Chapter 8

Alteration of cartilage degeneration and inflammatory markers in temporomandibular joint osteoarthritis occurs proportionally

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Abstract

Objective There is a growing interest in markers for cartilage degradation in synovial joints because of their potential diagnostic and prognostic value. Therefore, the aim of this study was to identify valuable degradation markers for temporomandibular joint (TMJ) osteoarthritis (OA) by comparing the relative concentrations of carboxyterminal telopeptides type I and II (CTX-I and II), cartilage oligomeric matrix protein (COMP) and prostaglandin E_2 (PGE_2) in synovial fluid (SF) of TMJs with OA to healthy, symptom-free TMJs.

Methods In this cross-sectional case-control study, participants were recruited from the University Medical Center Groningen (UMCG), the Netherlands. Cases were defined as patients with TMJ OA, and control subjects had symptom-free TMJs. The outcome variables were the relative concentrations of CTX-I, CTX-II, COMP and PGE_2 in osteoarthritic TMJ SF compared to symptom-free joints. An independent samples Mann-Whitney U-test was used to compare the relative concentrations.

Results A total of 30 cases (9 males, 21 females; mean age 40.1 with SD 15.3) and 10 control subjects (5 males, 5 females; mean age 30.3 with SD 10.8) were studied. No significant differences were found between relative concentrations of CTX-I ($p=0.548$), CTX-II ($p=0.842$), COMP ($p=0.140$) and PGE_2 ($p=0.450$). Unexpected low relative concentrations of CTX-I en high relative CTX-II concentrations were observed.

Conclusions Assumed changes in SF concentration of CTX-I, CTX-II, COMP and PGE_2 in TMJ OA seem to occur proportionally. Furthermore, the unexpected large contribution of CTX-II suggests that this marker may be useful to quantify cartilage degradation in TMJ OA.

Introduction

To date, sufficient diagnostic instruments to determine temporomandibular joint (TMJ) degeneration are lacking. Diagnosis and treatment are usually based on clinical and radiologic examination, whereby severity of the degradation and activity of the degenerative process remain largely unknown. The diagnostic instrument that is most commonly used for research purposes is the (revised) Research Diagnostic Criteria for Temporomandibular disorders (RDC/TMD). [1,2] This clinical instrument is supplemented with imaging when needed. The RDC/TMD aims at reducing inter-observer differences, but its correlation with TMJ degeneration is unknown since there is no golden standard. For imaging, conventional X-ray examinations such as orthopantomography (OPT), transpharyngeal recordings according to Parma, and transcranial radiographs according to Schüller may be applied, but computed tomographic or magnetic resonance imaging examinations are used more and more. Although these imaging techniques often provide additional information about the extent of the damage that has occurred, TMJ degeneration, especially severity and activity, is insufficiently displayed. In some severe cases arthroscopy may be applied in order to gain information about the extensiveness and activity of the degeneration process. However, this procedure involves surgery and is therefore not useful as diagnostic instrument in most cases.

There is a growing interest in markers for degeneration in synovial joints. Potentially these markers may be of diagnostic and prognostic value by determining degeneration severity and activity. [3,4] Markers of large synovial joint degeneration can be found in serum, urine or synovial fluid (SF). However, serum and urine concentrations of TMJ degeneration markers are mostly undetectable, since the TMJ is one of the smallest joints of the human body. [5] For small joints like the TMJ, assessment of the concentrations of these markers in SF seems to be more accurate. [6]

The serum level of cross-linked carboxy-terminal telopeptides of collagen type II (CTX-II) is believed to be an important marker for destruction of hyaline cartilage, which consists mainly of type II collagen. [7-9] However, with regard to SF CTX-II, the importance of this marker is still ambiguous. [9,10] Since the fibrocartilage lining of the articular surfaces of the TMJ predominantly consists of collagen type I, SF CTX-II concentration may be less accurate for TMJ degeneration. Furthermore, in synovial joints with hyaline cartilage covering the articulating surfaces, serum CTX-I is seen as an important marker for bone degradation when the underlying bone is affected. [11] Degeneration of the TMJ will probably result in higher concentrations of CTX-I in the SF. Especially in an early stage of the disease when bone involvement is not likely to occur, this elevation will be caused by cartilage degradation. However, when the disease progresses, elevation of SF CTX-I may also emanate from bone degradation.

Serum concentration levels of cartilage oligomeric matrix protein (COMP) are considered to be a biomarker of both hyaline and fibrocartilage degradation, and a prognostic indicator of joint osteoarthritis (OA). [12-14] However, it does not indicate which joint(s) is (are) affected. To establish that, SF from suspected joints has to be tested. During synovial inflammation, prostaglandin E_2 (PGE_2) is released by synoviocytes [15] and

is thought to be useful for estimating the degree of inflammation. [16] However, in other studies this has not always been confirmed. [17]

In TMJ OA there is usually no excessive accumulation of fluid in the joint cavity because of the chronic course of the disease. Isolation of SF from a joint without hydrops is cumbersome and requires injection of a physiologic buffer, which should be mixed with SF in situ and then retracted. This procedure results in dilution of the SF sample and inaccurate determination of marker concentrations. In order to overcome this problem, assessed concentrations of CTX-I, CTX-II, COMP and PGE_2 within the SF sample were added and the sum was set as 100%. Thus calculated relative concentrations are independent of the dilution factor.

The purpose of this study was to identify valuable degradation markers for temporomandibular joint (TMJ) OA and provide insight into cartilage degradation within the TMJ. The investigators hypothesized that relative SF concentrations of CTX-I, COMP and PGE_2 would be increased in TMJ OA compared to healthy TMJs, whereas the relative concentrations of CTX-II would be decreased. The specific aim of this study was to compare the relative SF concentrations of CTX-I, CTX-II, COMP and PGE_2 in osteoarthritic TMJs with healthy, symptom-free TMJs.

Materials and Methods

Study design

This cross-sectional case-control study was conducted at the University Medical Center Groningen (UMCG), the Netherlands, from June 2011 to June 2012. The ethical committee of the UMCG approved the research protocol prior to patient recruitment (METc 2010.131), and all subjects were informed according to the guidelines of this committee and signed informed consent. Based on findings of other substances in TMJ OA, [18] the clinically relevant effect size was estimated to be 1.2. In order to detect an effect size of 1.2, with alpha being 0.05 and a power of 0.8, 10 controls and 30 patients were needed. Thus a 1:3 matching ratio was used. To be included in the study, patients had to be 18 years of age or over and diagnosed with osteoarthritis according to the revised RDC/TMD. [1] Control subjects had to be patients with healthy, symptom-free TMJs who were scheduled for cosmetic orthognathic surgery. Patients were excluded as study subjects if they used medication that could interfere 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 had 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. The included patients were scheduled for arthrocentesis and no additional treatment was applied, since early lavage of the TMJ seems to be related to superior clinical outcomes. [19,20] NSAID treatment was stopped at least four weeks before the arthrocentesis was performed in order to eliminate the effect of NSAIDs on PGE_2 concentrations.

Variables

The predictor variable was disease state of the TMJ. 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 assay (ELISA) kits for CTX-I, CTX-II (Cusabio biotech co., ltd, Wuhan, China), COMP and PGE_2 (Abnova, Taipei City, Taiwan). The assays were performed in duplo according to the manufacturer's instructions. Demographic variables included age and gender.

Data collection

In both groups the same technique was used for SF collection. SF was collected from the TMJ using intra-articular puncture of the superior joint compartment. The joint was first infiltrated with 0,2 ml intra-articular anaesthesia (Ultracain forte, Aventis Pharma, Hoevelaken, The Netherlands) and subsequently distended with 2ml isotonic sodium chloride solution to facilitate collection of the fluid. As part of the orthognathic surgery, the control group first received general anaesthesia. After fluid collection, elimination of erythrocytes and large protein aggregates was established by immediate centrifugation (25200g, 10min., 4° C). The clean samples were stored at -80° C. All samples were simultaneously analyzed.

Statistical analysis

Relative 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. A graphical representation of one of the samples is given in figure 1. Distribution of the relative concentrations of CTX-I, CTX-II, COMP and PGE_2 in osteoarthritic TMJ SF were compared to the distribution of the relative concentrations of these substances in symptom-free joints using an independent samples Mann-Whitney U-test (SPSS 18.0).

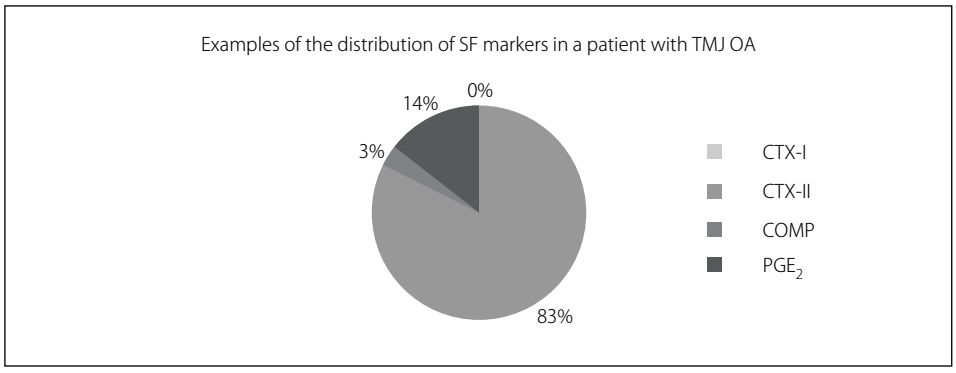

Figure 1, Distribution of the relative SF concentrations of CTX-I, CTX-II, COMP and PGE_2 in one individual patient with TMJ OA. Relative quantities of each marker were calculated by normalization (sum of the four markers = 100%).

Results

Because TMJ OA usually occurs as a mono-arthritis, samples were collected unilaterally. Consequently 30 consecutive patients with TMJ OA participated in this study, and 10 healthy control subjects (table 1). Concentrations of CTX-I, CTX-II, COMP and PGE_2 were measured regardless of the dilution of the samples. Detectable concentrations of CTX-I were found in four of the 30 SF samples obtained from arthritic joints, and in none of the healthy joint samples. CTX-II, COMP and PGE_2 were detectable in all samples. Age and gender did not influence the relative concentrations significantly ($P > 0.01$) (table 2). The relative concentration of each marker was determined for each sample. After normalization, median ratios and inter quartile range (IQR) were used to compare both groups. Differences in relative concentrations of each marker between the samples of the OA group and the control group were determined using an independent samples Mann-Whitney U test. No significant differences were found for CTX-I ($p = 0.548$) CTX-II ($p = 0.842$), COMP ($p = 0.140$) and PGE_2 ($p = 0.450$) (table 3).

Table 1. Patients' characteristics

Characteristics	TMJ OA	Healthy TMJ	P value
Sample size (n)	30	10	
Gender (female), n (%)	21 (70)	5 (50)	0.251
Age (years), mean (SD)	40.1 (15.3)	30.3 (10.8)	0.068

SD = standard deviation

Table 2. Correlations of patients' characteristics and relative concentrations of CTX-I, CTX-II, COMP and PGE_2

Characteristics	CTX-I	CTX-II	COMP	PGE_2
Sample size (n)	40	40	40	40
Gender, P value*	0.856	0.922	0.900	0.790
Age, Spearmen's ρ (P value)	0.078 (0.632)	-0.041 (0.802)	-0.090 (0.579)	-0.018 (0.913)

* independent samples Mann-Whitney U test

Table 3. Relative concentrations of CTX-I, CTX-II, COMP and PGE_2

Disease state	CTX-I	CTX-II	COMP	PGE_2
Relative concentrations in TMJ OA, % (IQR)	0.00 (0.00)	74.29 (62.06)	0.76 (3.37)	13.19 (32.20)
Relative concentrations in healthy TMJ, % (IQR)	0.00 (0.00)	63.52 (38.03)	4.50 (10.26)	32.05 (22.03)
P value	0.548	0.842	0.140	0.450

IQR = inter quartile range

Discussion

The purpose of this study was to identify valuable degradation markers for TMJ OA and

provide insight into cartilage degradation within the TMJ. The investigators hypothesized that relative SF concentrations of CTX-I, COMP and PGE_2 would be increased in TMJ OA compared to healthy TMJs, whereas the relative concentrations of CTX-II would be decreased. The specific aim of this study was to compare the relative SF concentrations of CTX-I, CTX-II, COMP and PGE_2 in osteoarthritic TMJs with healthy, symptom-free TMJs. The results of this study did not show any significant differences in the relative concentrations of CTX-I, CTX-II, COMP and PGE_2 between affected and symptom-free TMJs. Furthermore, unexpected low relative concentrations of CTX-I and high relative CTX-II concentrations were observed.

TMJ pain and inflammation seem to be related to elevated SF concentrations of PGE_2. [5,21] Furthermore, elevation of SF concentrations of COMP are likely to occur. [22] The results of the present study suggest that, if concentrations of PGE_2 and COMP were elevated, CTX-I and CTX-II were also proportionally elevated in SF of the OA group. Since the fibrocartilage lining of the articular surfaces of the TMJ predominantly consists of collagen type I, elevated concentrations of CTX-II were unexpected. However, the superficial layer of the articular fibrocartilage may contain more collagen type II than the inner part, especially with regard to the articular disc. [23] Degradation of this superficial layer may have contributed to the concentrations of CTX-II.

Several SF markers for cartilage degradation in the TMJ have been investigated in the past decade, such as different types of matrix metalloproteinase, aggrecanase and glycosaminoglycan components. [24,25] These markers were identified as potential markers for cartilage degradation. However, they did not provide insight into which type of collagen is mainly affected in TMJ OA. To the best of our knowledge there are no studies investigating CTX-I and/or CTX-II as SF markers for cartilage degradation in TMJ OA. The remarkable results of the present study suggest that degradation of collagen type II may play a more prominent role than was hypothesized based on the composition of TMJ fibrocartilage.

In this study, relative concentrations were determined, whereas most other studies measured absolute concentrations. [22,26-29] Assessment of relative concentrations allows for SF analysis independent of the sample dilution, and may be more suitable for routine clinical use. In order to obtain SF from the TMJ it is inevitable to dilute the fluid that is present within the joint cavity. 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 samples. By contrast, during injection and aspiration, contamination of the sample may have occurred due to micro bleeding. Elimination of contamination with regard to cells was obtained by centrifugation of the samples. However, markers measured in this study that may have been detectable in serum as well, were not eliminated from the samples by the centrifugation procedure. Therefore, the results in this study may provide some overestimation of the concentrations, due to minimal blood contamination.

In relation to the cartilage degradation markers, PGE_2 concentrations were changed proportionally. Because early cartilage degradation in the TMJ is difficult to determine with non-invasive techniques, the samples of the OA group were obtained from patients

with obvious joint pain, which did not improve after two weeks of NSAID treatment. Then NSAID treatment was stopped and patients were scheduled for arthrocentesis at least four weeks later. However, in the four weeks prior to arthrocentesis, patients may have used NSAIDs without reporting. Unreported NSAID use may have influenced in particular the PGE_2 concentrations.

As part of the orthognathic surgery, the control group first received general anesthesia where after the same technique was applied to collect the samples as in the OA group. General anaesthesia may have influenced the PGE_2 synthesis. However, this effect is detectable in post-operative serum concentrations, and the samples in this study were collected pre-operatively. [30,31] Therefore, it seems unlikely that general anaesthesia has influenced PGE_2 concentrations in the obtained TMJ SF samples.

There was no normal distribution of the relative concentrations. Possibly, inter patient variations, and fluctuations over time within patients, as well as possible variation 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

Assumed changes in SF concentration of CTX-I, CTX-II, COMP and PGE_2 in TMJ OA seem to occur proportionally. Furthermore, the unexpected large contribution of CTX-II suggests that this marker may be useful to quantify cartilage degradation in TMJ OA. Determination of the absolute concentrations of CTX-II in TMJ OA is needed to estimate its diagnostic and prognostic value as cartilage degradation marker.

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