



ORIGINAL ARTICLE

Impact of estrogen therapy on temporomandibular joints of rats: Histological and hormone analytical study

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KEYWORDS

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Abstract Objectives: This research aims to evaluate the effects of estrogen deficiency and replacement on the TMJ structures of rats. The considerable similarities in the anatomical features of rats and humans make rats a suitable model for human scientific studies.

Methods: A clinical trial was conducted on 18 female Sprague-Dawley rats grouped into three categories. The GI group included 6 female rats labelled as the control group, the GII group consisted of 6 females that received ovariectomies, and the GIII group had 6 ovariectomised females that were injected with estrogen replacement therapy in a science laboratory at King Abdulaziz University. ANOVA and Tukey HSD post hoc tests were used to determine any significant differences between the levels of estrogen among the three groups.

Results: The results indicate that some TMJ structures, including the articular disc and condylar cartilaginous layer, were degraded after estrogen deficiency. However, there was a slight improvement in the cartilaginous layer thickness and proliferation of chondroid cells after estrogen replacement therapy. Estrogen level was reduced in the ovariectomized rats, and while estrogen injections increased blood hormone levels, the levels did not reach those of the control group.

Conclusion: Estrogen deficiency degraded some TMJ structures, and there is only a slight recovery after estrogen replacement therapy.

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1. Introduction

Temporomandibular disorder (TMD) is an orofacial disorder that affects the masticatory muscles and temporomandibular joints. It is characterized by pain in the joints, muscle spasms, limited opening of the mouth, clicking and locking of the jaw, and deviation of the mandible (Cooper and Kleinberg, 2007).

The etiology of TMD is multifactorial, which contributes to induction, perpetuation, and even aggravation of TMD (Habib et al., 2015). Causes of TMD include traumatic injury, stress, immune disease, malocclusion, and parafunctional habits (Oliveira et al., 2006).

The prevalence and severity of TMD were reportedly higher in females than males, mainly between the ages of 20–40 years (Bagis et al., 2012; Wänman, 1996). Children, adolescents, and aging women were rarely affected (Kamisaka et al., 2000). The TMD symptom severity also depends on the patient's age, as the pain onset tends to occur after puberty and peaks in women's reproductive years. There is also an increased risk of TMD in women who have received exogenous estrogen (Bagis et al., 2012).

Estrogen is a female sex hormone that is responsible for the growth and development of the female genital system (Palmblad, 2019). It can be used as hormonal replacement therapy for postmenopausal women, and it can also serve as an oral contraceptive and as therapy for certain hormone-sensitive cancers, such as breast and prostate cancers (Girdler, 1993).

A rat's TMJ is a suitable model for human scientific studies since the shape of the disc, synovial membrane, temporal articular surface, and condyle are similar in humans and rats (Porto et al., 2010). Although rats have a differently shaped articular surface and their condyle axis has larger angles, a rat's TMJ is still considered a convenient model (Orset et al., 2014). Rats also have a short reproductive cycle of four days, which encompasses the metestrus, pro-estrus, and estrus cycles (Mechoulam et al., 2005). Estrogen starts to rise during the metestrus cycle, reaches its peak in the pro-estrus cycle, and then decreases in level during the estrus phase (Mechoulam et al., 2005).

Previous research has demonstrated the role of estrogenic hormones in the development of cartilage, pathologies, and osteoarthritis. A study of ovariectomized rats revealed that the thickness of the articular fibrous tissue was increased in the anterior and central parts of the condyle while the bone volume decreased in the anterior and posterior portions two weeks post-surgery (Yasuoka et al., 2000). A study by Kuroda et al. (2003) has shown that the bone mineral density of the condylar region and the trabeculae of the mandibular bone decreased in ovariectomized rats after 109 days. In contrast, a study was done of the condyles of rats that were sectioned and immersed in the 17 β -estradiol hormone. After four days, there was a decrease in the total thickness of the condylar cartilage and an inhibition of the chondrocyte's proliferation (Talwar et al., 2006).

Previous studies have shown that estrogen deficiency and replacement influence some TMJ structures differently and that the effects can vary depending on the time period. This study aims to investigate the effects of estrogen deficiency and replacement, in particular on the condyles of rats in four weeks.

2. Materials and methods

This study utilized 18 female Sprague-Dawley rats, albino laboratory rats that are used in medical experiments in the laboratory of science at King Abdulaziz University. The weight of the female rats ranged from 180 to 250 g at the time of their

arrival at the animal house at the age of 3–4 months, characteristics that were well-suited for these experimental animals. The rats mainly consumed stock water and soybean-free dry meals (since soy contains estrogen).

The rats were grouped into three categories: GI (6 control rats); GII (6 rats that received ovariectomies); and GIII (6 rats that received ovariectomies and estrogen replacement).

The rats were anaesthetised by intramuscularly injecting ketamine (55 mg/kg) mixed with xylazine (5.5 mg/kg). Bilateral flank incisions ovariectomies were performed. The ovarian bundles were removed after ligation with 4–0 silk sutures and closing the skin with 5–0 silk sutures.

According to Talwar et al. (2006) and Yasuoka et al. (2000), thirty days after surgery, the animals were treated as follows:

The 6 ovariectomised animals of GIII were intramuscularly injected with estradiol with a dosage of 0.5 mg/kg of body weight every 7 days in 4 weeks.

The serum estrogen levels were measured in (1) the ovariectomized rats, (2) the ovariectomized rats that received the hormone replacement therapy, and (3) the control rats at the pro-estrus phase of the estrus cycle according to Marcondes et al. (2002). Estrogen measurements were taken from the right and left sides of the TMJ, 7 days after the last injection. The data were recorded and statistically analysed using the one-way Analysis of Variance (ANOVA) to compare the levels of hormones in each group. When ANOVA yielded considerable variance in the hormone levels of the groups, the Tukey HSD Post-hoc test was applied.

3. Results

3.1. Histological studies

Histological analysis was conducted at the end of the fourth week for all groups. The control group TMJs was conducted to examine the condylar and glenoid fibrous layers, the condylar and glenoid cartilage layers, the temporodiscal and condylodiscal spaces, and the subarticular bone marrow. The fibrous layer that covered the condylar head included numerous fibroblasts that were scattered through dense parallel-arranged avascular layers of collagen fibers (Fig. 1).

In the group induced by ovariectomies, TMJ histological changes were noted in the articular disc such as the central fusion of the same to the fibrous and cartilaginous layers of the mandibular condyle, and irregular thickness of the condylar cartilaginous layer that was associated with chondrocyte condensation. (Fig. 2).

After the ovariectomies and estrogen replacement therapies, TMJ changes were noted in the condyle fibrocartilaginous layers of the rats. Besides, there was a lack of interface between the condylar cartilaginous and fibrous layers. In these rats, the cartilaginous layers had an increased thickness and included a proliferation of chondroid cells (Fig. 3).

3.2. Statistical analysis

It was established that the control animals have the highest estrogen level at 87.30 among the experimental ones, particularly the ovariectomised, which has an estrogen level of 47.80, and those undergone estrogen replacement therapy,

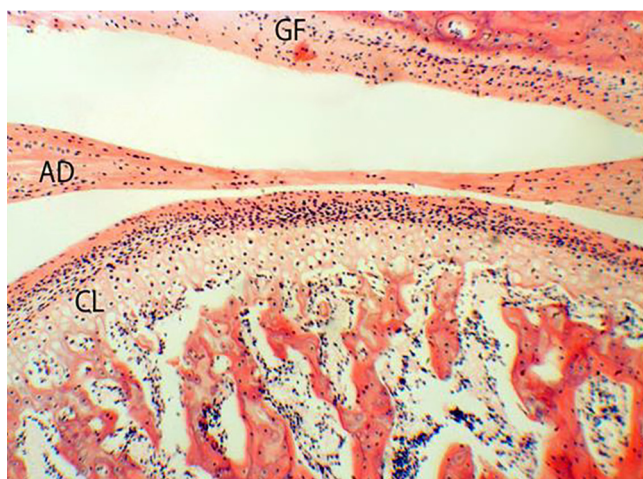


Fig. 1 Photomicrograph from the sagittal section of the temporomandibular joint (TMJ) from a female rat in the control group (GI) showing the histological components. Legend: roof of the glenoid fossa (GF); condylar bone (CL); articular disc (AD). (H & E stain, orig. mag. X100).

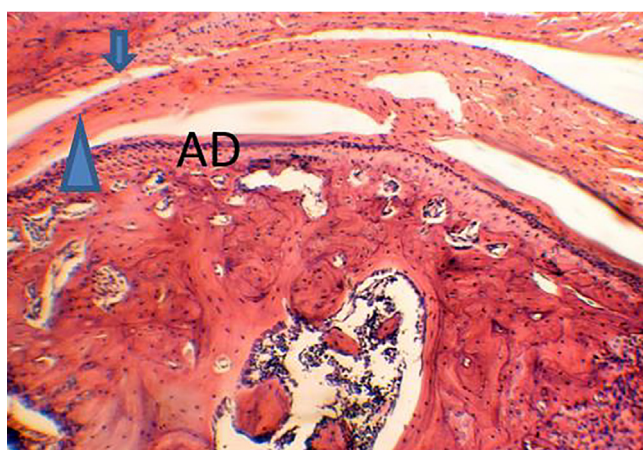


Fig. 2 Photomicrograph from the sagittal section of the TMJ from a female rat in the group (GII) showing the fragmentation of the articular disc (arrow) and the fusion of the central part of the disc with the fibrous layer of the condylar head (arrowhead), the irregular thickness of the condylar cartilaginous layer, and the approach of the bone marrow spaces to the condylar cartilaginous layer. (H& E stain, orig. mag. X100).

which has 60.20 estrogen level. Nevertheless, they have the same p-value of 0.001 (Table 1) and (Fig. 4).

A comparative study was conducted on the mean differences of the estrogen levels between the control and experimental animal group, which shows the following statistical data, viz: Control vs. Ovariectomy - the estrogen levels in the control animal groups is higher compared to the ovariectomised with a significant mean difference of 39.5; Control vs. Estrogen Replacement Therapy - the estrogen levels in control animals is likewise higher than those undergone replacement therapy with a mean-variance of 27.1; and Ovariectomy vs. Estrogen Replacement Therapy - the estrogen level in ovariectomised animals is relatively lesser than those undergone hormone

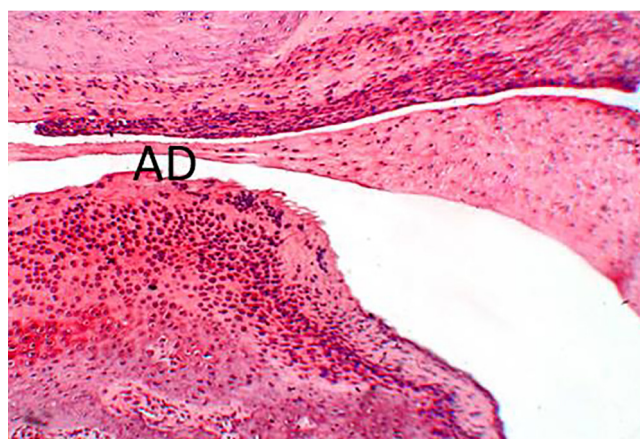


Fig. 3 Photomicrograph from the sagittal section of the TMJ from a female rat in the group (GIII) showing the changes in the condyle fibrocartilagenous layer and a lack of interface between the condylar cartilaginous and fibrous layers. The cartilaginous layer exhibited an increased thickness and a proliferation of chondroid cells. (H & E stain, orig. mag. X00).

replacement therapy with a mean difference of 12.4. However, both the animal and experimental animal groups garnered the same p-value of 0.001 (Table 2).

4. Discussion

TMDs encompass a range of pathological conditions that involve pain and dysfunction in the masticatory system. This multifactorial condition is recognized by symptoms in the joints and their structural components (Kapila and Xie, 1998). TMD may be caused by a variety of inflammatory disorders that trigger degenerative changes to the joints (De Leeuw and Kasser, 2008; Mercuri, 2008). This study researched the impact of the deficiency of estrogen on the TMJs of female rats and its subsequent replacement. Although rats do not have an articular eminence (Tanaka et al., 2008), rats were suitable experimental models for these TMJ studies due to the close morphological and histological similarities of their TMJ articular structures to those of humans.

4.1. Effects of decreased estrogen levels on the TMJ

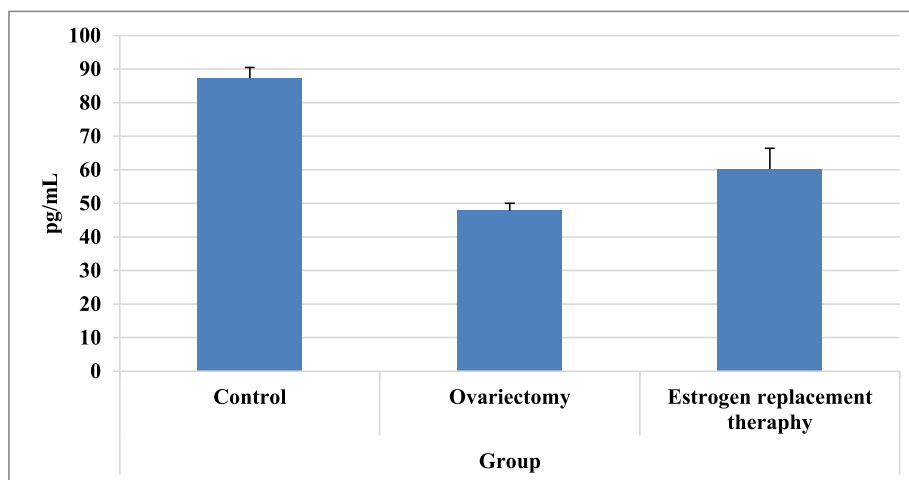
The present work demonstrated that the TMJs of ovariectomised rats underwent microscopic changes in response to estrogen deficiency. In these rats, there was a central fusion between the articular disc and the fibrous and cartilaginous layers of the mandibular condyle. Also, a chondrocyte condensation was associated with an irregular thickness of both the condylar and the glenoid cartilaginous layers. However, the chondrocytes in the condylar cartilage were atrophied, as evidenced by their decreased size and pyknotic nuclei.

The histological changes in the ovariectomised rats were accompanied by statistical data showing a substantial reduction in the hormone levels compared to the control rats. These observations were similar to previous studies that have explored the relationships between estrogen deficiency and TMJ histological changes (Porto et al., 2010). Those studies

Table 1 One-Way ANOVA test of estrogen level relative to control and experimental animal group.

Variables	Total	Estrogen level (pg/mL) Mean \pm SD	p-value
Group			
Control	6	87.30 \pm 3.2	< 0.001*
Ovariectomy	6	47.80 \pm 2.3	
Estrogen replacement therapy	6	60.20 \pm 6.2	

* $p > 0.05$ = statistically significant using One-Way ANOVA test.

**Fig. 4** Bar chart representing the mean estrogen levels in the control and experimental animal groups.**Table 2** Multiple comparisons of the estrogen levels between different groups with Tukey HSD Post-hoc test.

Group	Mean Difference	95% Confidence Interval		p-value
		Lower Bound	Upper Bound	
Control vs. Ovariectomy	39.5*	-45.847	-33.153	< 0.001
Control vs. Estrogen replacement therapy	27.1*	-33.447	-20.753	< 0.001
Ovariectomy vs. Estrogen replacement therapy	12.4*	6.053	18.747	< 0.001

* The mean difference is significant at the 0.05 level.

found that decreased serum estrogen levels after an ovariectomy caused the articular fibrous layer at the anterior (front) and central part of the condyle to increase in its thickness (Porto et al., 2010).

Recent studies have shown that estrogen can affect cartilage chondrocytes. This effect has been attributed to estrogen hormone receptors (ERs). ER α and ER β may indirectly modulate the production of cytokines, matrix metalloproteinases, and growth factors (Tanko et al., 2008). ER α and ER β are expressed in chondrocytes as well as in the subchondral bone (Ushiyama et al., 1999). Other studies have revealed that an ovariectomy can induce articular cartilage degradation; these studies have also demonstrated that the histopathological features of osteoarthritis can be found in the knee joint cartilage of both rats and cynomolgus monkeys (Oestergaard et al., 2008). Ovariectomies have also shown an increased thickness in the young rats' condylar cartilage (Okuda et al., 1996). Additionally, ER β -knockout mice exhibit cartilage thickening in the condyle, along with an increase in the number of carti-

lage cells, demonstrating estrogen's effect on the mandibulo-condylar cartilage (Kamiya et al., 2013).

An interesting discovery in this study reveals the bone marrow's frequent approach to the mandibular condyle near the condylar surface and the widening of the condylo-discal space. These results are comparable with those noted in another study that found trabecular bone invasion into the posterior portion of the articular soft tissue 2 weeks after ovariectomy (Yasuoka et al., 2000), indicating that the involvement of the subchondral bone may occur at a relatively preliminary stage. The skeletal response to estrogen deficiency with site-specific differences can be associated with differences in the metabolic activity, osteoprogenitor cell populations, supply of blood, and the standard bone turnover levels experienced by the cells in the bone (Yasuoka et al., 2000).

Accordingly, the impact of an ovariectomy may differ among anatomical placements and these variances may signify improper bone turnover rate that occurs in the post-ovariectomy (Yasuoka et al., 2000). Another histological study

reported that increased condylar cartilage thickness in ovariectomised rats was caused by a change in the number and/or the activity of osteoclasts at the cartilage-bone interface. The condylar cartilage thickness increased when there were fewer osteoclasts, resorbing the less densely mineralized matrix of the hypertrophic layer, invading the blood capillaries from the lower underlying bones, and resulting in slower bone replacement of the cartilage (Orajärvi, 2015). These reports contradict our findings that irregular thickness of the condylar and glenoid cartilaginous layers was associated with chondrocyte condensation.

4.2. Effects of estrogen replacement on the TMJ of ovariectomised rats

The ovariectomised rats that received estrogen replacement had a dense condylar fibrous layer and an increased thickness in the condylar cartilage layer that was accompanied by atypical chondrocytes. The cortification of the subarticular condylar bone was associated with the development of densely arranged haversian systems and a few bone marrow spaces. Most of the articular soft tissues and subchondral bones, however, have appeared to be near-normal. Although statistical data shows a substantial decrease in the hormone levels after the ovariectomies, this reduction was reversed after the estrogen replacement therapy. The highest mean estrogen levels were found in the control group (GI), followed by the group that received the estrogen replacement after their ovariectomies, and the lowest mean estrogen levels were found in the ovariectomised animals.

In this study, estrogen levels which increased in the ovariectomised rats after exogenous hormone replacement therapy did not reach or equal to the levels in the control animals. Previous *in vitro* studies have investigated that in both anterior and posterior regions there was an increase of articular soft tissue thickness (Yasuoka et al., 2000).

The last study noted in the impact of estradiol in the proliferation and metabolism of the mandibular condylar cartilage cells in rabbits; these reports found that estradiol administration was accompanied by the erosion of the articular cartilage at a greater rate and gravity compared to those placebo-controlled (Kamisaka et al., 2000). The effects in the estradiol-treated rabbits were presumably caused by a reduction in sulphated proteoglycan synthesis in the articular cartilage (Kamisaka et al., 2000). Similarly, in group GII the present study found the erosion of the condylar cartilaginous layer and the development of atypical chondrocytes. The estradiol effect on the joint disorder was accompanied by an increase the cartilage metabolism.

This study was limited to data collected from rats; hence the results of this study are limited to the non-human tissue structure. For instance, studies in the animal model sometimes give results that are not compatible with human subjects. Also, there was no sample size calculation and larger sample size may give different findings. However, in this study, the sample was divided into three groups with defined controlling factors

5. Conclusion

Consistent with previous reports, the present study demonstrated that harmful histological changes in the TMJ's struc-

tures occur with decreased estrogen levels. Estrogen replacement does not completely remediate the harmful histological changes to the TMJs after ovariectomy. Moreover, the ovariectomized animals that were injected with exogenous estrogen had high blood hormone levels, but these levels were still lower than the estrogen level in the control groups.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical consent

Ethics approval has been obtained from the institutional ethical committee of Alfarabi colleges in Jeddah, Kingdom of Saudi Arabia (Reference no. 19-10/4) and from Research Ethical Committee of King Abdulaziz University (Reference no. 687-19).

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