

Differences of mandibular condylar cartilage thickness of Mus musculus after given different intensities of physical stress

Rizentya Salsabila ¹, Rachma Fadhilah ¹, Dafif Ayatillah ¹, Ni Putu Mira Sumarta ^{2,*}, Muhammad Subhan Amir ² and Sisca Meida Wati ³

¹ Undergraduate Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

² Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

³ Department of Oral and Maxillofacial Pathology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

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Abstract

Objective: This study aimed to evaluate the differences in the thickness of Mus musculus mandible cartilage after being given low, moderate, and high-intensity physical stress.

Materials and Methods: This research is an experimental laboratory study. A total of 12 Mus musculus were divided into four groups, which are the control group (C), low (LS), moderate (MS), and high-intensity physical stress group (HS). The physical stress group was given treatment with swimming exercises using 3%, 6%, and 9% of body weight with a duration of 40%, 70%, and maximum swimming time calculation. After the experiment was carried out, the animal was decapitated and deparaffinized using xylol to dissolve paraffin in tissue to make histopathology preparations that were stained with hematoxylin-eosin. The thickness of mandibular condylar cartilage was calculated under a light microscope with a magnification of 400x and analyzed using the One Way ANOVA test to determine if the relationship between the sets of data in the treatment group is statistically significant ($p < 0.05$).

Results: The thickness of the mandibular cartilage was measured to be thinner in the MS group (56.30 ± 6.11) and the HS group (78.04 ± 19.31) compared to the C group 85.85 ± 7.20 . Statistical analysis with One Way ANOVA test showed that all groups had a significant difference in the thickness of mandibular condylar cartilage ($p = 0.049$).

Conclusions: There was a difference in the thickness of the mandibular cartilage after giving low, moderate, and high-intensity physical stress.

Keywords: Articular Condyle; Human and health; Mandibular condyle; Physical Stress; Temporomandibular Joint

1. Introduction

The mandibular condylar cartilage is crucial for efficient jaw movement and disc articulation in the temporo mandibular joint (TMJ). TMJ problems are much more common in stressed people than healthy people, according to the Prospective Evaluation and Risk Assessment of Orofacial Pain (OPPERA) study [1]. Stress is a normal component of human physiology. Still, when it is too strong for the body to handle, stress can disrupt the body's homeostasis by disrupting the immunological, hormonal, and autonomic nervous systems [2][3].

Stress is a physical condition of the brain and body that develops when physical stimuli influence homeostasis, these situations or stimuli are considered as a trigger for stress is known as a stressor. Stress is divided into psychological and physical stress. Physical stress is defined as force applied to a specific area of biological tissue. Physical stress is

* Corresponding author: Ni Putu Mira Sumarta

processed by the brainstem and hypothalamus[4]. Psychological stress is an adaptation to the fight-or-flight response over the course of evolution, can lead to a constellation of physiological responses (including systems nervous, endocrine, and immune) which can be harmful in some condition [5].

Psychological stress dilates medial and posterior condylar cartilage and produces isolated lesions of the bone under the condylar [6]. Condylar cartilage is divided into four zones, which are grouped distal-proximal subchondral condyles bones: fibrous zone, proliferation zone, mature zone, and hypertrophic zone [7]. Osteoarthritis (OA), which is characterized by cartilage degeneration, is one of the degenerative changes in the TMJ condylar cartilage [8].

TMJ will decrease gradually if the pressure on the chewing muscle exceeds the load on the mandibular condyle. Its hallmarks are marginal proliferation, proteoglycan loss, early cartilage fibrillation, and subchondral bone changes. Stress can cause TMJ anomalies and histological changes and damage the ultrastructure of the articular cartilage. Remodeling of condylar cartilage occurs when animals are stressed and are advised by changes over time [9].

Excessive levels of physical stress can make tissues more resistant to more stress.[7] Physical stress has been widely used to examine physiological changes and the ability of organisms to cope with stress [10]. According to previous research, high- intensity physical stress increases antioxidant defenses, which cause oxidative stress. Exercise volume (length and intensity) may be a major modulator of exercise-induced oxidative stress, according to recent research showing that low to moderate intensity can cause oxidative stress [11].

The thickness of condylar cartilage is influenced by physical and mental stress. Based on the background above, researchers conducted a study of differences in the thickness of the condylar cartilage of the temporomandibular joint in rats subjected to low-moderate and high-intensity physical stress using histopathological examination with a light microscope.

2. Material and methods

2.1. Experimental Design

This was a laboratory experiment study with a posttest-only control group design. The ethical clearance committee approved this research of the Faculty of Dental Medicine, Airlangga University, Indonesia (No.521/HRECC.FODM/VIII/2022).

2.2. Experimental Animal Treatment

A total of 12 *Mus musculus*, aged 2-3 months, weight 20-30 grams, and healthy mice (characterized by active movement) were included in the study. The first two days are cage optimization time. The experimental animal was introduced to the watery environment on the third day but was not given swimming treatment. Days four and five are acclimatization time for light swimming. Weight training was given on the sixth day, and rest time was given on the seventh day.

The samples were categorised into four groups, each comprising three samples. The C group solely engaged in routine activities, such as eating and drinking, and did not undergo any physical stress in the form of swimming exercises. The LS group consisted of *Mus musculus* subjected to swimming for a duration equivalent to 40% of their average maximum swimming time, with an additional ballast load equivalent to 3% of their body weight. The MS group consisted of *Mus musculus* subjected to swimming for a duration equivalent to 70% of their average maximum swimming time, with an additional ballast load equivalent to 6% of their body weight. The HS group consisted of *Mus musculus* were subjected to swimming with the maximum count of swimming time, with an additional ballast load equivalent to 9% of their body weight.

2.3. Histopathological Preparation

After being treated, the experimental animal was decapitated and deparaffinized using xylol to dissolve paraffin in tissues. Then rehydration is done using absolute alcohol, 90% alcohol, and 80% alcohol to enter water into the tissue. The first stained use 0.6% HCl and then 0.5% lithium carbonate; the second stained uses eosin. Dehydration is repeated using 80% alcohol, 90% alcohol, and 100% alcohol to remove water from the tissue. mounting is done using Entellan to preserve the stained tissue. The thickness of the mandibular condylar cartilage is measured based on the results of photos taken using the Nikon Disk Element software on a computer connected to light microscope with 400x magnification. The data of the mandibular condylar cartilage thickness was taken on 5 different sections.

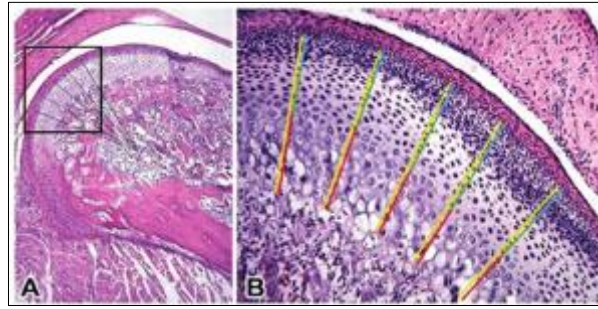


Figure 1 Measurements of histological sections of the rat mandibular condyles were carried out in each group with 400x magnification [12]

2.4. Statistical Analysis

The research data were analyzed with descriptive statistics to describe variable characteristics, the Shapiro-Wilk normality test to determine whether the data obtained came from a normal population, the data homogeneity test, and the Levene test to determine whether the conditions before the treatment were the same for all groups, and the ONE Way ANOVA Test to determine whether there was a difference in the thickness of mandibular condylar cartilage after being given physical stress with a rating of $p < 0.05$ to be considered significant. Data were analyzed using Statistical Product and Service Solution (SPSS) for Windows version 20.0.

3. Results

The Control group (Fig. 2.a) shows a well-formed mandibular cartilage condyle and fibrous zones with a regular arrangement of connective tissue. There can be seen the fibrous, proliferative, mature, and hypertrophic zones showed by colored arrows. In the hypertrophic zone, it can be seen that there are quite a lot of chondrocyte cells, flat and have fairly large sizes. In the LS group (Fig. 2.b), condylar cartilage is not composed as well as in the control group. Conversely, in the anterosuperior part, there is damage, the fibrous zone is looks more thicker than the other group. In this zone, dense and coarsely arranged connective tissue is visible. The hypertrophic zone showed the chondrocytes still looked dense as in the control group. The MS group (Fig. 2.c) shows an irregularly arranged cartilage of the condyles, and the fibrous zone is very thin even though the connective tissue is almost invisible. Chondrocytes in medium intensity group appear less dense and spread out in the hypertrophic zone. In the high-intensity group (Fig. 2.d), condylar cartilage is not arranged as well as in other groups, while only parts are damaged and not intact and have very thin fibrous zone. The hypertrophic zone appears to be damaged, indicated by fragmented tissue. Chondrocytes in high intensity group appear more less dense than in medium intensity group and spread out in the hypertrophic zone.

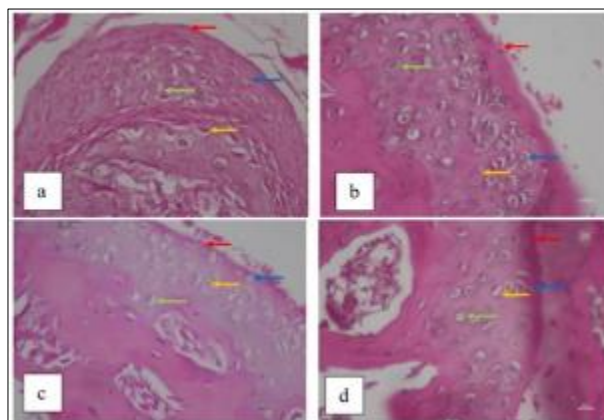


Figure 2 Thickness histological picture of the condylar cartilage of temporomandibular joint with hematoxylin-eosin staining under a light microscope with 400x magnification. There can be seen the fibrous zone showed by red arrows, proliferative zone showed by blue arrows, mature zone showed by green arrows, and hypertrophic zones showed by orange arrows. (a) control group, (b) low-intensity group, (c) medium intensity group, and (d) high-intensity group

Table 1 Result of the study

Groups	Number of samples	Average \pm Standard Deviation	Shapiro-Wilk test	Levene test	One Way ANOVA test
Control Group	3	85.85 \pm 7.20	0.290	0.195	0.049
Low -intensity	3	86.47 \pm 10.93	0.077		
Moderate-intensity	3	56.30 \pm 6.11	0.324		
High-Intensity	3	78.04 \pm 19.31	0.678		

The increased in the thickness of the condylar cartilage can be seen in the low- intensity physical stress group compared to the control group (Table 1). There was also a decrease in the thickness of the condylar cartilage in the moderate and high- intensity physical stress group compared to the control group.

The normality test using Shapiro-Wilk probability value p for mandibular condylar cartilage thickness in C, LS, MS, and HS groups showed that all groups had a value of $p > 0.05$, so it consider that data was normally distributed. The homogeneity test result for all groups has $p = 0.195 > 0.05$, so it consider data has homogeneous variance. The One Way ANOVA test showed cartilage thickness results based on four groups with $p = 0.049 > 0.05$, so it consider as significant.

4. Discussion

Physical stress is defined as a force applied to certain areas of biological tissue or a response to environmental pressures and demands. The level of physical stress that exceeds the maintenance range, i.e., overload, increases tissue tolerance to the next stress so that any applied force can cause a change in the physiological response to being counted as physical stress [13]. In this study, Mus musculus were used as experimental animals by given swimming treatment as indirect physical stress for 6 weeks to consider a chronic stress.

Based on the study's results, indirect physical stress was shown to cause changes in cartilage thickness compared to the control group. Histological examination showed the thickest thickness of the mandibular cartilage in the low- intensity group and the lowest in the medium-intensity group. This proves that the administration of physical pressure with different intensities causes changes in the thickness of the condyles cartilage in the temporomandibular joint. In histopathological observations, fibrous zones in the moderate and high-intensity stress groups were thinning. In addition, chondrocytes were seen less dense in Mus musculus given low, moderate, and high-intensity physical stress as in the control group. This happened because stress could cause chondrocyte apoptosis and cartilage damage through the overactivation of endoplasmic reticulum stress and hypoxia [14]. In the high-intensity stress group, thicker fibrillation was seen compared to other groups. Fibrillation is an early sign of degeneration in cartilage and histologically, the surface of the cartilage appears irregular.[15]

The thickness of the mandibular cartilage in the moderate physical stress group proved to be lower compared to other groups. Decreased cartilage thickness is associated with the pathogenesis of TMJD. Specific cellular signaling induced by mechanical stress, or mechanotransduction, plays an important role in normal development, tissue dysfunction, and degeneration.[16] Physical stress does not directly stimulate the HPA axis to release stress hormones that damage cartilage[17]. In contrast, direct physical stress will increase levels of inflammatory mediators and degradative enzymes that are directly present in cartilage.[18]

Based on research that conducted by Chen et al (2017) demonstrate a relationship between basal HPA axis activity and inflammation, as well as responsiveness and habituation of the HPA axis. It has been shown that chronic activation of the inflammatory and HPA axis systems can have unfavourable long- term repercussions. In animal studies, it was found that excessive cortisol release negatively impacted brain systems, particularly hippocampal glucocorticoid receptors, which decreased the HPA axis's feedback sensitivity. Inflammation has also been shown to have a feed-forward impact on the HPA axis, which can be stimulated by peripheral inflammatory substances to produce more cortisol, which has an anti- inflammatory effect. [19] When the organism is burdened by cumulative stress, cortisol will increased. Due to prolonged exposure to the catabolic effects of glucocorticoids, stress peptides, and proinflammatory cytokines, the organism becomes worn out. This burden strains the body and may affect the emergence of metabolic and neuropsychiatric problems. Understanding the mechanisms that control cortisol production is crucial. [20]

Stress in the form of swimming and loading given to animals tries to affect muscles, joints, and bones in other body parts, such as knees and thighs. There is some evidence that TMJ pain is associated with chronic pain in other joints. Recurrent and persistent pain can be associated with peripheral and central nervous system changes. In addition, genetic factors such as COMT gene polymorphism (catechol-O- methyltransferase) and ADRB2 (beta-2 adrenoceptor) increase in sensitivity to pain and chronic conditions of musculoskeletal pain. The ADRB2 receptor is a ligand coupled to G-protein and expressed in peripheral sites of the spinal cord involved in pain transmission, producing allodynia through the activation of intracellular kinases and facilitates the transmission of pain through the release of pro-inflammatory molecules. As a result, stress on joints in other parts of the body can indirectly cause pain and osteoarthritis in TMJ joints.[21]

Cartilage degradation occurs due to an imbalance between mechanical stress on the joint and cartilage absorption. Cartilage remodeling is maintained by a balance between catabolic and anabolic processes, a compensatory mechanism in response to pressure or stress to maintain homeostasis in normal and healthy individuals. This creates an imbalance between renovation and degradation, so low-intensity stress leads to more renovation than degradation. In contrast, moderate and high-intensity groups experienced greater degradation than remodeling.[18]

Changes in TMJ are related to position, especially the position of the pelvis, lumbar and thoracic spine, head, and mandibles. This disorder seems to strengthen the relationship between the position of TMJ and other parts of the body. Disorders in one joint subunit can cause compensation in another joint. [15] Mechanical stimuli modulate the thickness of the mandibular cartilage and the proliferation of chondrocytes. Hypertrophic chondrocytes play a role in mechanoresponsive mechanisms and cause increased metabolic activity and activation of pathological processes that can cause irreversible cartilage degradation.[22]

In the research results, there were differences in cartilage thickness in each group due to indirect physical stress through swimming time to see the changes that occurred in the TMJ. Indirect physical stress stimulates the HPA axis to secrete stress hormones which trigger damage to cartilage.[23] In direct physical stress, there is an increase in inflammatory mediators and degraded enzymes which occur directly in the cartilage.[18]

In addition, the results showed that the thickness of the mandibular cartilage in the low-intensity physical stress group was thicker than the control group, medium, and high-intensity groups. It happens because mandibular condyle cartilage is cartilage that is able to withstand loads and functions as a template for longitudinal growth and bone remodeling will occur through The TAZ, Wnt, MAPK pathways. [24]

5. Conclusion

There is a difference in the thickness of the mandibular cartilage after giving low, moderate, and high-intensity physical stress.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The ethical clearance committee approved this research of the Faculty of Dental Medicine, Airlangga University, Indonesia (No.521/HRECC.FODM/VIII/2022).

Statement of Authorship

All authors participated in data collection and analysis, and approved the final version submitted.

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