed the best correlation with gross pathologic findings. However, they did not evaluate the specimens by histologic changes which could reveal early changes of OA cartilage.

In the present study, we included all US changes of articular cartilage in the grading system. Further, the histologic changes evaluated by pathologists were used for comparisons which were more related with the deterioration of the arthritic cartilage. We found that the US grades correlated

<table>
<thead>
<tr>
<th>Grade</th>
<th>Anterior area</th>
<th>US</th>
<th>Middle area</th>
<th>Histology</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>L</td>
<td>Total</td>
<td>M</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>24</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>6</td>
<td>13</td>
<td>4</td>
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<tr>
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<td>13</td>
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<td>15</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1</td>
<td>11</td>
<td>15</td>
</tr>
</tbody>
</table>

M: specimen of medial femoral condyle; L: specimen of lateral femoral condyle.
well with histologic grades (Rho = 0.78, 0.89, both P < 0.001). The correlation of US findings and histologic changes demonstrated that well controlled ultrasonography might be a valid tool for evaluation of arthritic cartilage changes in OA.

The weight bearing portion of the femoral condylar cartilage was supposed to change first in a case of knee OA. This was the area we were interested in and had to evaluate. The weight bearing portion of femoral condylar cartilage could be well evaluated in patients who could flex their knees adequately\textsuperscript{11,18,22}. However, limited range of knee joint motion which made weight bearing portion visualization difficult was quite common in patients with knee OA. Thus, we chose an anterior area of our specimen to represent the anterior sub-weight bearing portion of the femoral condyle where visualization was much easier, \textit{in vivo}. There was moderate correlation between US grading over anterior area and histologic grading over middle area (Rho = 0.70, P > 0.001). This implied that the US findings from the sub-weight bearing region might be reliable for the evaluation of the arthritic cartilage changes of the weight bearing region in clinical practice. However, several limitations would be met when we evaluated the femoral condylar cartilage, \textit{in vivo}. The US beam would be interfered with by the osseous portion of the patella\textsuperscript{12}. Moreover, visualization of the interface of synovial space–cartilage might be impaired in patients with synovitis\textsuperscript{12}. Further study on \textit{in vivo} ultrasonographic examination of femoral condylar cartilage is warranted.

In conclusion, this preliminary work revealed good correlation of \textit{in vitro} US grading and histologic grading of femoral condylar cartilage. A moderate correlation was found between US grading over sub-weight bearing areas and histologic grading over weight bearing areas.

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References


