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# Synovial TGF-β1 and MMP-3 levels and their correlation with the progression of temporomandibular joint osteoarthritis combined with disc displacement: A preliminary study

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**Abstract.** Osteoarthritis (OA) is a slow progressing degenerative disease that affects the joints, including the temporomandibular joint. In the present study, transforming growth factor-β1 (TGF-β1) and matrix metalloproteinase 3 (MMP-3) in synovial fluid (SF) were examined in detecting cartilage synthesis and degradation in progression of temporomandibular joint osteoarthritis (TMJ OA) combined with disc displacement (DD) diseases. SF was obtained from 16 patients with TMJ OA combined with DD and 10 normal volunteers. TGF-β1 and MMP-3 levels were measured by enzyme-linked immunosorbent assay. In addition, TMJ OA combined with DD was classified into three stages based on radiographic signs on the preoperative tomograms and surgical findings at operation, and different treatment options were administered according to the stages. SF from TMJs with TMJ OA combined with DD showed higher levels of TGF-β1 and MMP-3 compared with the asymptomatic control TMJs. With the progression of TMJ OA combined with DD, TGF-β1 levels in SF were lower, while MMP-3 levels in SF were significantly higher. In conclusion, these data suggest that MMP-3 is not only involved in the pathological destruction process of TMJ OA combined with DD initially, but also has a positive correlation with the degree of pathological changes. Furthermore, a significant increase of TGF-β1 levels was found in the SF that were able to counteract the deleterious effects of MMP-3 at the early stage of TMJ OA combined DD, providing the scientific basis on repositioning displaced disc as early as possible for these patients.

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*Key words:* temporomandibular joint, osteoarthritis, transforming growth factor-β1, matrix metalloproteinase 3, synovial fluid, opening disc displacement

## Introduction

Osteoarthritis (OA) is a slow progressing degenerative disease characterized by cartilage damage, synovial fibrosis and osteophyte formation which may involve several joints, including the temporomandibular joint (TMJ) (1,2). Once the TMJ is involved, severe handicap and significant pain generally occur. Clinically, temporomandibular joint osteoarthritis (TMJ OA) is characterized by joint pain, crepitus, restricted motion and eventually loss of joint function (1-3).

Recent advances in imaging, particularly magnetic resonance imaging (MRI) and arthroscopy have contributed greatly to the understanding of the intra-articular lesions of the disc and condyle (4,5). Pathological changes, such as disc displacement (DD) may be involved in the development of TMJ OA. In their studies, Macher *et al* (6) and Ali *et al* (7) demonstrated that the surgical induction of anterior DD in rabbits might lead to OA. Long *et al* (8) showed that the degree of anterior DD of TMJ is correlated with OA in rabbits. However, a correlation between the change and detailed pathogenesis remains to be adequately elucidated.

Transforming growth factor-β1 (TGF-β1) is an important inducer of cartilage extracellular matrix (ECM) production and is suggested to be a potential tool to enhance cartilage repair upon damage in OA (9-11). By contrast, matrix metalloproteinases (MMPs) are a large group of matrix degrading enzymes that contribute to joint destruction in OA. High activities of diverse MMPs, especially of MMP-3 are believed to be highly involved in matrix breakdown (12-14). These cytokines can infiltrate the synovium, which may alter the synovial fluid (SF) viscosity and lead to impairment in the lubrication and nutrition of articular cartilage and disc.

The aim of the present study was to investigate the levels of TGF-β1 and MMP-3 in SF to evaluate the initiation and progression of TMJ OA combined with DD and provide a scientific basis for the assessment of treatment effectiveness for these patients.

## Patients and methods

*Patients*. Consecutive patients referred to our department and who had been selected for TMJ surgical treatment were studied.

Table I. Staging of TMJ OA combined with DD.

| Staging (TMJ OA combined with DD) | Imaging findings | Surgical findings |
|---|---|---|
| Early | Anterior disc displacement, disc deformed moderate to marked disc thickening, abnormal bone contours | Disc deformed disc displaced anteriorly, variable adhesion |
| Intermediate | Anterior disc displacement, Disc deformed, Marked disc thickening, Abnormal bone contours | Degenerative remodeling of condylar cartilage surface, Adhesion, deformed disc without perforation |
| Late | Anterior disc displacement with disc perforation, Disc deformed badly, Degenerative osseous changes | Gross degenerative changes of disc and hard tissue, Disc perforation, Multiple adhesion |

TMJ OA, temporomandibular joint osteoarthritis; DD, disc displacement.

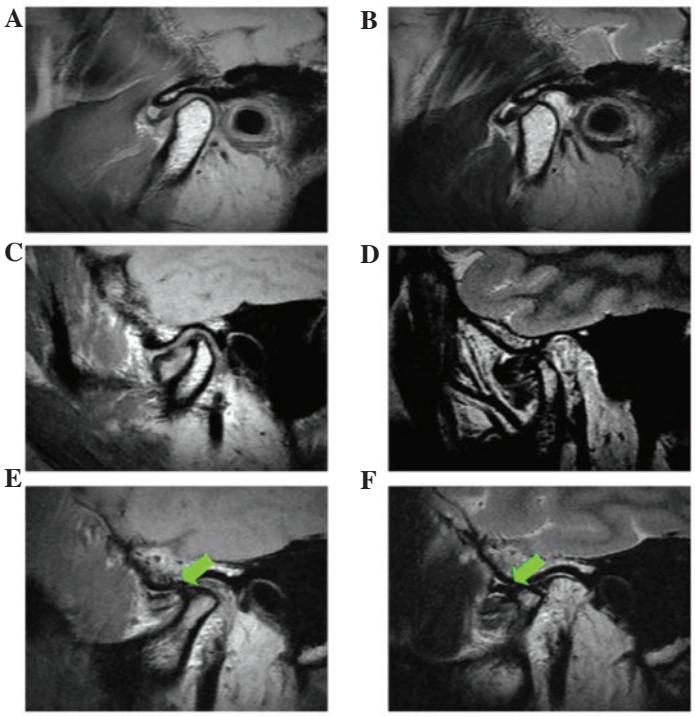


Figure 1. Magnetic resonance imaging (MRI) oftemporomandibular joint osteoarthritis (TMJ OA) combined with disc displacement (DD) is shown. (A and B) showed early TMJ OA combined with DD; (C) and (D) showed intermediate TMJ OA combined DD; (E and F) showed late TMJ OA combined with DD; (A, C and E) closed position; (B, D and F) open position. Green arrow, disc perforation.

Patients with inflammation or arthritic diseases other than osteoarthritis and patients who had had traumatic events were excluded. The criteria for surgical treatment were unsuccessful non-surgical treatment and a clinical diagnosis of TMJ OA. The surgical technique has been thoroughly described elsewhere (15-17). The inclusion criteria for TMJ OA combined DD staging were a painful impaired TMJ mobility, radiographic signs on the preoperative tomograms and surgical findings at operation (Table I, Fig. 1). The mean age for these patients was 36.75 years (range, 18-58). There were 15 females and 1 male with a mean duration of symptom of 1.6 years (range, 4 months-5 years) (Table II). The present study was approved by the ethics committee of Shanghai Ninth People's Hospital affiliated to Shanghai JiaoTong University, School of Medicine. Informed consent and written agreement was obtained from each patient.

*SF samples from TMJ*. The experimental SFs were collected from >16 patients during surgery. The control SFs were collected from 10 healthy check-up examinees with no clinical and radiological evidence of TMJ OA. The TMJ was punctured with a standard disposable needle (diameter of 0.65 mm)

Table II. Patient characteristics.

| No. | Gender | Age (years) | Staging | Surgical treatment |
|---|---|---|---|---|
| 1 | Female | 20 | Intermediate | Open disc repositioning |
| 2 | Female | 50 | Late | Shave the osteophyte combined with open disc repositioning |
| 3 | Female | 53 | Intermediate | Open disc repositioning |
| 4 | Female | 21 | Early | Open disc repositioning |
| 5 | Male | 53 | Late | Condylectomy and reconstruction with costochondral graft |
| 6 | Female | 58 | Early | Open disc repositioning |
| 7 | Female | 52 | Late | Shave the osteophyte combined with open disc repositioning |
| 8 | Female | 18 | Late | Condylectomy and reconstruction with costochondral graft |
| 9 | Female | 28 | Intermediate | Open disc repositioning |
| 10 | Female | 22 | Late | Open disc repositioning |
| 11 | Female | 25 | Intermediate | Open disc repositioning |
| 12 | Female | 43 | Late | Condylectomy and reconstruction with costochondral graft |
| 13 | Female | 23 | Early | Open disc repositioning |
| 14 | Female | 51 | Intermediate | Open disc repositioning |
| 15 | Female | 51 | Intermediate | Open disc repositioning |
| 16 | Female | 20 | Early | Open disc repositioning |

Table III. TGF-β1 levels in synovial fluid of TMJ OA combined with DD in patients and healthy volunteers.

| Group | Cases | TGF-β1 (pg/ml) |
|---|---|---|
| Control | 10 | 121.6628±15.20046 |
| TMJ OA combined DD[a] | 16 | 391.0205±130.6354 |

[a]P<0.05. TGF-β1, transforming growth factor-β1; TMJ OA, temporomandibular joint osteoarthritis; DD, disc displacement.

Table IV. MMP-3 levels in synovial fluids of TMJ OA combined with DD in patients and healthy volunteers.

| Group | Cases | MMP-3 (pg/ml) |
|---|---|---|
| Control | 10 | 660.3411±110.9955 |
| TMJ OA combined DD[a] | 16 | 4074.418±933.0046 |

[a]P<0.05. MMP-3, matrix metalloproteinase 3; TMJ OA, temporomandibular joint osteoarthritis; DD, disc displacement.

inserted into the posterior part of the upper joint compartment. TMJ SF samples were obtained by washing the joint cavity with saline using the push and pull technique. SFs were then centrifuged for 20 min at 2,000 x g using serum monovettes (Sarstedt, Nümbrecht, Germany) and stored at -80˚C until use.

*Enzyme-linked immunosorbent assay (ELISA).* Commercially available human DuoSet ELISAs (R&D Systems, Inc., Minneapolis, MN, USA) were used to estimate the concentrations of TGF-β1 and MMP-3 in appropriately diluted SFs. Ninety-six-well flat plates were coated overnight with the primary antibody at room temperature (HCl 1 N) for 10 min, following reneutralization (NaOH 2.7 N/HEPES 1 M). Samples and controls of known concentration were added in wells for 2 h and were incubated with the secondary antibody for an additional 2 h. Conjugation with horseradish peroxidase and addition of tetramethylbenzidine and $H_2O_2$ produced light emission at 450 nm. Results were corrected by subtraction of light emission at an aborbance value of 540 nm.

*Statistical analysis.* Data were presented as the mean ± standard error of the mean. The Statistical Package for Social Science (SPSS) software version 11.5 (SPSS, Inc, Chicago, IL, USA) was used for statistical processing. The non-parametric Mann-Whitney U Test was used. P<0.05 was considered to indicate a statistically significant difference.

## Results

*TGF-β1 levels in SF.* The median value of TGF-β1 in SF was three times higher when comparing patients with TMJ OA combined with DD to the healthy volunteers. (391.0205 pg/ml vs. 121.6628 pg/ml, P=0.000, z=-4.217) (Table III).

Of the patients, 4 had TMJ OA combined with DD of early staging, 6 had TMJ OA combined with DD of intermediate staging and 6 had TMJ OA combined with DD of late staging (Table II). Notably, the SF from patients with TMJ OA combined with DD of late staging had lower TGF-β1 levels compared with patients of early or intermediate staging (Fig. 2).

*MMP-3 levels in SF.* The median value of MMP-3 in SF was six times higher when comparing patients with TMJ OA combined with DD to the healthy volunteers (4047.418 pg/ml vs. 660.3411 pg/ml, P=0.000, z=-4.216) (Table IV).

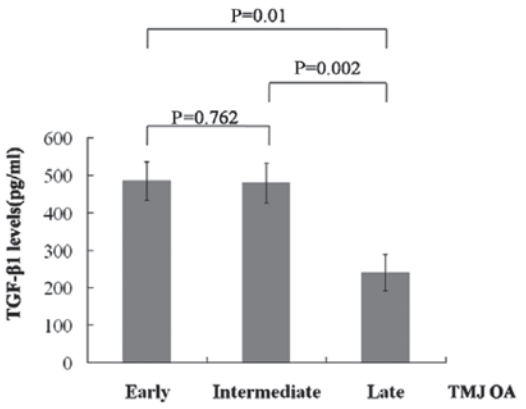


Figure 2. Transforming growth factor-β1 (TGF-β1) levels in synovial fluid from patients with temporomandibular joint osteoarthritis (TMJ OA) combined with disc displacement (DD) of varying staging are shown.

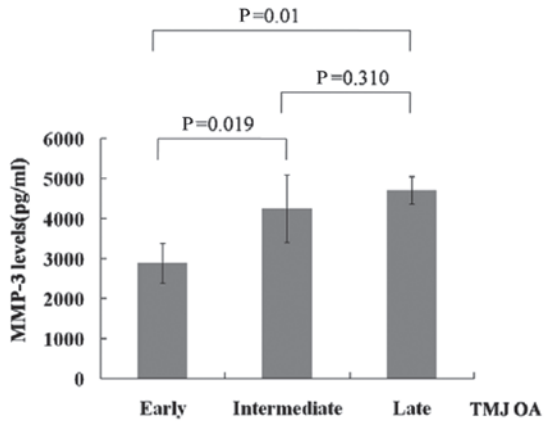


Figure 3. Matrix metalloproteinase 3 (MMP-3) levels in synovial fluid from patients with temporomandibular joint osteoarthritis (TMJ OA) combined with disc displacement (DD) of varying staging are shown.

Moreover, SF from patients with TMJ OA combined with DD of early staging had lower MMP-3 levels compared with patients of intermediate or late staging (Fig. 3).

## Discussion

SF produced by TMJ synovium normally functions as a biological lubricant as well as a biochemical pool through which nutrients and regulatory cytokines traverse (18,19). The cytokines are secreted by chondrocytes in articular cartilage and synoviocytes in synovium and concentrated in the synovial space by the semi-permeable synovial lining (20,21). Synovial membrane permeability can be altered by inflammation, which produces diseased SF because of alterations to biochemical mediators. Therefore, measuring the change of cytokines in TMJ may contribute to understanding the disease process of TMJ OA combined with DD.

The present study has shown that TGF-β1 and MMP-3 in SF are potential markers in assessing microenviroment inflammation or cartilage change in TMJ OA combined with DD. TGF-β1 is mainly released by articular chondrocytes in TMJ and secreted as an inactive complex comprising a TGF-β1 dimer, its propeptide latency-associated peptide (22). Active TGF-β1 stimulates chondrocytes responding with ECM synthesis from cartilage (23,24) and stabilizes the phenotype of the prehypertrophic chondrocytes (25,26). MMP-3 is secreted from synoviocytes and articular chondrocytes in a latent form, and binds with the tissue inhibitor of the metalloproteinase. MMP-3 is reported to crucial in matrix breakdown since it is capable of degrading a number of cartilage components, including proteoglycan, fibronection and collagens (12-14). Thus, in normal TMJ, small quantities of active TGF-β1 and MMP-3 are well-known in the development and homeostasis of cartilage and bone tissues (27-29).

At the initial stage of TMJ OA combined DD, TGF-β1 and MMP-3 levels in SF were increased while the destruction of bone and cartilage was not evident, which manifested as a reparative response at an early stage. Previous studies have shown TGF-β1 to be an important anabolic with proven beneficial effects on cartilage repair in the initiation of OA (30-32). In their study, van der Kraan *et al* (10) have demonstrated that an upregulated expression of TGF-β1 in early OA is found to be accompanied by increased synthetic activity. Blaney *et al* (31) have shown that TGF-β stimulates ECM, and counteracts the main catabolic roles at the early stage of murine knee joints OA. Takahashi *et al* (32) show that TGF-β1 counteracts the interleukin-1 (IL-1) upregulation of MMPs.

In the process of TMJ OA combined with DD, MMP-3 concentrations in SF were higher while TGF-β1 concentrations in SF showed a gradual decrease. MMP gene expression can be enhanced by pro-inflammatory cytokines, such as IL-1 and tumor necrosis factor-α (TNF-α) (33). Previous studies have shown a high prevalence of synovial inflammation in TMJ OA (34,35). Therefore, with the progression of TMJ OA combined DD, MMP-3 may be activated, while an excessive amount of active MMP-3 directly degrades the cartilage matrix. In addition, MMP-3 is able to activate other MMPs, such as MMP-1, MMP-7 and MMP-9. Thus, a significant increase of MMP-3 was not counteracted by insufficient amounts of TGF-β1, which aggravates the destruction of cartilage that results in the release of osteoclasia of cartilage and TMJ disc perforation.

Therefore, TMJ OA combined with DD is not considered to be a simple and unavoidable result of wear and tear, and sustaining or even enhancing the initial process may be an option. In our study, DD on MRI was observed in the 12 patients. This obvious change in TMJ anatomical structure may affect the homeostasis of joint cartilage. Thus, TMJ open disc repositioning procedures were performed on the 10 patients in the early and intermediate stages of TMJ OA, as well as another 2 patients in the late stage of TMJ OA combined DD, who had small disc perforation. Postoperative MRIs after three or six months showed that: i) the displaced disc was placed in its normal anatomical location; ii) preoperative degenerative changes did not exhibit any evident further development. Moreover, physiological remodeling, such as new bone formation was observed. These findings suggest that the displaced disc should be repositioned as early as possible in patients with TMJ OA combined with DD, in order that the aggravation of OA might be terminated and good condylar remodeling be achieved after disc repositioning, since sufficient amounts of TGF-β1 may be able to counteract the destructive effects of MMP-3 at an early stage.

To elucidate the cause of TMJ OA combined with DD, further investigation regarding the regulatory mechanism of TGF-β1 and MMP-3 in pre- and postoperative periods is required in animals and humans.

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