Chapter 7

Inflammation is more distinct in osteoarthritis of the temporomandibular joint compared to the knee joint

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Abstract

Objective Most of the current understanding of articular cartilage maintenance and degradation is derived from large, load bearing synovial joints, in particular the knee joint. The aim of this study was to identify valuable degradation markers for cartilage degradation in the temporomandibular joint (TMJ) by comparing the relative concentrations of Carboxyterminal telopeptides of collagen type I, and II (CTX-I and II) cartilage oligomeric matrix protein (COMP) and prostaglandin E₂ (PGE₂) in synovial fluid (SF) of TMJ and knee joints with cartilage degradation.

Methods In this cross-sectional comparative study, participants were recruited from the University Medical Center Groningen (UMCG), the Netherlands. Patients with TMJ osteoarthritis were compared to patients with knee osteoarthritis. The outcome variables were the relative SF concentrations of CTX-I, CTX-II, COMP and PGE₂. An independent samples Mann-Whitney U-test was used to compare the relative concentrations.

Results 30 consecutive patients (9 males, 21 females, mean age 40.1, SD 15.3) with TMJ osteoarthritis, and 31 consecutive patients (20 males, 11 females, mean age 37.4, SD 13.7) who were scheduled for arthroscopy of the knee joint participated in this study. Significant differences were found between relative concentrations of COMP (p = 0.000) and PGE₂ (p = 0.005), and no significant differences between relative concentrations of CTX-I (p = 0.720) and CTX-II (p = 0.242).

Conclusions Relative SF concentrations of COMP and PGE₂ showed significant differences between the TMJ and the knee joint, suggesting that there are differences in pathophysiology and that the inflammatory component may be more distinct in the TMJ.
Introduction

Most of the current understanding of articular cartilage maintenance and degradation is derived from large, load bearing synovial joints, in particular the knee joint. Like the knee joint, the temporomandibular joint (TMJ) is a complex synovial joint with an intra-articular disc and comparable mechanical features. Furthermore, the cartilage degenerative processes are believed to develop similarly.

An important difference between these joints is the type of cartilage that forms the articular lining. In the knee joint, articular surfaces are covered with hyaline cartilage, whereas in the TMJ this lining consists of fibrocartilage. In both joints, the cartilage lining consists mainly of collagen. In the TMJ the fibrocartilage lining predominantly contains collagen type I, whereas hyaline cartilage in the knee joint mainly consists of collagen type II.

The serum level of cross-linked carboxy-terminal telopeptides of collagen type II (CTX-II) is believed to be a valid marker for destruction of hyaline cartilage. Although with regard to synovial fluid (SF) CTX-II, the importance of this marker is still ambiguous. However, because the lining of the articular surfaces of the TMJ is fibrocartilage, this marker may be less accurate for cartilage destruction within the TMJ. Cross-linked carboxy-terminal telopeptides of collagen type I (CTX-I) is a degradation product of collagen type I. Therefore, degradation of the fibrocartilage lining within the TMJ is likely to result in higher concentrations of CTX-I in the SF. On the other hand, in synovial joints with hyaline cartilage, CTX-I is considered an important marker for bone degeneration. Taken together, in the TMJ CTX-I may be an accurate marker for both fibrocartilage degeneration and bone destruction.

Serum cartilage oligomeric matrix protein (COMP) is considered a biomarker of cartilage degradation of hyaline as well as fibrocartilage, and a potential prognostic indicator of cartilage damage. Besides, COMP is found in the SF of affected joints. In small joints, such as the TMJ, SF COMP may be more accurate as a prognostic indicator than serum COMP. During synovial inflammation, prostaglandin E₂ (PGE₂), which is the best-known lipid mediator that contributes to inflammatory pain, is released by synoviocytes. It is thought to be useful for estimating the arthritic intensity, although in other studies this was not always confirmed.

Isolation of SF from the TMJ is cumbersome and requires injection of a physiologic buffer, which should be mixed with SF and then retracted. This procedure results in dilution of the SF sample and inaccurate determination of marker concentrations, and therefore incomparability with undiluted knee joint samples. In order to overcome this problem, assessed concentrations of CTX-I, CTX-II, COMP and PGE₂ were added and the sum was set as 100%. Thus calculated relative concentrations are independent of the dilution factor. The relative contribution of each marker was then determined within the SF samples obtained from TMJs and from knee joints.

Due to the different types of articular cartilage, it can be hypothesized that clinically relevant differences occur and may be detectable in the SF. The relative SF concentrations of CTX-I, CTX-II, COMP and PGE₂ in TMJs compared to knee joints have not been
determined before. Insight in differences and similarities between these two joints may be helpful in determining to what extent research outcomes obtained from one joint type can be applied to other joints.

The purpose of this study was to identify valuable degradation markers for cartilage degradation in the TMJ and gain insight in the differences and similarities between the TMJ and the knee joint. The investigators hypothesized that the contribution of COMP and PGE₂ would be comparable in both joints, that in the TMJ CTX-I would be increased and CTX-II decreased, whereas in the knee the opposite would occur. The specific aim of this study was to compare relative concentrations of CTX-I, CTX-II, COMP and PGE₂ in SF of patients with TMJ osteoarthritis with patients with knee joint osteoarthritis.

Materials and Methods

Study design

This cross-sectional comparative study was conducted at the University Medical Center Groningen (UMCG), the Netherlands, from June 2011 to June 2012. Patients with TMJ osteoarthritis were recruited from the department of Oral and Maxillofacial Surgery, and patients with knee joint arthralgia from the department of Orthopaedic Surgery. Prior to patient recruitment, the research protocol was approved by the ethical committee of the UMCG (METc 2010.131). All participants were informed according to the guidelines of the ethical committee, and provided signed informed consent. No studies of sufficient quality were found that compared SF concentrations of markers in the TMJ with markers in the knee. Based on findings of other markers in the TMJ and the assumed small differences between the TMJ and the knee joint, an effect size of 0.5 was considered clinically relevant. In order to detect an effect size of 0.5, with alpha being 0.05 and a power of 0.9, two groups of 30 patients were needed. To be included in the study, patients had to be 18 years of age or over. In the TMJ group patients had to be diagnosed with osteoarthritis (OA) according to the revised RDC/TMD (pain on palpation and during mouth opening and/or lateral excursions, with crepitation during mandibular movement). Additionally, for each patient an orthopantomogram (OPT), a transpharyngeal recording according to Parma, and a transcranial radiograph according to Schüller were recorded. In the knee joint group, patients had to have significant pain and function impairment of the affected joint, which was supported by visual cartilage destruction on CT or MRI. Patients were excluded as study subjects if they already had used NSAIDs (including cyclooxygenase 2 inhibitors) or corticosteroids less than four weeks before consultation because of their possible interference with one of the markers under study, if they suffered from systemic diseases like rheumatoid arthritis, if they had a history of surgical intervention of the respective joint or in case of pregnancy. Prior to inclusion, patients with TMJ OA received NSAID treatment (Ibuprofen 600mg three times daily) for two weeks. If thereafter the joint pain was still present, than the inclusion and exclusion criteria were applied. All included patients were scheduled for arthrocentesis or arthroscopy and no additional treatment was applied. NSAID treatment was stopped at least four weeks before arthrocentesis or arthroscopy.
was performed in order to minimize the effect of NSAIDs on PGE$_2$ concentrations.

**Variables**
The predictor variable was the joint type, i.e. TMJ or knee joint. The primary outcome variables were the relative concentrations of CTX-I, CTX-II, COMP and PGE$_2$ which were measured using commercially available enzyme-linked immunosorbent assays (ELISA) for CTX-I, CTX-II (Cusabio biotech co., ltd, Wuhan, China), COMP and PGE$_2$ (Abnova, Taipei City, Taiwan). These assays were performed in duplo according to the manufacturer’s instructions. Demographic variables included age and gender.

**Data collection**
In all patients only the affected joint was sampled. Consequently 30 SF samples of the TMJ and 31 of the knee joint were collected. Three different operators collected the samples prior to the arthrocentesis or arthroscopy procedure. SF was collected from the TMJ using intra-articular puncture of the superior joint compartment, which is part of the standard procedure prior to TMJ arthrocentesis or arthroscopy. To that end, after injection of approximately 0.2 ml intra-articular anaesthesia (Ultracain forte, Aventis Pharma, Hoevelaken, The Netherlands) into the joint, the joint cavity was filled with 2ml isotonic sodium chloride solution, and homogeneously mixed with the SF using a syringe with pumping motion. Thereafter, 2 ml of the mixture was aspirated and the syringe was removed from the joint. Patients undergoing arthrocentesis or arthroscopy of the knee joint received general or spinal anaesthesia. Prior to intervention and independent of the final surgical procedure, SF was obtained by intra articular puncture using dry arthroscopy to locate SF accumulations. Only when needed for aspiration, SF was minimally diluted with isotonic sodium chloride solution. After fluid collection, elimination of erythrocytes and large proteins was established by immediate centrifugation (25200g, 10min., 4°C). Thereafter, the samples were stored at -80°C. All samples were analysed simultaneously.

**Statistical analysis**
Relative SF concentrations of each marker were calculated by normalization (sum of the four markers within a SF sample = 100%). Hereby the dilution factor plays no role since the relative concentrations of the markers within a sample remain the same: with dilution, all markers within a sample change equally. Distribution of the relative concentrations of CTX-I, CTX-II, COMP and PGE$_2$ in TMJ SF was compared to the distribution of the relative concentrations of these markers in knee joints using an independent samples Mann-Whitney U-test (SPSS 18.0).

**Results**
30 consecutive patients, who were scheduled for arthrocentesis or arthroscopy of the TMJ, and 31 consecutive patients who were scheduled for arthrocentesis or arthroscopy of the knee joint met the inclusion criteria and participated in this study (table 1). There
was a significant difference with regard to gender between the two groups (P = 0.007). However, age and gender did not influence the relative concentrations significantly (P > 0.01) (table 2). Concentrations of CTX-I, CTX-II, COMP and PGE₂ were measured regardless the dilution of the samples. An example of the distribution of the relative concentrations is provided in figure 1. Detectable concentrations of CTX-I were found in four of the 30 TMJ samples, and in six of the 31 knee joint samples. CTX-II, COMP and PGE₂ were detectable in all samples. The relative concentration of each marker was determined for each sample. After normalization, median relative concentration and inter quartile range (IQR) were used to compare both groups. Significant differences were found between relative concentrations of COMP (p<0.0001) and PGE₂ (p=0.005), and no significant differences between relative concentrations of CTX-I (p=0.720) and CTX-II (p=0.242) (table 3).

Table 1. Patients’ characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TMJ</th>
<th>Knee joint</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>30</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Gender (female), n (%)</td>
<td>21 (70)</td>
<td>11 (34)</td>
<td>0.007</td>
</tr>
<tr>
<td>Age (years), mean (SD)</td>
<td>40.1 (15.3)</td>
<td>37.4 (13.7)</td>
<td>0.457</td>
</tr>
</tbody>
</table>

SD = standard deviation

Table 2. Correlations of patients’ characteristics and relative concentrations of CTX-I, CTX-II, COMP and PGE₂

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CTX-I</th>
<th>CTX-II</th>
<th>COMP</th>
<th>PGE₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>61</td>
<td>61</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>Gender, P value*</td>
<td>1.000</td>
<td>0.603</td>
<td>0.076</td>
<td>0.279</td>
</tr>
<tr>
<td>Age, Spearmen’s ρ (P value)</td>
<td>-0.211 (0.103)</td>
<td>-0.096 (0.460)</td>
<td>0.045 (0.733)</td>
<td>0.188 (0.146)</td>
</tr>
</tbody>
</table>

* independent samples Mann-Whitney U test

Figure 1. Distribution of the relative SF concentrations of CTX-I, CTX-II, COMP and PGE₂ in two individual patients. Relative concentrations of each marker were calculated by normalization (sum of the four markers within the sample were set at 100%).
Table 3. Relative concentrations of CTX-I, CTX-II, COMP and PGE$_2$

<table>
<thead>
<tr>
<th>Joint type</th>
<th>CTX-I</th>
<th>CTX-II</th>
<th>COMP</th>
<th>PGE$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative concentrations in the TMJ, % (IQR)</td>
<td>0.00 (0.00)</td>
<td>74.29 (62.06)</td>
<td>0.76 (3.37)</td>
<td>13.19 (32.20)</td>
</tr>
<tr>
<td>Relative concentrations in the knee joint, % (IQR)</td>
<td>0.00 (0.00)</td>
<td>82.92 (26.54)</td>
<td>6.62 (7.65)</td>
<td>2.82 (9.68)</td>
</tr>
<tr>
<td>P value</td>
<td>0.720</td>
<td>0.242</td>
<td>0.000</td>
<td>0.005</td>
</tr>
</tbody>
</table>

IQR = inter quartile range

Discussion

The purpose of this study was to identify valuable degradation markers for cartilage degradation in the TMJ and gain insight in the differences and similarities between the TMJ and the knee joint. The investigators hypothesized that the contribution of COMP and PGE$_2$ would be comparable in both joints, that in the TMJ CTX-I would be increased and CTX-II decreased, whereas in the knee the opposite would occur. The specific aim of this study was to compare relative concentrations of CTX-I, CTX-II, COMP and PGE$_2$ in SF of patients with TMJ osteoarthritis with patients with knee joint osteoarthritis. The results of this study show significant differences in the relative concentrations of COMP and PGE$_2$ between the TMJ and the knee joint, suggesting differences in pathophysiology between the two joints. The inflammatory component seems to be more distinct in the TMJ. Furthermore, high levels of COMP may be associated with disease progression, which seems to be more distinct in the knee joint. Based on the composition of fibrocartilage and hyaline cartilage, a significant difference was expected between the two joints with regard to the contribution of CTX-I and CTX-II. However, according to the results of this study, CTX-II seems to be the most prominent cartilage degradation product in both joints, whereas CTX-I was not detectable in most samples.

In this study, relative SF concentrations were determined, whereas in most other studies only absolute concentrations have been measured. Since similar concentrations would suggest similar pathophysiology, the results of this study suggest differences in pathophysiology between the TMJ and the knee joint because the relative quantities of some of these markers appear to differ significantly. In particular the contributions of COMP and PGE$_2$ differ between the two joints, suggesting that the inflammatory component in the pathophysiology of cartilage destruction may be more distinct in the TMJ. PGE$_2$ is produced by synovial fibroblasts and is a potent mediator of pain, which may be an explanation for the prominent role of pain in the clinical features of TMJ OA. The limited contribution of COMP in TMJ OA may support the assumption that in contrast to OA of the knee joint, articular temporomandibular disorders often appear to be self-limiting. The hypothesis that there are indeed pathophysiological differences in cartilage destruction between the TMJ and the knee joint is supported by the findings of Kapila et al. who investigated the effect of relaxin induced matrix metalloproteinases on the cartilage matrix.
CTX-I was detected in only four of the 30 TMJ samples obtained from diseased joints. CTX-I was assumed to be a detectable SF marker for fibrocartilage degradation, because fibrocartilage consists mainly of collagen type I, of which CTX-I is a degradation product. However, possibly the detectable concentrations of CTX-I in these four cases were due to degradation of underlying bone rather than fibrocartilage, since CTX-I is considered as an important marker for bone degeneration. With regard to CTX-II, the unexpected high relative concentrations in the TMJ may indicate that the superficial layer of the articular fibrocartilage contains more collagen type II than the inner part. This is supported by Kondoh et al. who found a significantly higher collagen type II synthesis in the articular surfaces, than in the inner part of the TMJ disc.

In order to obtain SF from the TMJ, it is inevitable to dilute the fluid that is present in the joint cavity. In few cases this applies to the knee joint as well. In this study, isotonic sodium chloride solution was used as a physiologic buffer. Dilution of the SF may have been responsible in particular for the non-detectability of CTX-I in most of the TMJ samples. However, since hyaline cartilage in the knee joint predominantly consists of type II collagen, high relative concentrations of CTX-I were expected mainly due to bone and/or meniscus involvement.

Assessment of relative concentrations allows for SF analysis independent of the sample dilution, and may be more suitable for routine clinical use. Furthermore, this method facilitates comparison with other joints because of its independence of the dilution factor. However, this method is not commonly used in research, therefore comparison of the results of this study with the available literature is indirect. The relative concentrations indicate the contribution of the markers, but do not determine the absolute increase or decrease as in most other studies.

Because cartilage degeneration in the TMJ is often difficult to detect with non-invasive techniques, the samples were obtained from patients with obvious joint pain and impaired mandibular movement, with crepitus (osteoarthritis according to the Research Diagnostic Criteria for Temporomandibular Dysfunction (RDC/TMD IIIb)\textsuperscript{17,27}), which did not improve after two weeks of treatment with NSAID treatment (exclusion acute arthritis). Subsequently, the diagnosis was confirmed by the X-ray recordings. Knee joint samples were obtained from patients with significant pain, function impairment, and cartilage destruction visible on CT-scan or MRI. However, notwithstanding accurate patient selection, heterogeneity may have been introduced in both groups due to individual fluctuations in the course of the disease after inclusion and prior to sample selection.

There was no normal distribution of the relative concentrations. Possibly, inter-patient variation and fluctuations over time within patients, as well as possible differences in disease classification as mentioned above, may have contributed to the large variation. With regard to this variation, the sample size of recruited patients may have been too small to reflect an assumed normal distribution.
Conclusion

Relative SF concentrations of COMP and PGE$_2$ showed significant differences between the TMJ and the knee joint, suggesting that there are differences in pathophysiology and that the inflammatory component may be more distinct in the TMJ.


