

Etiological factors associated with temporomandibular joint disorder – study on animal model

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Abstract

The etiology of temporomandibular disorder (TMD) is multidimensional: biomechanical, neuromuscular, bio-psychosocial and biological factors may contribute to the disorder. The main objectives of our study were investigation and analysis of the degree of involvement for several presumptive etiological factors of TMD: biomechanical stress (BS), estrogen hormones (EH) and emotional stress (ES). Six groups ($n=10$) of mature female Wistar rats were included in the study. Single presumptive etiological factor was applied in three groups (BS, EH and ES groups) and also association of presumptive etiological factors were applied in two groups (BSEH and BSES groups). No etiological factor was applied for the control group. Animals were sacrificed after a 60 days period and histological analysis of the temporomandibular joint (TMJ) tissues was performed. The changes in the mandibular cartilage, articular disc, temporal bone and synovial tissue were observed under optical magnification and quantified. All samples developed changes in the thickness of the condylar cartilage comparing to control group. The reduction was highly statistical significant for the EH, ES and BSES groups ($p<0.001$) and statistical significant for the BS and BSEH groups ($p<0.05$). The most important modifications with severe cartilage thickness reduction have been obtained in case of BSES group. In conclusion, biomechanical, emotional stress and estrogens can be considered as possible etiological factors in TMD.

Keywords: temporomandibular joint, stress, biomechanical, emotional, estrogens.

Introduction

The frequency of severe disorders that are accompanied by headache and facial pain and that are characterized by urgent need of treatment is 1–2% in children, about 5% in adolescents and 5–12% in adults [1].

It is evident from the numerous epidemiological studies on the occurrence of temporomandibular disorders (TMDs) in the general population that there are a number of consistent findings. Firstly, signs of temporomandibular disorders appear in about 60–70% of the general population and yet only about one in four people with signs are actually aware of or report any symptoms [2].

The most disturbing feature in TMD is pain, followed by restricted mandibular movement, which can cause difficulty eating or speaking, and noises from the temporomandibular joint (TMJ) during jaw movement [3]. TMD can detriment quality of life, because the symptoms can become chronic, difficult to support, affecting professional performances and vitality. Their etiology and pathogenesis are poorly understood, so control of TMJ diseases is difficult and symptomatic treatment is usually recommended. In diagnosed cases, invasive surgical therapy has too many risks to become a current treatment solution [4, 5]. Therefore, understanding the etiology of TMD is extremely important in identifying and avoiding potential pathological factors.

Three major causes have been proposed as etiological factors for TMD: mechanical stress, female hormones and psychological factors. In this article, we tested on mature

female Wistar rats these etiological factors separately in three groups and also in association in two groups.

Most authors consider occlusal abnormalities to be a fundamental factor in the onset of TMD's symptoms, whereas other studies suggest that they only represent one of the many factors associated with this condition [6, 7]. Past studies have suggested that many occlusal factors have a relationship with the TMD development. However, until now, none of them have been proved to be sufficient to cause TMD [8].

From the epidemiological point of view, TMD prevalence is much higher in women (ratio 2:1) than in men (10:1) [6]. Epidemiological data show that women have a higher risk than men to develop TMD, due to influence of estrogen hormones and different behavior in inflammation modulation [9, 10].

The role of emotional stress and personality in the etiology of the TMD syndrome has also undergone extensive scrutiny. Psychological studies have shown that patients with functional disorders of the temporomandibular region have similar psychological profiles and psychological dysfunction, as well as other chronic musculoskeletal pain disorders, such as tension type headache and back or arthritic pain [11].

TMJ in animal models proved to be similar to the human joint and researchers have been using this model for their studies [12–15]. This study on animal model allows to assess the histological changes in TMJ after several etiological factors' application and to identify the degree of involvement of various factors in the occurrence of TMD [14–16].

Materials and Methods

Sixty white adult female Wistar rats (weight 150 g) were divided into six study groups. Three groups were exposed separately to an etiological factor for TMD: biomechanical stress (BS), overdose of estrogen hormones (EH) or emotional stress (ES). Two groups were exposed to a combination of two factors (BSEH and BSES). The control group received no etiological factor. The experiment lasted for 60 days.

BS was achieved by metal crowns in occlusal interference cemented on first mandibular right molar.

In order to control the amount of estrogen hormones in the general circulation, ovariectomy was performed in the study groups which received a hormone overdose (EH and BSEH groups) calculated based on the body weight (b.w.) of each animal (50 µg/kg normal dose, 80 µg/kg overdose, values based on previous research) [4]. Hormonal manipulation was performed by daily injection with 17β-estradiol. Hormones were dissolved in propylene glycol.

ES was induced by a ringing bell for 10 minute every hour (100 decibels) [17].

During the period of study, the animals were maintained in a temperature-controlled room (23°C) and were housed in plastic cages with soft bedding on a 12:12 light cycle with food and water available *ad libitum*.

All the animals and the experimental protocols were approved by the Ethical Committee of our University, in accordance with international guidelines for the study on animals.

Animals were sacrificed (in accordance with Bioethics Committee guidelines) by decapitation, after anesthesia induced by hypodermic injection with Narcoxyl 0.1 mg/g b.w. and Ketamine 0.3 mg/g b.w.

Whole rat heads were sectioned and TMJ on each side was removed in one piece. A cube (0.5 cm width) containing the condyle was prepared from each TMJ of decapitated rats, than samples were fixed in 10% formalin solution and than demineralized. For the demineralization process, a solution (pH 7–7.2) with 250 g EDTA (ethylenediaminetetraacetic acid), 1750 mL distilled water and 25 g sodium hydroxide was used. All samples were introduced in a volume 20 times larger their own. During one month, every four days freshly prepared solution was used to immerse the samples. After complete decalcification, all samples were introduced for 24 hours in 10% formalin solution, than rinsed with water for one hour and prepared for paraffin embedding. The decalcification process allowed seriate sagittal sections realization of the samples through bone.

The tissue samples were passed in a series of graded ethanol, and embedded in paraffin. Seriate sagittal sections 5–7 µm thick (10 sections for each TMJ) were realized and Hematoxylin–Eosin (HE) staining was performed. Histopathological samples were examined and captured using a digital camera (Olympus) and evaluated with a computerized image analysis system (Cell B, Olympus). Significant sections for each sample were identified and photographed with ×10, ×20 and ×40 magnification lens. Changes in mandibular condyle, articular disc, temporal bone and synovial tissue were identified and analyzed.

Histomorphometric evaluation of the condylar cartilage was performed by adapting previously used techniques [18, 19]. Thickness of the inferior margin of the hypertrophic zone on the external margin of the fibrous layer was measured for each zone (anterior, central and posterior). Minimum 10 measurements for each sample were performed and the average cartilage thickness for the field and for the group was calculated.

Statistical analysis

After quantification of cartilage thickness in mandibular cartilage, all data were centralized for each separated group and a statistical analysis was realized using R software. Data normality was verified with Shapiro–Wilk test. Average comparing was performed with the Kruskal–Wallis test followed by the Wilcoxon signed-rank test with the Bonferroni's correction.

Results

By the comparative analysis of the results obtained from the measurements on the condylar cartilage, in all five study groups reduced average values have been observed in comparison with the control group. This observation indicates that the experimental model has functioned properly and all different factors applied have had a certain effect on the thickness of the condylar cartilage.

The dimension of the condylar cartilage has uniformly reduced, with similar values (between 119.56 µm and 126.75 µm) for the BS, EH, BSEH and ES groups.

The most important modifications have been observed in the BSES group, where the average dimension of the condylar cartilage has been the lowest (Table 1).

Table 1 – Condyle cartilage thickness quantification for the six study groups. Results are expressed in µm

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	Control	BS	EH	BSEH	BSES	ES
Average	134.24	122.09	123.07	119.56	92.39	123.13
SD	16.72	31.07	22.54	13.58	18.53	37.03
SEM	4.32	10.98	5.04	2.72	3.71	7.26

BS: Biomechanical stress; EH: Estrogen hormones; ES: Emotional stress; SD: Standard deviation; SEM: Standard error of mean.

By applying the Kruskal–Wallis test, statistically significant results have been obtained in experimental groups ($p < 0.001$). The experimental groups were then compared in pairs using the Wilcoxon statistical test with Bonferroni's correction.

Therefore, the thickness of the condylar cartilage was significantly reduced in case of the BSES group. Nevertheless, the reduction was highly statistically significant for the EH, ES and BSES groups ($p < 0.001$) and statistically significant for the BS and BSEH groups ($p < 0.05$) (Figure 1).

The histopathological analysis was then performed in order to observe the TMJ modifications in rats for the six study groups. There were no histological modifications in the control group on serial transversal sections in TMJ (Figure 2). For the rats in the BS group, at the end of the experiment, especially in the anterior and central zone of the mandibular condyle (areas under functional loading), a multifocal chondrocyte loss was noticed in all cartilage

layers with chondrocyte nests development. In only one animal, there was a complete destruction of the cartilage in the TMJ (Figure 3).

At the TMJ level, for the animals in the EH group, in the central and anterior areas of the condyle a hypocellular aspect was recorded with a pale aspect and zonal loss of chondrocyte. In the same areas of the cartilage, multifocal development of chondrocyte nests was observed (Figure 4).

All modifications in the BSEH group were observed in the mandibular condyle area. As in the other groups, the most important lesions appeared in the anterior and central zones: pale hypo-cellular aspect, multifocal chondrocyte

nests. The reduction of the articular disc was significant ($p < 0.05$).

In the ES group, the chondrocyte loss was more important in the central zone of condyle cartilage, villous proliferations in synovium and reduced inflammatory cells were observed.

The most severe modifications were observed in the BSES group. The same types of changes with similar distribution were recorded, but their intensity was significantly higher: pale hypo-cellular aspect, chondrocyte nests development, multifocal cell loss. The articular disc had a hypo-cellular aspect and reduced central thickness (Figure 5).

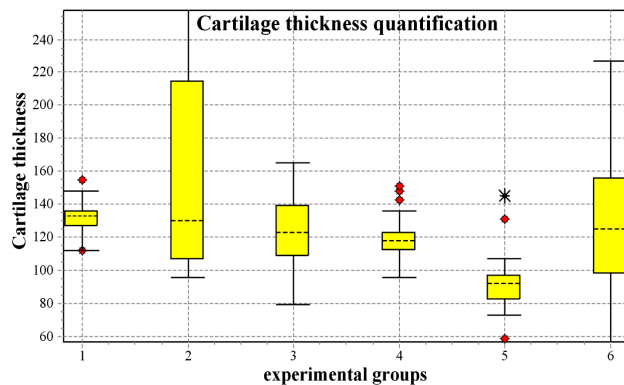


Figure 1 – The box plot diagram is showing the results of cartilage thickness measurements at condyle cartilage level. The circles represent abnormal results. Star indicates significant differences between groups 1, 3 and 4, in contrast with group 6 ($p < 0.001$). Groups: 1: Control; 2: BS; 3: EH; 4: BSEH; 5: BSES; 6: ES.

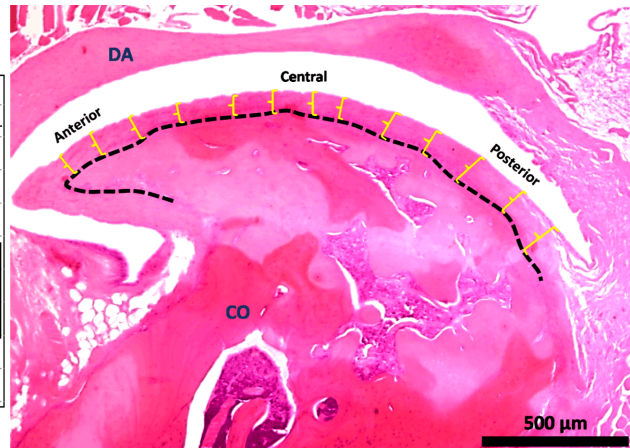


Figure 2 – Sagittal sections of the TMJ in Wistar rats in control group. HE staining, $\times 100$. The areas from the mandibular condyle where measurements were performed (anterior, central and posterior). DA: Articular disc; CO: Mandibular condyle.

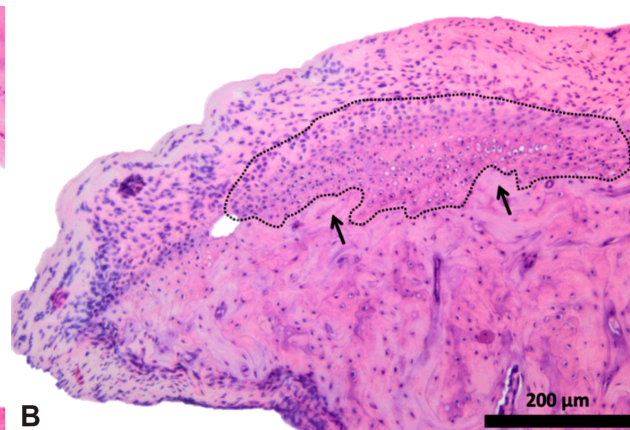
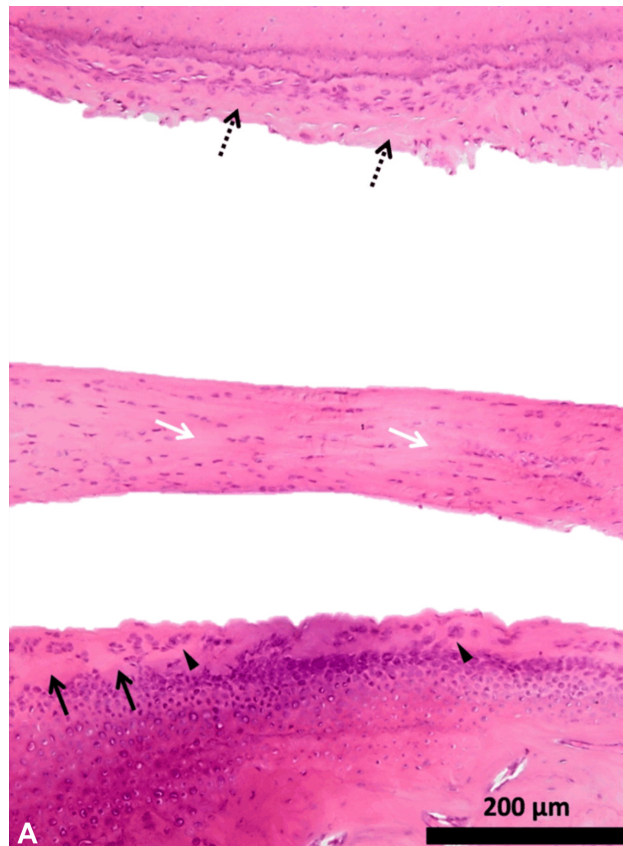


Figure 3 – Sagittal sections of the TMJ in rats of the BS group. HE staining, $\times 200$. (A) Temporal fosse cartilage destruction (dotted arrows), hypocellularity on articular disc (white arrows), regional chondrocyte loss (black arrows) and chondrocyte nests development (arrow tip) on condylar cartilage. (B) Antero-central focal chondrocyte proliferation (area limited by dots), subchondral sclerosis (arrows).

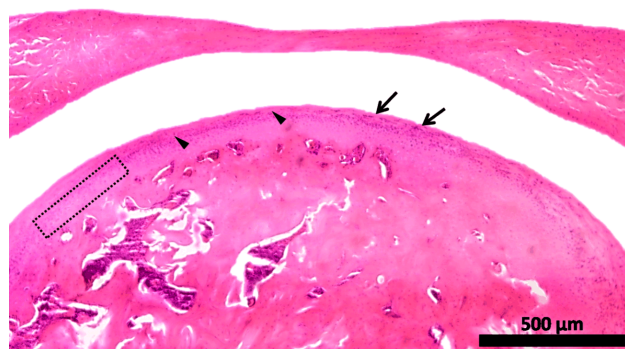


Figure 4 – Histopathological changes in rat's TMJ from the EH group. Sagittal sections in HE staining, $\times 100$. Proliferative villous can be observed in the synovium of the posterior area, chondrocyte hypocellularity in the anterior zone of the condylar cartilage with pale aspect (dotted squares). A regional loss of chondrocytes was observed in the condylar cartilage with chondrocyte nests development (arrow tips).

Discussion

Previous animal model studies have already proved that, in certain experimental conditions, which induce the articular disc displacement, functional and histomorphological modifications are recorded.

Nicoll *et al.* (2010) observed in a study realized on Holzman rats, which after mechanical maxillary overloading carried out one hour per day for a seven days period, the articular disc significantly reduces its thickness and zonal cellular arrangements appear [20].

A recent study used as animal model for Sprague–Dawley female rats showed that TMJ osteoarthritis was induced by intra-articular monosodium iodoacetate injection and various doses of 17β -estradiol supplements. After 28 days, they obtained results showing that estradiol increased the articular cartilage degradation and sub-chondral bone erosion [16].

Our results of the histomorphometric analysis showed similar articular disc thickness reduction for the groups where individual factors (BS, EH, ES) were applied.

Biomechanical stress obtained by occlusal overloading increases muscular activity and intra-articular pressure, producing disk modifications. In accordance with other studies, we observed histomorphometric changes at the articular tissues [18].

Psychological stress also plays a major role in TMD development. Research findings have supported a relationship between anxiety, muscular tension, and TMD symptoms. De Leeuw & van der Meulen consider that muscle dysfunction and accompanying pain are very often the result of stress induced muscular hyperactivity. Stress-induced muscular dysfunction may induce secondary changes in the TMJ [21]. Our results were similar to previous studies, showing articular disk reduction within the ES group.

Endogenous estrogen affects the remodeling processes within the TMJ possibly by changing the extracellular matrix in the joint or by changing bone volume [20]. Such changes have been observed in our study with increased degree of articular disc degradation and proliferative cell reduction when overdose of estrogen hormones was administered.

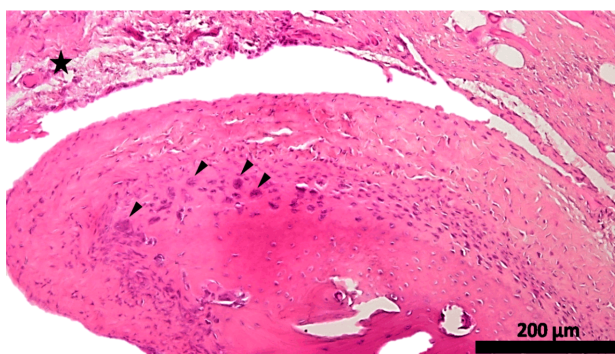


Figure 5 – Histological changes in rat's TMJ from the BSES research group. Sagittal sections in HE staining, $\times 200$. Proliferative villous can be observed in synovium (stars) and focal loss of chondrocytes in the central zone of the condyle (arrow tip).

The most severe modifications that we have recorded were in the group exposed to biomechanical and emotional stress.

There are several limitations to our experimental model. Due to the involvement of multiple factors and the complexity of underlying mechanisms, it is difficult to extrapolate these results to other models.

Conclusions

All presumptive etiological factors of our study determine articular modifications confirmed by histomorphometric results. The association of etiological factors significantly increased the severity of articular lesions. Emotional stress associated with biomechanical stress have the most destructive potential of inducing TMD. As with all animal models, there are difficulties in the transition to humans and clinical care, so further research is necessary.

Conflict of interests

The authors declare that they have no conflict of interests.

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