Serum keratan sulfate is a promising marker of early articular cartilage breakdown

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Objectives. To find serum markers that may serve as indices for an early diagnosis of degeneration or damage of the articular cartilage.

Methods. Twenty-four healthy volunteers, 19 individuals with knee trauma (KT) and 31 with knee osteoarthritis (OA) were evaluated. KT patients were divided into a group (n=5) with an injury <2 months old (recent KT) and a group (n=14) with that >2 months old (old KT). Articular cartilage damage was assessed using either arthroscopy or direct observation. Serum concentrations of hyaluronic acid (HA), cartilage proteoglycan aggrecan turnover epitope (CS846) and cartilage oligomeric protein (COMP) were measured using enzyme-linked immunosorbent assay kits and those of keratan sulfate (KS) and chondroitin-6-sulfate (C6S) using high-performance liquid chromatography.

Results. Serum KS in the recent KT group (2095 ± 594 ng/ml) was significantly higher than that in the old KT group (1373 ± 418 ng/ml; P=0.021), and serum COMP in the recent KT group (1572 ± 182 ng/ml) showed a tendency that was higher than that in the old KT group (1350 ± 250 ng/ml; P=0.079).

Serum KS in OA patients with Kellgren and Lawrence (KL) grades 0 and I (1456 ± 334 ng/ml) showed a tendency that was higher than that in OA patients with KL grades II, III and IV (1248 ± 220 ng/ml; P=0.084).

Conclusions. The serum concentration of KS correlated with the damage of the articular cartilage and it was significantly increased even at an early stage after the injury.

KEY WORDS: Keratan sulfate, Glycosaminoglycan, Cartilage oligomeric protein, Cartilage injury, Osteoarthritis, Serum marker.

Introduction

The prevalence of patients with articular cartilage defects among patients with symptomatic knees requiring arthroscopy has been reported as 5-20% [1–3]; when left untreated, osteoarthritic changes are observed on X-rays taken after 10–20 yrs [4, 5]. Thus, articular cartilage injury is considered a cause of osteoarthritis (OA). Even if there is no articular cartilage injury, degeneration of the articular cartilage is considered to begin in humans at a young age, and articular cartilage changes, such as changes in colour and fibrillation, can occur. Injury or early-stage alterations of the articular cartilage in OA cannot be detected using X-ray examination. Magnetic resonance imaging (MRI) can detect articular cartilage defects and cartilaginous quality changes to some extent, but this technique is not sensitive enough to detect early OA changes and is expensive to be used as a routine examination. Serum markers, on the other hand, are suitable as screening tests, and only patients with high values of serum markers should be subjected to MRI or arthroscopy to detect articular cartilage degeneration. If it were possible to detect OA or articular cartilage damage at an early stage, patients could be educated to prevent the progression of OA. Moreover, it would be useful to monitor the natural course of articular cartilage damage or repair after, for instance, autologous chondrocytes implantation, whose effectiveness is still controversial because there is no method to effectively evaluate cartilage repair.

In 1985, Thonar et al. [6] measured serum keratan sulfate (KS) using an enzyme-linked immunosorbent assay (ELISA) by anti-KS antibody (1/20/5-D4), and suggested its usefulness as a marker of OA. However, the correlation was weak and it did not correlate with X-ray grading [7]. Many researchers have tried to detect the metabolic products of articular cartilage components (proteoglycan, type II collagen and non-collagenous proteins) in joint fluid or blood and thereby a marker of OA [8–11]. As reported by Okumura et al. [12], early OA articular cartilage destruction begins with a loss of glycosaminoglycans (GAGs) from articular cartilage surfaces, followed by collagenolysis. Thus, the first event in OA or articular cartilage damage is the release of GAGs, which play an important role in maintaining articular cartilage function. Consequently, early markers of articular cartilage damage or OA change might be among GAG metabolic products. We selected KS, chondroitin 6-sulfate (C6S), cartilage proteoglycan aggrecan turnover epitope (CS846) and hyaluronan (HA) as candidate markers, and cartilage oligomeric protein (COMP), which is not a component of GAGs but has been reported as a marker of OA [9]. These components have been reported to correlate with OA, to some extent, but not with cartilage damage caused by degradation and/or injury. These metabolic products can be measured in joint fluid, serum and urine, but we measured them in serum because it is easy to collect.

We measured KS using high-performance liquid chromatography (HPLC), which has been reported to be more accurate than ELISA [13]; C6S using HPLC, and CS846, HA and COMP using ELISA. We measured these markers in healthy volunteers and in patients with knee trauma (KT) or OA, who were subjected to knee surgery and whose articular cartilage was optically assessed (by arthroscopy or direct observation). We examined the correlation of these markers with the articular cartilage assessment to evaluate their usefulness as markers of early articular cartilage breakdown caused by degeneration and/or injury but that showed no change by X-ray examination.

Patients and methods

This study was approved by the institutional Review Board of Marunouchi Hospital and was conducted in accordance with the Helsinki Declaration of 1975, revised in 1983. Written informed consent was obtained from the healthy volunteers and patients prior to their participation in the study.
Blood collection from healthy volunteers

Ten men and 14 women (23–52 yrs old) volunteered to participate in the study. The volunteers were healthy with no gross obesity, inferior limb malalignment, history of knee injury or knee disorders. Sera were collected and stored at −80°C.

Patients with KT or knee OA

Nineteen KT patients (11 men and 8 women; 20–54 yrs old) and 31 patients with knee OA (11 men and 20 women; 40–80 yrs old) who were diagnosed to undergo knee surgery participated in the study. X-rays of knee and lumbar spine were available for all the patients. Sera samples were collected before surgery and stored at −80°C. The condition of the knee articular cartilage was observed at the time of surgery either arthroscopically or by direct observation. Among the 19 KT patients, two had meniscal injuries, 12 had ligament injuries and five had both meniscal and ligament injuries. KT patients were divided into a group of 5 patients with injuries <2 months old (recent KT) and a group of 14 patients with injuries >2 months old (old KT). Among the 31 OA patients, eight underwent total knee replacement, one underwent a high tibial osteotomy and 22 underwent arthroscopic debridement.

Assessment of articular cartilage surfaces by X-ray and visual inspection

X-ray images were assessed using the Kellgren and Lawrence (KL) grading scale [14]. All the KT patients were KL grade 0. Seven of the OA patients were KL grade 0, seven were KL grade 1, four were KL grade II, six were KL grade III and seven were KL grade IV. Articular cartilage damage was assessed using the Société Francaise d’Arthroscopie (SFA) scaling system [15]. In brief, the degree of articular cartilage damage was estimated at the time of surgery either arthroscopically or by direct observation. The width of the damaged area was evaluated as a percentage of the damaged area in the medial and medial femoro-tibial and patello-femoral areas, separately. The SFA score was then calculated using a coefficient. The SFA score represents not only the degree of articular cartilage surface damage, but also the width of the damaged area.

Determination of the serum markers

Keratan sulfate was determined by HPLC after digestion with keratanase II (Seikagaku Corporation, Tokyo, Japan) according to the method of Tomatsu et al. [13] Each serum sample (0.2 ml) was treated with a protease (actinase E: Kaken Pharmaceutical Co. Ltd., Tokyo) and the negatively charged substance containing KS was fractionated by Q sepharose and digested by keratanase II. The KS-derived β-galactosyl-1(4)-6-O-sulfo-N-acetylglosamine (m-ks) and β-6-O-sulfo-galactosyl-(1-4)-6-O-sulfo-N-acetylglosamine (d-ks) were contained in the solution that was digested by the enzyme and were measured using HPLC. Standard KS derived from bovine cornea (Seikagaku) was used to measure KS under identical conditions; and the quantity of KS in each serum sample was calculated as the sum of m-ks and d-ks. To determine C6S concentration, 0.2 ml of each serum sample was first treated with chondroitinase ABC (Seikagaku). The quantity of unsaturated disaccharide contained in the digested fluid was determined and C6S was detected by HPLC [16]. For the determination of CS846, COMP and HA, the Aggrecan Chondroitin Sulfate 846 Epitope ELISA Kit (IBEX Technologies, Inc., Montreal, Quebec, Canada), Human COMP ELISA Kit (Kamiya Biomedical Company, Seattle, WA, USA) and Hylauronan Assay Kit (Seikagaku Corporation) were used respectively.

Statistical analysis

To determine the statistical significance of inter-group differences, Steel’s multiple comparison test for patient group vs control and Wilcoxon rank-sum test for inter-patient group were conducted, and the P-level was set at <0.05.

Results

Arthroscopic findings in KT patients and serum concentrations of KS, C6S, CS846, HA and COMP

All KT patients had articular cartilage damage. Their cartilaginous damage scores (SFA) for the recent KT group and the old KT group were 1.2 ± 0.7 and 3.8 ± 3.9, respectively. On X-ray examination, no changes were noted in the knee or intervertebral joints (Fig. 1).

The serum concentrations of KS, C6S, CS846, HA and COMP in KT patients are shown in Table 1. KS, C6S, CS846 and COMP were significantly higher in the recent KT group (P = 0.001, P = 0.047, P = 0.022 and P = 0.001, respectively), and KS and COMP higher in the old KT group (both P < 0.001) than in controls.

X-ray and arthroscopic examination of OA patients and serum concentrations of KS, C6S, CS846, HA and COMP

The SFA scores of OA patients distributed by their KL grade are presented in Table 2. The SFA score increased in relation with the OA patients were KL grade 0, seven were KL grade I, four were KL grade II, six were KL grade III and seven were KL grade IV. Articular cartilage damage was assessed using the Société Francaise d’Arthroscopie (SFA) grading system [15]. In brief, the degree of articular cartilage damage was estimated from 0 to IV according to the SFA grading scale. The width of the damaged area was evaluated as a percentage of the damaged area in the medial and medial femoro-tibial and patello-femoral areas, separately. The SFA score was then calculated using a coefficient. The SFA score represents not only the degree of articular cartilage surface damage, but also the width of the damaged area.

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X-ray and arthroscopic examination of OA patients and serum concentrations of KS, C6S, CS846, HA and COMP

The SFA scores of OA patients distributed by their KL grade are presented in Table 2. The SFA score increased in relation with...
increased KL grade. Even in patients with KL grade 0 OA, degeneration or damage of the articular cartilage surface was observed by direct optical methods. In such patients, no X-ray findings were detected in the knee nor in the intervertebral discs (Fig. 2).

Serum concentrations of KS were significantly higher in most OA stages (KL grade 0: P=0.004, I: P<0.001, III: P=0.004 and IV: P=0.008) and serum COMP were significantly higher in all OA stages (KL grade 0: P=0.004, I: P=0.002, II: P=0.008, III: P=0.002 and IV: P<0.001) than in controls. C6S and HA

**Comparison of the serum markers between patient groups**

Since the serum concentrations of KS and COMP were higher in most stages of KT and OA than in controls, those differences between stages were compared in Fig. 3. The serum KS in the recent KT group (2095±594 ng/ml) was significantly higher than that in the old KT group (1373±418 ng/ml; P=0.021) and those in OA patients with KL grades 0 and I (1456±334 ng/ml) showed a tendency that was higher than that in patients with KL grades II, III and IV (1248±220 ng/ml; P=0.084). The serum concentrations of COMP in the recent KT group (1572±182 ng/ml) showed a tendency that was higher than that in the old KT group (1350±250 ng/ml; P=0.079), but those in OA patients showed no difference between the patient group with KL grades 0 and I (1639±434 ng/ml) and the patient group with KL grades II, III and IV (1731±355 ng/ml).

**Discussion**

This study showed that the serum concentration of KS was high in patients with early-stage damage of the articular cartilage undetectable by X-ray imaging. Serum KS may be suitable as a screening test for articular cartilage damage and to monitor the natural course of articular damage or repair.

In the KT patients with recent injuries, KS was significantly higher than those with old injuries, suggesting that serum

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**Table 1. Serum concentration of markers of cartilage degeneration or damage in patients with knee trauma**

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th>KT patients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Recent trauma</td>
<td>Old trauma</td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>24</td>
<td>5</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>KS (ng/ml)</td>
<td>910±145</td>
<td>1.2±0.7</td>
<td>3.8±3.9</td>
<td></td>
</tr>
<tr>
<td>C6S (ng/ml)</td>
<td>97±28</td>
<td>122±10</td>
<td>104±22</td>
<td></td>
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<tr>
<td>CS846 (ng/ml)</td>
<td>137±24</td>
<td>214±77</td>
<td>142±46</td>
<td></td>
</tr>
<tr>
<td>COMP (ng/ml)</td>
<td>1030±150</td>
<td>1572±182</td>
<td>1350±250</td>
<td></td>
</tr>
<tr>
<td>HA (ng/ml)</td>
<td>41±15</td>
<td>44±19</td>
<td>39±12</td>
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</tr>
</tbody>
</table>

The values are the mean±s.d.

*Patients evaluated within 2 months after the injury.

**Table 2. Serum concentration of markers of cartilage degeneration or damage in OA patients**

<table>
<thead>
<tr>
<th>X-ray grade</th>
<th>Healthy subjects</th>
<th>OA patients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>7</td>
<td>4</td>
<td>6</td>
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<tr>
<td>SFA</td>
<td>2.4±2.1</td>
<td>5.1±3.5</td>
<td>28.8±47.5</td>
<td>7&gt;100</td>
</tr>
<tr>
<td>KS (ng/ml)</td>
<td>910±145</td>
<td>1501±360</td>
<td>1411±326</td>
<td>1253±241</td>
</tr>
<tr>
<td>C6S (ng/ml)</td>
<td>97±28</td>
<td>116±18</td>
<td>115±15</td>
<td>102±22</td>
</tr>
<tr>
<td>CS846 (ng/ml)</td>
<td>137±24</td>
<td>147±67</td>
<td>151±108</td>
<td>140±70</td>
</tr>
<tr>
<td>COMP (ng/ml)</td>
<td>1030±150</td>
<td>1710±550</td>
<td>1570±310</td>
<td>1580±200</td>
</tr>
<tr>
<td>HA (ng/ml)</td>
<td>41±15</td>
<td>80±65</td>
<td>72±21</td>
<td>76±68</td>
</tr>
</tbody>
</table>

The patients were grouped by their X-ray grade.

The values are the mean±s.d.

*Patients evaluated within 2 months after the injury.

SFA, Société Française d’Arthroscopie score; KS, keratan sulfate; C6S, Chondroitin-6-sulfate; CS846, cartilage proteoglycan aggrecan turnover epitope; COMP, cartilage oligomeric protein and HA, hyaluronic acid.
KS might indicate the release of cartilaginous GAG in the early stage after injury in spite of moderate cartilage damage. In OA patients, the serum concentrations of KS, C6S, HA and COMP were significantly higher than in healthy controls as reported previously [6, 17–19]. Among these parameters, KS was high in patients with KL grades 0 and I, indicating that KS might serve as a marker of early-stage OA. The KS concentrations in OA patients tended to decrease as the KL grade increased from 0 to IV, which reflects disappearance of the joint space. It may mean that in OA, a greater quantity of the cartilage matrix is released when the joint space has not yet narrowed. On the contrary, COMP was high in KL grade IV. This can be explained by the fact that COMP is a non-collagen protein that exists in the synovial membrane, meniscus and tendon, as well as in the cartilage and its increase is most likely related to the inflammation of various intra-articular tissues. The changes in C6S and HA were marked in KL grade IV, indicating that these are not markers of early-stage cartilage destruction.

KS is a component of proteoglycans found in the articular cartilage, intervertebral discs and corneas. Because corneas are relatively small tissues, serum KS mainly originates from articular cartilage and intervertebral discs. Thus, the serum concentration of KS is not only a marker of knee articular cartilage, but also of other joints and intervertebral discs. Therefore, before concluding that the elevated serum concentration of KS originated from the knee joint articular cartilage, the possibility of spondyloarthropathy and OA in other joints must be examined. We verified that there were no X-ray changes in the lumbar spine nor symptoms caused by lumbar spinal abnormalities in KT patients (Fig. 1), although spondyloarthrotic changes existed in OA patients because most of these patients were of advanced age (Fig. 2). We verified that no OA symptoms were observed in joints other than the knee in these patients. We are planning to investigate serum KS in patients with spondyloarthropathy or intervertebral disk herniation in the future.

KS is considered to reflect the normal metabolism of cartilage, and KS increases in case of mechanical injuries within a few months after injury. Budsberg et al. [20] found that serum KS increased 1–3 months after resection of the anterior cruciate ligaments of dog knees. In our report, KT patients who were evaluated within 2 months after the injury exhibited an acute release of KS. Although the SFA score of KT patients was very small, indicating that damage was confined, serum KS was high (Table 1). After the rapid release of KS ends, release from the injured surfaces continues at a relatively high rate. This phase is considered to continue for a few years to a couple of decades as in KT evaluated >2 months after the injury and in early-stage OA patients (KL grades 0 and I). The persistence of this condition leads to OA in a few decades. This phase corresponds to advanced OA (KL grades II, III, IV).

This report is the first study to show that serum KS increases early after an injury causing small articular damage and in patients with early-stage OA undetectable by X-ray imaging. As only a small volume of blood is required for the measurement of serum KS, this parameter may serve as a screening test to detect articular cartilage injury and it is expected to contribute greatly to the decision on a therapeutic strategy for the management of OA or cartilage injury.

**Rheumatology key messages**

- Serum keratan sulfate correlates with damage of the articular cartilage.
- It may serve as a screening and monitoring test of the natural course of articular cartilage damage or repair.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**