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MMP-3 and -8 expression is found in the condylar surface of temporomandibular joints with internal derangement

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BACKGROUND: Internal derangement is one of the most common disorder of the temporomandibular joint (TMJ). The aim of this study was to investigate the associations of matrix metalloproteinase (MMP)-3 and -8 expression in articular condylar surface with different stages of TMJ internal derangement according to Wilkes (*Minn Med*, 1978; 61: 645–52) and osteoarthrosis (OA) according to Dijkgraaf et al. (*J Oral Maxillofac Surg*, 1995; 53: 1182–92).

METHODS: The study was based on 54 condylar specimens obtained during TMJ surgery. Immunohistochemistry using antibodies specific to MMP-3 and -8, represented in cartilage destruction, was carried out.

RESULTS: In all tissue specimens, MMP-3 expression was intense in the surface layer but showed less intensive staining in the deeper layers. Some MMP-8 expression was also seen. The severity of TMJ internal derangement, however, did not seem to have a statistically significant correlation ($P < 0.05$) with the expression of these enzymes.

CONCLUSION: The study confirms that distinct expression of MMP-3 and -8 is found in the condylar surface of TMJs with internal derangement.

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Keywords: internal derangement; mandibular condyle; matrix metalloproteases; osteoarthrosis; temporomandibular joint

Introduction

Internal derangement consists of disc dysfunction and damage of the temporomandibular joint (TMJ) (1). It is considered to be a risk factor for developing osteoarthrosis (OA) with remodelling of the condyle and the mandibular fossa (2). More than two-third of patients with clinical TMJ symptoms have internal derangement (3).

Osteoarthrosis of the condylar cartilage of the TMJ results from an imbalance between the predominantly chondrocyte-controlled anabolic and catabolic processes and is characterized by progressive degradation of the extracellular matrix (ECM) (4, 5). The TMJ behaves as a complex organ, in which biomechanical and biochemical processes regulate the physiology of cartilage, bone, synovium, ligaments and synovial fluid (6–8). In this concept, TMJ OA is an organ failure involving all relevant structures. Both the development and the reversal of the pathological process appear to be intimately related to the adaptive capacity of the tissues that make up the joint organ (8).

Matrix metalloproteinases (MMPs) appear to be major factors in the pathological destruction of articular cartilage (9, 10). Altogether 23 human MMPs are known at present, and at least collagenases (MMP-1, -8, 13) (11), gelatinases (MMP-2, -9) (11, 12) and stromelysins (MMP-3, -19) (13) are involved in different stages of TMJ pathogenesis. MMPs in the cartilage or synovium of individuals with joint diseases are thought to be induced by proinflammatory cytokines produced by, for example, synoviocytes (14).

The MMP-3 is secreted by fibroblasts, synovial cells and chondrocytes (15, 16), and it is considered to be the most important proteinase responsible for cartilage matrix degradation (17). It also activates other MMPs, including MMP-2 and MMP-9 (18). MMP-8 has been shown to be involved in the cleavage and denaturation of type II collagen in articular cartilage (19), and positive expression has been shown in OA cartilage specimens (20). The expression of MMP-3 and MMP-8 has been reported to increase in damaged cartilage (21).

Only a few studies using human material have been made to examine the histopathological findings of TMJ tissues and to evaluate these findings related to surgically confirmed cases of TMJ internal derangement. The aim of this study was to investigate the associations of MMP-3 and -8 expression in articular condylar surface with different stages of TMJ internal derangement and OA.

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Materials and methods

This study was based on 54 TMJs (24 right, 30 left) of surgically treated patients (11 male and 43 female; mean age 36.3 years, range 16–76). The specimens were taken during TMJ surgery at Oulu University Hospital, Finland, in 1986–1997. The diagnosis was based on clinical and computed tomography (CT) and/or magnetic resonance imaging (MRI) examinations and confirmed by surgical findings (22). The duration of symptoms in the operated patients ranged from a couple of months up to over 10 years. Extrinsic trauma (e.g. a blow in the face, a car accident) was present in 15 of the 54 cases. The main indications for surgery were subjective symptoms i.e. TMJ pain, locking of the TMJ and markedly restricted mouth opening despite conservative stomatognathic treatment. Surgical procedures were performed by the same oral surgeon (HP) on patients under general anaesthesia and nasal intubation. A modified preauricular skin incision was made. Surgical operations included high condylectomy, repositioning of the disc, discectomy and deliberation of adhesions. The ethical Committee of Oulu University Hospital approved the investigation. The TMJs were classified into two groups according to the disc position diagnosed clinically and radiologically and confirmed at surgery. The first group was diagnosed to have reducing anterior disc displacement (ADD-r) (15/54TMJs) (Fig. 1a,b), while the second group had non-reducing anterior disc displacement (ADD-nr) (39/54TMJs), (Fig. 2a,b). In TMJs with ADD-nr, perforation of the disc was recorded in 10 of 39 and perforation of the posterior attachment in three of 39 cases.

The stages of TMJ internal derangement were classified into mild, intermediate and severe according to Wilkes (23) classification based on clinical, surgical and pathological stages. Mild internal derangement (I) is characterized by simple disc displacement without any morphological alteration of the disc and with or without osseous remodelling. The intermediate stage (II) is characterized by disc displacement and morphological deformity and/or osseous remodelling changes. Severe derangement (III) is characterized by perforations of the disk attachments and osseous remodelling and/or osteoarthritic changes (sclerosis, osteophyte formation, articular surface flattening, depression and/or cystic alterations).

In all cases, tissue specimens included articular condylar surface (cartilage and subchondral bone). The size of specimens varied, however, due to the degree of condylar surface pathology seen at surgery. The tissues were fixed in 4% buffered formalin, and the specimens were decalcified in 5% formic acid. The tissues were embedded in paraffin and cut into 5 μm sections. Paraffin-embedded sections were stained with haematoxylin and eosin for general morphology.

The pathogenesis of TMJ OA in these specimens was examined and classified, using light microscopy (Leica Leitz DM RB/E, Germany), into four stages according to Dijkgraaf et al. (4): (1) initial and repair stage – proliferation and increased metabolic activity of chondrocytes, (2) early stage – swelling, proliferation and increased metabolic activity of chondrocytes, collagen network with signs of disorganization, (3) intermediate stage – fibrillation, detachments, thinning, cluster formation, chondocyte degeneration and necrosis, collagen network is markedly disorganized, (4) late stage – extensive fibrillation, possibly denudation, violation tidemark, chondrocyte necrosis, collagen network severely disorganized.

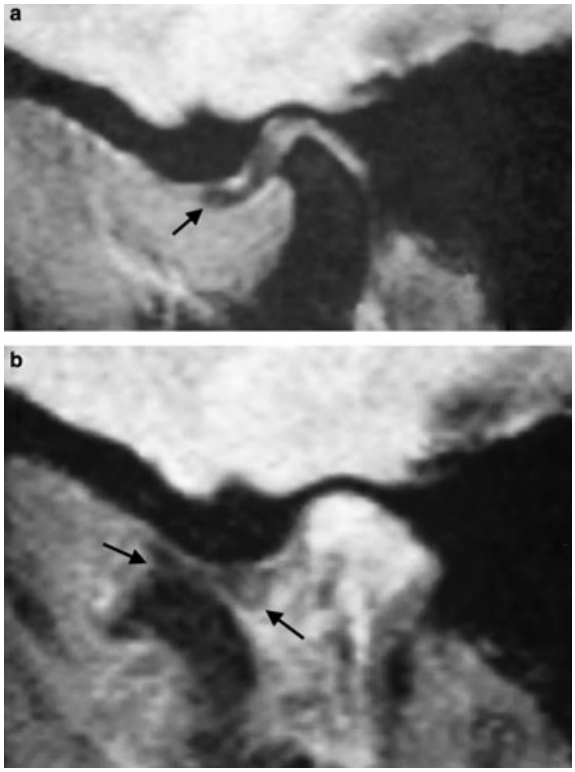

Figure 1 (a,b) Sagittal MRI images of left TMJ in a woman aged 20 years (same patient as in Fig. 5a,b) showing anterior disc dislocation with reduction (ADD-r), surgically confirmed. (a) In a mouth closed position, the disc (arrow) is seen anterior to the condyle. (b) In a mouth open position, the disc is seen in the normal position between the articular surfaces (arrows).

For immunostainings, deparaffinized sections were pre-treated with 0.4% pepsin for 30 min at 35°C. Endogenous peroxidase activity was quenched by treatment with 3% H_2O_2 for 10 min. Non-specific binding of antibodies was blocked by normal horse/goat serum treatments [diluted 1:20 in phosphate buffered saline (PBS), 0.1% bovine serum albumin (BSA)]. The sections were incubated with diluted monoclonal MMP-3 antibody (Oncogene Research Products, San Diego, CA, USA) (diluted 1:10 in PBS, 0.1% BSA) and polyclonal MMP-8 antibody (Chemicon International Inc, Temecula, CA, USA) (diluted 1:4000 in PBS, 0.1% BSA) overnight at +4°C. For negative controls, monoclonal non-immuno mouse serum for MMP-3 and polyclonal non-immuno rabbit serum for MMP-8 were used. The secondary antibody, biotinylated anti-mouse/anti-rabbit IgG was applied (for 30 min, at RT), followed by the

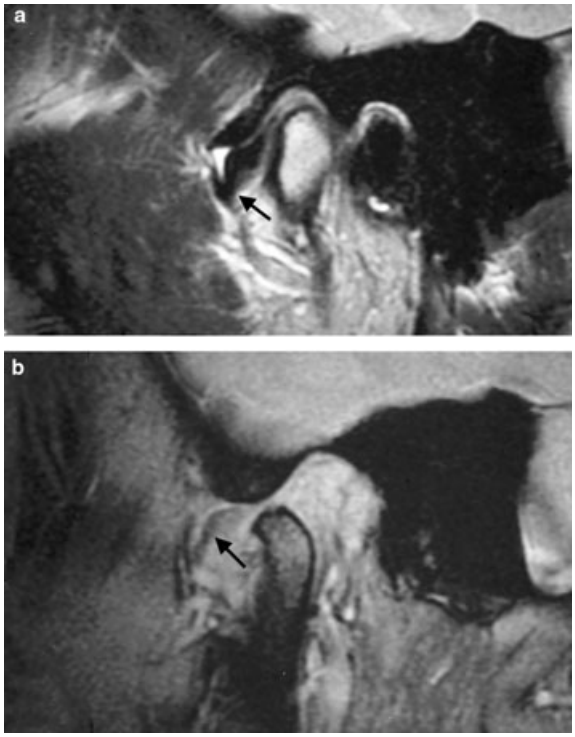

Figure 2 (a,b) Sagittal MRI images of left TMJ in a woman aged 28 years (same patient as in Fig. 5c,d) showing anterior disc dislocation without reduction (ADD-nr), surgically confirmed. (a) In a mouth closed position, the deformed disc is seen anterior to the condyle (arrow). (b) In a mouth open position, the disc is still situated anterior to the condyle (arrows). Note the restricted movement of the condyle.

avidin-biotin-peroxidase complex (Vector Elite Kit Abbott, Chicago, IL, USA, for 30 min, at RT). The sections were counterstained with Mayer's haematoxylin (Histolab Products AB, Göteborg, Sweden) and finally mounted in GVA mount (Zymed, Laboratories Inc., San Francisco, CA, USA). (24) Immunostaining was considered to be specific to MMP-3 and MMP-8 because immunoreactivity was not observed in the negative controls.

The intensity of immunostaining in condylar surface, including cartilage area, was analysed. Chondrocytes and the surrounding ECM were estimated as (0) for no staining, (+) for mild staining and (++) for intense staining. The whole sample was microscopically examined to identify the condylar surface area at ×50 and ×100 magnification.

The area of examined articular condylar surface was defined from the surface of the specimen to the border between subchondral bone and mineralized cartilage. The surface layer in the specimens was determined to constitute one-fourth of the total thickness of the examined area, with the deep layer accounting for the remaining three-fourth of the area. The calibration of observations was performed by two researchers unaware of each other's results.

Statistical analysis

The count of positively and negatively stained areas of MMP-3 and -8 expressions in specimens of articular condylar surface showing different stages of internal derangement, OA and disc position (ADD-r and ADD-nr) was analysed by Fisher's exact test. A difference of $P < 0.05$ was considered significant.

Results

The internal derangement of the TMJ was classified as Wilkes stage I in 13 of 54 (ADD-r eight of 13, ADD-nr five of 13), stage II in 27 of 54 (ADD-r six of 27, ADD-nr 21 of 27) and stage III in 14 of 54 (ADD-r one of 14, ADD-nr 13 of 14) specimens.

In condylar specimens of TMJs with ADD-r (Wilkes class I-III) MMP-3 immunostaining was intense in the surface layer in all cases but less intensive in deeper layer (Figs 3a and 5a). The intensity and distribution of MMP-8 immunostaining were between no/mild staining and intense staining in the surface and deeper layers in the mild (I) and intermediate (II) stages, while no intense staining was seen in the severe stages (Figs 3a and 5b).

In the condylar specimens of TMJs with ADD-nr (Wilkes class I-III), MMP-3 immunostaining in the condylar surface layer was intense in all stages of internal derangement (Figs 3b and 5c). In the deeper layers, MMP-3 immunostaining was less intensive (Fig. 3b). The distribution of MMP-8 immunostaining was relatively less intensive in the deeper layer compared with the surface (Figs 3b and 5d).

The articular condylar surface of all specimens showed signs of histopathological changes of OA according to Dijkgraaf et al. (4). Typical findings included general loss of cartilage and fibrillation, detachment, thinning and disorganization of the collagen network. Most of the osteoarthritic cartilage was cracked and partially desquamated. Underlying subchondral bone showed signs of eburnation. Using Dijkgraaf's classification, the OA seen in the mandibular condylar specimens was classified as stage 2 in nine of 54 (ADD-r three of nine, ADD-nr six of nine) and as stage 3 in 45 of 54 (ADD-r 12 of 45, ADD-nr 33 of 45).

The specimens with ADD-r (OA stage 2 and 3) had intense MMP-3 immunostaining in condylar surface layer in all specimens (Figs 4a and 5a). In the deeper condylar layers the intensity of immunostaining varied between no/mild and intense in both stages of OA (Fig. 5a). MMP-8 expression in the surface and deeper condylar layers showed intense staining only in stage 3 specimens (Figs 4a and 5b).

The specimens with ADD-nr (OA stage 2 or 3) had intense MMP-3 immunostaining in the condylar surface layer, and immunostaining in this area was equally intensive in both stages of OA (Figs 4b and 5c). In the deeper layers immunostaining was less intensive (Fig. 4a). MMP-8 staining was seen in the surface layers, but in the deeper condylar surface layers intense MMP-8 staining was only seen in stage 3 specimens (Figs 4b and 5d).

Specimens with ADD-nr had relatively more intense immunostainig of MMP-3 and -8 compared with the

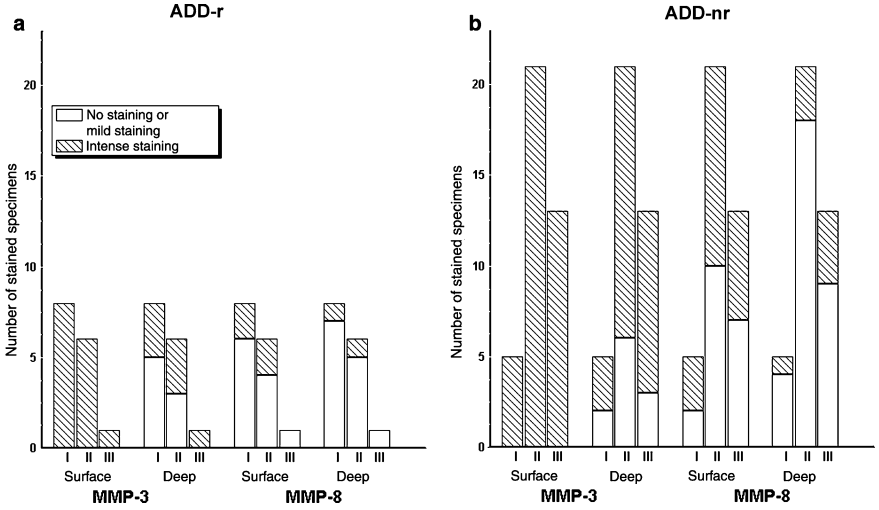

Figure 3 (a) Relative distributions and intensity of MMP-3 and -8 immunostainings in the surface and deeper layers of articular condylar specimens of TMJs with ADD-r and (b) with ADD-nr in different stages of internal derangement according to Wilkes (23). There were no statistically significant differences ($P > 0.05$) between the specimens with ADD-r and ADD-nr.

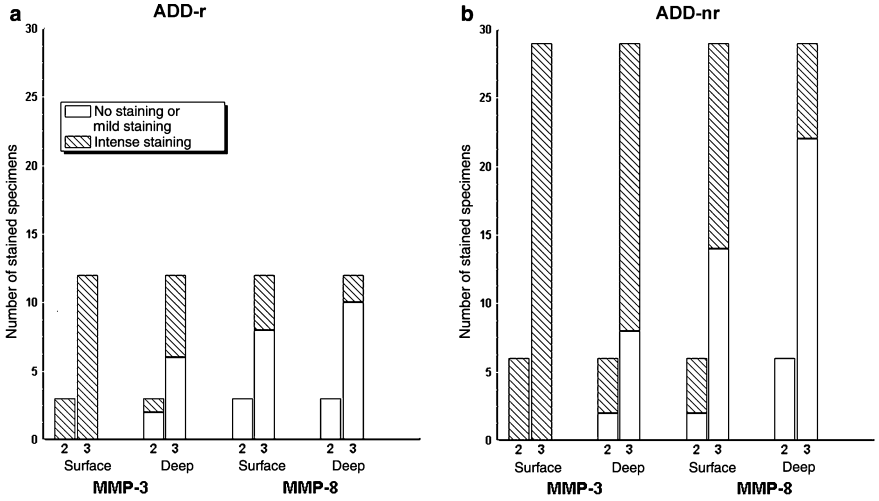

Figure 4 (a) Relative distributions and intensity of MMP-3 and -8 immunostainings in the surface and deeper layers of articular condylar specimens of TMJs with ADD-r and (b) with ADD-nr in OA stage 2 and 3 according to Dijkgraaf et al. (4). There were no statistically significant differences ($P > 0.05$) between the specimens with ADD-r and ADD-nr.

specimens with ADD-r. However, the comparison of the distribution and intensity of MMP-3 and -8 immunostaining with the severity of TMJ internal derangement according to the Wilkes (23) and OA stage according to Dijkgraaf et al. (4) revealed no statistically significant differences ($P > 0.05$). Also the differences in MMP expression between the groups of ADD-r and ADD-nr in different stages were not statistically significant (Figs 3a,b and 4a,b).

Discussion

In this study, the diagnosis of TMJ internal derangement was based on clinical signs and symptoms and CT and/or MRI findings and it was surgically confirmed. Based on the immunostained specimens, it seems that MMP-3 and MMP-8 expression in the articular condylar surface is associated with progression of the pathological changes in the TMJs with ADD-r and ADD-nr. A comparison between the immunostaining of these enzymes and the severity of TMJ internal derangement classified according to Wilkes (23) or the stage of OA according to Dijkgraaf et al. (4) did not, however, reveal a statistically significant correlation. More intense MMP-3 immunoreactivity was seen in the surface layers of all specimens, while MMP-8 immunoreactivity was found to be relatively uniform. Semiquantitative immunohistochemical analysis of the results revealed MMP-8 immunostaining to be relatively more intense in the specimens of TMJs with ADD-nr than in those with ADD-r. The tendency of MMP-8 expression hence seems to be related to the severity of TMJ internal derangement.

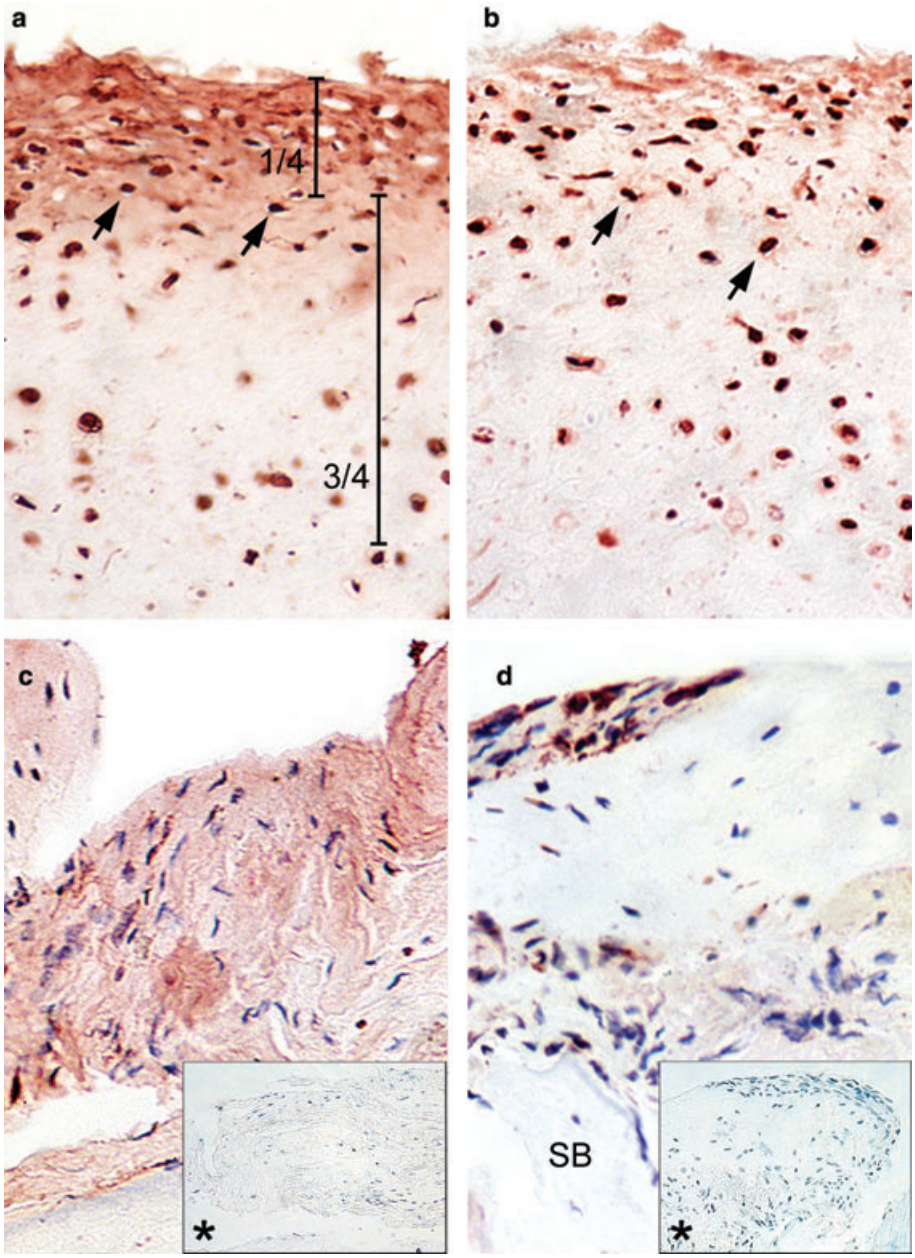

Figure 5 Immunostaining of MMP-3 and -8 in articular condylar surface (cartilage and subchondral bone, SB). Specimens presenting TMJs with ADD-r (a and b; same patient as in Fig. 1) and TMJs with ADD-nr (c and d; same patient as in Fig. 2). (a) Intense immunostaining for MMP-3 in the surface layer. Surface layer (1/4) and deeper layer (3/4). (b) MMP-8 immunostaining is intense in the surface and mild in the deeper layers. (c) MMP-3 immunostaining showing intense staining in the surface layer (d) MMP-8 immunostaining is mild. Chondrocytes (arrowheads) show positive immunostaining. (Original magnification ×250) (Haematoxylin counterstain) *Insets of negative control.

In the study of synovial fluid in the operated TMJ patients Srinivas et al. (11) noted the levels of several MMPs, including MMP-8, to be elevated in patients with mild and severe signs and symptoms of TMJ dysfunction. They found a trend of predominantly, but not statistically significant, elevated levels of MMP-8 even in mild TMJ dysfunction. This was assumed to reflect the active tissue destruction occurring during painful periods of inflammatory response in the TMJ. Their findings cannot be directly compared with ours, as the classification of patients into mild and severe TMJ dysfunction was based on the dysfunction index by Helkimo (25) and the MMP-8 analysis of synovial fluid was quantitative.

The major TMJ structures, including condylar cartilage, synovial tissue and fluid, intra-articular disc and retrodiscal tissue, have been shown to express several MMPs during tissue destruction caused by internal derangement of the joint. Articular cartilage contains a large amount of matrix macromolecules such as proteoglycan and type II collagen (26–28). These molecules contribute to the flexibility of cartilage and the

protection of the joint components from various mechanical stimulations, such as compression, shearing and stretching loads. Mechanical stress exceeding the adaptive capacity of the TMJ is considered a risk factor for OA. The imbalance between MMPs and their inhibitors may be involved in the breakdown of the articular cartilage matrix of the TMJ. A strong association between the OA-active joints and the presence of biologically active forms of known tissue degradation enzymes has been shown (13, 11). Tanaka et al. (29) recognized disc perforation and suggested that a local mechanical excess stress eventually leads to disc perforation and includes cartilage matrix degradation. MMP-3 has been shown to be synthesized and secreted from chondrocytes and synovial lining cells (16) and also to take part in the degradation of cartilage (17). MMP-3 has also been shown to have increased expression and activation after experimentally altered loading of TMJ structures (30). These findings support the assumption of MMP-3 being a key enzyme in cartilage degradation.

Collagenases have been detected in the synovial fluid of patients with traumatic arthritis, rheumatoid arthritis and OA (31). MMP-8 is known to be expressed by circulating polymorphonuclear leucocytes, but human chondrocytes (32), rheumatoid synovial fibroblasts (33) and plasma cells also express MMP-8 (34). MMP-8 has the highest activity against cartilage collagen type II, and in patholophysiological conditions, MMP-8 is regarded as playing a central role at sites of matrix degradation.

Based on the available evidence of MMP-3 and -8 expressions in different cell types, it can be suggested that cartilage degradation occurs as a result of extrinsic and intrinsic enzyme activity. Dijkgraaf et al. (4) found that, in OA cartilage, degenerative changes are mainly intrinsic. The source of other MMPs in TMJ OA have been suggested to be both intrinsic, based on positive immunostaining of chondrocytes, and extrinsic, because of a loss of integrity of articular cartilage, which enables the MMPs to penetrate the cartilage and to accelerate the degradation. In our material the integrity of the articular condylar surface was also lost because of OA. We suggest that MMP-3 and MMP-8 staining in the matrix may not be secreted from chondrocytes alone but also from synoviocytes and inflammatory cells. On the other hand, MMP-3 also appears to be produced by disc cells, and it has been noted that disc cells may possibly play a role in cartilage destruction as the primary cellular source of ECM-degrading enzymes with an intense chronic inflammatory response developing after cartilage or disc injury (35). This finding may explain the intense MMP-3 staining in the articular surface in all specimens of our material.

Only a few human *in vivo* studies have been performed where the histopathology of TMJ internal derangement in surgically confirmed cases has been evaluated, and where specimens of the articular condylar surface have been taken in open TMJ surgery. Two different methods used to classify the pathologic condition did not reveal a statistically significant correlation between MMP-3 and -8 expression and the stage of TMJ pathology. They did reveal, however, positive expression of these enzymes. This finding may be related to the fact that the degradating enzymes are readily active even in the early stages of joint destruction. To understand TMJ pathology, to improve diagnosis and to target the treatment accordingly, more specific methods must be developed in examining these enzymes and their contribution to the complex pathological processes specific to TMJ tissues.

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