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NFκB and MMP13 expression in condylar cartilage of temporomandibular joint with occlusal disharmony in vivo

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ABSTRACT

Background: *Temporomandibular disorder (TMD) is a collection of symptoms that causes pain and disturbs a person's life quality. One of the trigger factors is mechanical overloading. Mechanical overloading in occlusal disharmony conditions will lead to an inflammatory reaction in the temporomandibular joint (TMJ). This condition will induce nuclear factor Kappa Beta (NFκB) activation to transcribe proinflammatory cytokines and matrix metalloproteinase-13 (MMP13) and will also degrade condylar cartilage as a major factor in strengthening the TMJ.* ***Purpose:*** *The aim of this study was to analyze the expression of NFκB and MMP13 in the condylar cartilage of TMJ with occlusal disharmony.* ***Methods:*** *This research was an experimental study with post-test-only control group design. Twenty Rats (Rattus norvegicus) were divided into four groups: One control group without any intervention and three experimental groups. Occlusal-reducing intervention was due on the right molar of the experimental groups, which were divided into the 7th, 14th, and 21st days. Immunohistochemical staining was performed to determine the expression of NFκB and MMP13 in the condylar cartilage. The data were analyzed by using the Welch test and independent t-test.* ***Results:*** *There were significant differences in NFκB and MMP13 expression between the control and experiment experimental groups ($p<0.05$). NFκB expression increased on the 7th, 14th, and 21st days of observation. The MMP13 expression showed a significant difference between the control and experimental groups ($p<0.05$).* ***Conclusion:*** *Occlusal disharmony increases NFκB and MMP13 expression and could affect TMJ integrity and induce TMD. These findings are important for describing the mechanism of TMJ damage and developing potential alternative therapies to prevent further TMD.*

Keywords: *condylar cartilage; dentistry; medicine; occlusal disharmony; temporomandibular disorders*
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INTRODUCTION

The temporomandibular joint (TMJ) is a synovial joint that functions to help the speaking process, opening and closing the mouth, and the process of chewing and swallowing food.[1] Damage to the TMJ is called temporomandibular disorder (TMD). It often occurs without the person realizing it and is assumed to be a common disease that does not require intervention. The prevalence of TMD is quite high in the world. Around 60–70% of the population of India experience TMD, but only 5–7% seek treatment.[2] In Indonesia, the prevalence of TMD was 23.4% among children aged 7–12 years and 36.9% among adolescents aged 13–18 years.[3] The other study was conducted on dental students consisting of 58.8% of TMD in the moderate category.[4] TMD is the second most common musculoskeletal disorder (after chronic back pain) that causes pain and disability and therefore influences the quality of life.

TMD describes a group of common musculoskeletal dysfunctions that affect TMJ or masticatory muscles.[1] The symptoms and signs include such things as clicking sounds and crepitus during the chewing process, which can be followed by joint pain, muscle pain, headache, ear pain, limitation of mandibular movement, deviation and deflection.[1,5] Habitually chewing on one side, bruxism and

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stress are cause factors of TMJ disorders.[6] Previous studies have mentioned that occlusal interference also causes TMD, but as yet, no clear understanding of this matter has been arrived at.[7] Occlusal interference caused by occlusal reduction without any correction will lead to an imbalance of occlusion or occlusal disharmony.[8,9]

Occlusal disharmony is caused by premature contact of the teeth, which will cause excessive occlusion pressure.[7] This occlusion is the primary factor in the onset of TMD symptoms.[8] However, if this condition is not treated, it will result in pressure on the TMJ articular and lead to inflammation and, potentially, joint damage.[10] There is an occlusion pattern among occlusal disharmony patients, and this condition will create obstacles or disturbances in the jaw movement process. In an unbalanced state of occlusion, functional movement will also change, which will increase the biomechanical enhancement of the TMJ.[10,11]

The over-biomechanical enhancement causes occlusion trauma to periodontal tissue and the TMJ, soft tissue damage, inflammatory reactions and cell damage.[1,11] Cell damage causes the appearance of damage-associated molecular patterns (DAMPS), which will activate the tool-like receptor 4 (TLR-4) in the inflammatory process. TLR4 activation will induce the activation of transcription factors, namely Nuclear Factor Kappa Beta cells (NFκB).[11] NFκB is a transcriptional protein for producing proinflammatory cytokines. Pro-inflammatory cytokines consist of interleukins (IL) such as IL-6, tumor necrosis factor-alpha (TNF-a), IL-1, IL-8, and degradation enzymes. These cytokines are known to be associated with pathological conditions of TMJ.[12] On the other hand, the degradation enzyme will be induced by cytokines. One of the degradation enzymes is MMP13, which is a collagenase degradation enzyme that degrades type II collagen as the main constituent of condylar cartilage.[13] MMP13 degrades the extracellular matrix by breaking collagen and proteoglycans in peptide bonds in target proteins.[14]

NFκB and MMP13 have an important role in the pathogenesis of TMJ destruction. The function of NFkB as a transcription factor for pro-inflammatory mediators and MMP13 degrades the extracellular matrix in condylar cartilage. However, the mechanism of inflammation caused by occlusal disharmony is still not fully understood or clearly described. Therefore, the aim of this study was to analyze the effect of NFκB and MMP13 expression on the condylar cartilage of the TMJ with occlusal disharmony in Wistar rats (*Rattus norvegicus*), an *in vivo* study.

MATERIALS AND METHODS

The research was carried out in an experimental laboratory with post-test-only control group design. All research procedures have been approved by the Research Ethics Commission of the Faculty of Dentistry, Airlangga University (No. 750/HRECC.FODM/XI/2019). The research sample consisted of 20 male rats (*Rattus norvegicus*) Wistar strain weighing 200–250 grams.

The samples were divided into two large groups, namely one normal group as a control group and an experimental group, subdivided into three further groups, each group comprising five male rats.[15] The experimental groups were subject to the intervention of occlusal reduction. Occlusal reduction was performed on the maxillary and mandibular right posterior teeth. Occlusal reduction used a fissure diamond bur low speed with a stopper at 1 mm, so all the occlusal surface was flat (±1 mm).[9] The procedure of occlusal reduction did not cause dental pulp perforation. The experimental groups were the 7th, 14th, and 21st day groups. However, the control group was not subject to any intervention.

The TMJ tissue of the rats was taken on the 7th, 14th and 21st day after the experimental procedure.[16] Furthermore, the process of histological preparation process employed immunohistochemical methods using a monoclonal antibody to investigate the expression of NFκB (anti-NFκB p65 [f-6:sc8008], Santa Cruz Biotechnology, Inc., USA) and MMP13 (Anti-MMP13 antibody [EPR21778], Abcam, Massachusetts, USA) on the condylar cartilage. The positive results of immunohistochemical staining were shown as brown. The x200 magnification digital

Table 1. The rate value of NFκB expression between the control and experimental groups

Group	Mean ± Standard Deviation			p
	Day-7	Day-14	Day-21	
Experimental	0.27^{b} ±0.58	0.62^{c} ±0.94	0.20^{c} ±0.94	0.000*
Control		0.13^{a} ±0.14		

Notes: * the significance of α=0.05 (Welch test). The differences between a, b, and c superscript showed that there were differences among each group using the independent t-test.

Table 2. The rate value of MMP13 among the control and experimental groups

Group	Mean ± Standard Deviation			p
	Day-7	Day-14	Day-21	
Experimental	1.49^{b} ±0.43	2.69^{b} ±1.94	2.25^{b} ±1.52	0.001*
Control		0.11^{a} ±0.11		

Notes: * Significant value of p<0.05 (Welch test). The differences between a, b, and c superscript showed that there were differences among each group using the independent t-test.

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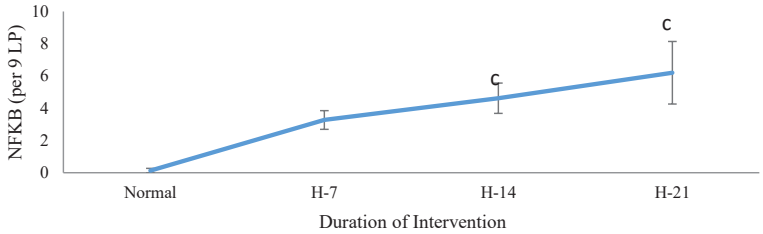

Figure 1. The increase in the NFκB expression rate between the control and experimental groups.

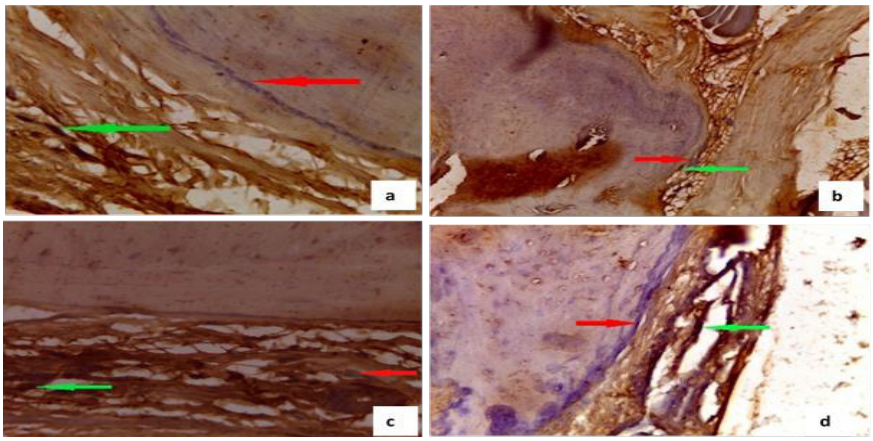

Figure 2. The expression of NFκB in the cartilage of the TMJ among the control group (a), the 7th day (b), the 14th day (c), and the 21st day (d). The expression of NFκB is shown in brown (green arrow) and the cells that did not express NFκB are shown in purple (red arrow) (x200).

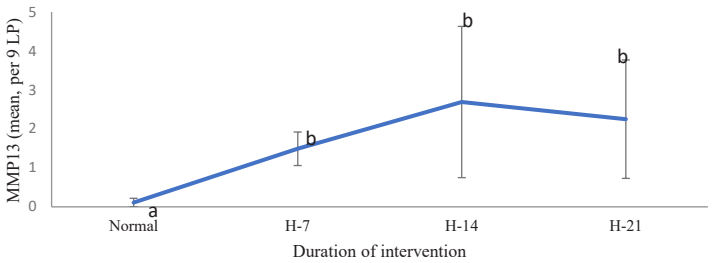

Figure 3. The increase in the MMP13 expression rate in the control and experimental groups.

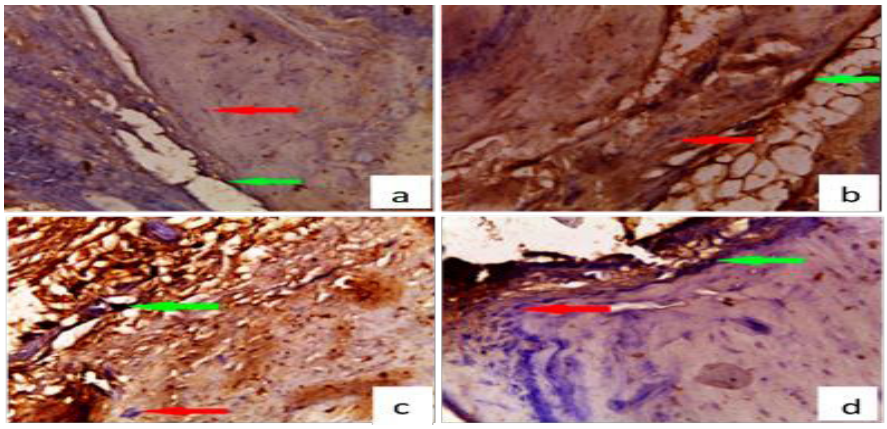

Figure 4. The expression of MMP13 in the cartilage of the TMJ among the control group (a), the 7th day (b), the 14th day (c), and the 21st day (d). The expression of NFκB is shown in brown (green arrow) and cells that did not express NFκB are shown in purple (red arrow) (x200).

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images analyzed the average optical density of each slice. The data of NFκB and MMP13 was analyzed by using the Welch test and was followed by an independent t-test ($p<0.05$).

RESULTS

The results of this study showed that the expression of NFκB was high in the experimental group (Table 1). NFκB expression grew as observation time increased, namely the observations on the 7th, 14th, and 21st days (Figure 1). NFκB was expressed in chondrocytes on the mandibular condyle of the TMJ (Figure. 2). The independent t-test showed that there was a significant difference between the control and experimental groups ($p<0.05$), and there was a significant difference between the 7th, 14th, and 21st days of observation among the experimental groups.

The expression of MMP13 in this study was shown to be higher in the experimental group (Table 2). MMP13 expression increased along with the observation time (Figure 3). The increase in MMP13 expression was seen in both groups (experimental and control). The period from the 7th to the 14th day also resulted in an increase in MMP13 expression. However, the MMP13 expression decreased from the 14th to the 21st day. MMP13 was expressed in the cartilage of the TMJ (Figure 4).

DISCUSSION

NFκB is a transcriptional protein that produces proinflammatory cytokines and an extracellular matrix of degradation enzymes.[13,17] In this study, NFκB was expressed in chondrocyte cytoplasm cells in the condylar cartilage of the TMJ. The expression of NFκB indicates an inflammation process that induces the pro-inflammatory mediators.[17] The previous study stated that pro-inflammatory cytokines such as IL-6, TNF-, IL-1 and IL-8 are associated with TMJ pathological conditions. Another study also stated that the function of NfκB is to regulate the inflammation process, immune response and wound healing, as well as apoptosis and helping cell function.[17,18] In autoimmune diseases, such as rheumatoid arthritis (RA), NFκB has an important role in the differentiation, development stage, activation and development of osteoclasts.[19]

This study showed that the expression of NFκB was carried out in chondrocytes in the condylar cartilage of the TMJ. NFκB is a transcription factor located in the cytoplasm and will translocate to the nucleus after activation. The activation process was induced by various agents including stress, mechanical stress, cigarette smoke, viruses, bacteria, inflammatory stimuli, cytokines, free radicals, carcinogens, tumor promoters and endotoxins.[20] The results of this study showed that the expression of NFκB in the experimental group on the 7th, 14th, and 21st days of observation was significantly higher than that of the control group. The function of NFκB is crucial to induce inflammation, which leads to a chronic systemic inflammatory state. NFκB is a transcriptional activator of stronger inflammatory response genes. The excessive and deregulated expression of NFκB leads to uncontrolled inflammation. Systemic and regulated inflammatory responses are part of the body's defense against injury, infection and disease.[18] Several studies mentioned that NFκB is the main inflammatory mediator in rheumatoid arthritis and cancer, cervical cancer, nasopharyngeal carcinoma, periapical lesions and TMJ osteoarthritis.[19–23] Activation of NFκB in the synovial layer also occurred among mice that had synovitis caused by a mechanical stress response due to excessive pressure.[24]

NFκB expression was shown to be higher on the 7th and 14th days and was at its highest on the 21st day. This indicates that an excessive mechanical load was able to induce the NFκB pathway in the experimental group. These results were similar to previous studies that showed that NFκB expression was detected on the 15th day of observation and played a role in TMJ cartilage damage.[19,21] The occlusal reduction on the right molar in animal groups made premature contact on the collateral side. The occlusal disharmony caused by occlusal reduction induces the excessive stimulation of mechanic sensors at the periodontal ligament and is related to an increase in mastication activities, including in the muscles and the TMJ.[25] Maxillomandibular changes due to occlusal disharmony cause overload articular burden and will lead to TMJ changes.

The mechanical overloading caused by occlusal disharmony induces cell damage and results in the appearance of DAMPS, which will activate the TLR-4 in an inflammatory process and induce the activation of NFκB to induce the release of pro-inflammatory cytokines.[26] The pro-inflammatory cytokines consist of IL-6, TNF-, IL-1, and IL-8 as well as degradation enzymes. These cytokines are known to be associated with pathological conditions of the TMJ.[13,26] The degradation enzyme responsible for degrading collagen is MMP13.[14] MMP13 is a collagenase degradation enzyme, which degrades type II collagen as the main constituent of the condylar cartilage. MMP13 degrades the extracellular matrix by breaking collagen and proteoglycans in peptide bonds in target proteins.[13,27] Extracellular matrix degradation will occur and cause cartilage damage, so the function of the TMJ will be disrupted.

The results of this study show that the expression of MMP13 in the experimental group was higher than that in the control group. The results also indicate that occlusal disharmony in the experimental group induced MMP13 secretion, which means that it is associated with TMJ damage. MMP13 expression increased on the 7th day of observation and reached its highest level on the 14th day of observation. The increase in MMP13 in the homeostatic process stimulates the secretion of regenerative enzymes, namely TIMP-1, PAI-5 and growth factors such as IGF-1,

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TGFβ, and FGF.[14,27] This process caused a reduction of MMP13 expression on the 21st day. This result was supported by a previous study that there was a reduction in MMP13 on the 21st day as a homeostatic response, but there was an increase on the 28th day.[14] In addition, MMP13 expression is dependent on the degree of transcription and other inflammatory mediators and endogenous inhibitors. The magnitude of the mechanical load not only affected the changes in cytokines but also affected the tissue response to cytokines.[28] A high level of MMP13 was found among the patients who experienced mild to severe disorders of the TMJ.[29]

Another study showed that the expression of MMP13 was highest during the second week among the experimental group with occlusal reduction on the anterior teeth and MMP13 expression showing on the chondrocytes in the cartilage.[30] The increase in MMP13 expression started on the 3rd day and increased on the 7th and 14th days. On the 7th day, MMP13 expression showed an increase. By the 14th day, the hypertrophic zone and proliferative layer had increased as had the hypertrophic layer by the 21st day.[16]

MMP13 is expressed in synovial joints and articular cartilage among patients with TMJ disorders and is almost undetectable in adult tissue. This enzyme is known as an extracellular matrix (ECM) and is involved in the degradation of joint damage such as osteoarthritis. The occlusal interference causes abnormal masticatory function and induces the deterioration of TMJ tissue homeostasis.[31] In another study, MMP13 was linked to the presence of pathological conditions of chondrocyte cells and occurred in the early phase of osteoarthritis.[27] MMP13 is a major enzyme that degrades the extracellular matrix by breaking peptide bonds in target proteins, so it will break down collagen and proteoglycans.[13,32] However, the research conducted still has its limitations due to the lack of samples of TMJ disorders. Therefore, further research on the specific effects and role of MMP is needed.

In conclusion, this study has shown that occlusal disharmony could induce an increased level of NFκB and MMP13 expression and lead to temporomandibular disorders. These findings provide the basis to customize and enhance the quality of the treatment of patients with TMJ disorders. Therefore, in future studies, it will be necessary to study the specific impact of MMP on the TMJ to provide a better understanding of TMJ destruction and develop a valuable therapy for TMD.

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