Histological Evaluation of the Temporomandibular Joint after Producing Artificial Traumatic Occlusion in Dogs

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KEY WORDS	ABSTRACT
Dental occlusion; Traumatic; Histology; Temporomandibular joint Second August 2010; Received August 2010; Received in revised form Nov. 2010; Accepted Jan. 2011	Statement of Problem: Traumatic occlusion can have an adverse effect on the temporomandibular joint tissue.Purpose: This study aims to determine the histological alteration in dogs' temporomandibular joints due to production of artificial traumatic occlusion.
	 Materials and Methods: Twenty dogs were divided into a control gro- up of four and two equal experimental groups. Unilateral occlusal interf- erences were created on the mandibular right first molars in the experim- ental groups. We established two experimental periods: one week and 45 days. After vital perfusion, the TMJs were separated for preparation, pig- mentation, and examination by a pathologist. Descriptive statistics, Chi- square and Fisher's exact test were used to analyze the data. P.value less than 0.05 was considered as significant. Results: The findings showed no inflammatory changes in the TMJ. Increased fibroris was found in 56 20% and was planiation in 28 12% of
	 the disks. Fibrotic changes were detected in 46.87% and vascularization in 28.12% of the condyles. The glenoid fossas suffered from fibrosis in 15.62% of the cases. Conclusion: Traumatic occlusion leads to histopathological alterations including an increase in the fibrosis tissue and vascularization in the
	condyle and disk.

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Introduction

The temporomandibular joint (TMJ) is a complex and highly refined unit. Health and normal function of this joint is affected by many factors. One controversial factor contributing to temporomandibular disorders (TMDs) is the occlusal condition.

Moteghi found a statistically significant relationship between occlusion and disease symptoms in TMJ in a study on 7337 patients [1]. The occlusal interferences, especially non-working premature contacts and lateral slides, are considered as the main factors in traumatic occlusion. The recent epidemiologic and clinical studies, however, do not show such a significant relationship. The role of occlusal factors as the etiologic factors in temporomandibular disorders (TMD) is not known yet. In some cases of malocclusions, the condyle is pushed toward the back of the glenoid fossa, causing pressure on the posterior band of the disk. This might in turn cause a thinning of the disk over a long period, resulting in its displacement [2].

Marzooq suggested overbite, occlusal interferences on the non- working side, and discrepancy between the intercuspal and retruded contact position as effective factors for TMD. Fuji studied the occlusal conditions of 52 patients suffering from TMD. 27 patients suffered from masticatory muscle pain, and 25 patients did not have any symptoms. To avoid partial judgment, he reduced the patients' symptoms by using the Bite Plan. He suggested that the only occlusal factor related to pain in that study had been lack of canine contact on the working side in the lateral movement [4].

Taskayayilmaz studied the relationship between the condyle position and TM joint disk, and occlusal contacts in lateral movements of the mandible. He concluded that premature contacts of the nonworking side have certain effects on the condition of the disk in TMD, and that it can cause anterior disk displacement, although neither the canine raised occlusion nor group function can affect TMD. The non-working side contacts have no effect on the intensity of disk displacement and condition of the condyle. The findings of this study indicate that the occlusal contacts of the non-working side cannot be considered as the primary reason for the posterior disk displacement [5].

Dervis studied TMD and denture relationship and found that 3 months of denture use in edentulous patients reduced the symptoms of TMD. But there was no significant relationship between TMD and improper occlusion of dentures [6]. Moreover,

Semkin studied 47 TMD sufferers diagnosed with TMD after pathologic occlusion and its treatment. He suggested prosthetic treatments for making TMJs internally stable [7].

Wang dealt with the relationship between the missing teeth and TMD. He believed that there was a relationship between some symptoms of TMD and secondary occlusal alteration caused by missing teeth [8].

Few researchers have studied the histopathologic alterations of TMJ following occlusal trauma. For example, Zhang used one-way occlusal splints for 1, 3, or 5 months on the lower left molars of rats to cause occlusal trauma in their temporomandibular joints. Electron microscopic evaluation showed that the surface layer of the disk and articular cartilage were damaged. Severe fibrillation with fiber sheets, collagen fibrils and bundles inside the fissures and craters were observed. The results of this study indicated the destructive effects of the joints due to occlusal trauma [9].

In a study of histopathologic evaluation of massetter alteration due to induction of occlusal trauma on the posterior maxilla teeth in rats, Nishide reported such alterations as increase in the connective tissues and the presence of inflammatory cells in the muscular fibers [10].

Chaves inserted occlusal interferences on the right posterior teeth of rabbits to observe their temporomandibular joints' microscopic alteration. He observed the presence of intra-articular hemorrhage in the supra- and infra-disk compartments and in the retrodiscal zone. No inflammatory cells were detected. The condylar fibrocartilage presented significant thickness alterations in the animals of the experimental groups [11].

There is an attempt in the present study to determine histological alteration in dogs' temporomandibular joints due to creation of artificial traumatic occlusion.



Figure 1 a Normal connective and cartilage tissue of the condyle head in control group 40X Magnification. **b** Normal and natural tissue of disc in control group 40X magnification. **c** Hemorrhage on condyle head 100X magnification. **d** Increase in fibrosis tissue on the surface of glenoid fossa 100X magnification. **e** Increase in fibrosis tissue in disc 40X magnification. **f** Increase in blood vessels in disc (v= vessel) 100X magnification

Materials and Methods

In this experimental study, the methods of the laboratory data collection were conducted through observation. The experimental procedures were performed in the Dental Research Center, Mashhad University of Medical Sciences. Twenty healthy 3-year old and mature male Stray dogs, were used as the specimens of the study. Four dogs were used as the control group, and the remaining 16 were divided into two groups of eight. Both groups were treated for occlusal trauma, one for a week, and the other for 45 days. Posterior composite, (B#1370B2) 3M-P60 (MN,U.S.) was placed on the first right lower molar occlusal surface, after treatment of the enamel by orthophosphoric acid 37% (B#B275) Ultra Etch (Ultradent Products, UT,U.S.) and the use of margin bond (B#MB011) (Colten, Swiss) composite.

The dog's right first molar teeth's occlusion were raised with a composite equal to the size of two layers of articulation GHM (GHM® articulating paper (GHM Hanel Dental GmbH, D-72622 Nurtingen, Germany) rimas each with a thickness of $40 \,\mu$ m, in order to separate the posterior second molar.

Thus the right TMJ was near to the trauma site and the left TMJ was located far from it. Eight dogs received perfusion after a period of one week and the other eight did so after 45 days. The 45-day cases were evaluated fortnightly with the existing composite. After perfusion, the TMJs of the cases were separated for preparation and pigmentation by H&E. First, they were soaked in formalin (10%) and then the specimens were placed in EDTA for 50 days for decalcification and after preparing the slices and pigmentation by H&E, they were eventually sent to a pathologist for analysis.

The cross-tab test and the Fisher's exact test were used to compare the alterations among the study groups (α =0.05). The statistical analysis was performed by SPSS version 11.0.

Results

After examination of the specimens, no inflammat-

ion of the TMJs was detected. There were no alterations of the condyle, glenoid fossa, joint disk, synovial membrane, and articular eminence. The most significant alteration was observed between the previously mentioned locations in the joint disk in the form of an increase in the fibrosis tissue (Figures 1-d, 1-e).

The frequency of the alterations could be ranked as: disk fibrosis, condyle fibrosis, disk vascularization (Figure 1-f), condyle vascularization and hemorrhage (Figure 1-c) and glenoid fossa fibrosis (Figures 2 and 3). The most frequent variations in the 1-week and 45-day cases, disregarding the right and left sides, were condyle and disk fibrosis, respectively.

Figure 4 shows the percentile frequency of the alterations in all of the groups (right TMJ 1-week cases, left TMJ 1-week cases, right TMJ 45-day cases, and left TMJ 45-day cases). This graph clarifies the higher frequencies of disk fibrosis respectively in the right TMJ 45-day cases, left TMJ 45-day cases, left TMJ 1-week cases, and right TMJ 1-week cases. The greatest amount of disk vascularization was detected in the left TMJ 45- day and 1 week cases. The result of Fisher's exact test between the control and experimental groups showed a significant increase in the fibrosis tissue by the condyle in the 1-week group (p = 0.007) and 45-day group (p = 0.002). Although the increase of fibrosis tissue in the 45-day group was more than that



Figure 2 Variables abundance in all of the specimens

of 1-week group, the difference was not significant.

The comparison between the increase of fibrosis tissue in the right and left condyle of 45-day and 1-week cases did not show any significant differences (p = 0.07). The increase of condyle vascularization in the 1- week cases did not show a significant difference with that of the control group (p = 0.101). In the 45-day cases, however, the amount of vascularization was significant (p = 0.043). There was no significant difference between the amount of the vascularization in the right and left condyle of 1week cases (p = 0.077), but there was quite a significant difference in the right and left condyle in the 45-day cases (p = 0.026).

The differences between the increase of the glenoid fossa fibrosis in the 1-week experimental group, and control group, and the 45-day experimental and control group were not significant. No signify-cant differences were observed between 1week and 45-day glenoid fossa fibrosis increase (p =1) and the difference was not significant between the right and left cases (p = 0.26). In disk examinations, considering vascularization, there were no differences between 45-day and 1-week cases (p=1). Also, in disk vascularization of the left and right cases of both 45-day and 1-week, there were no significant differences (p = 1). As compared to the control group (Figures 1-a, 1-b), the increase of disk fibrosis in 1-week and 45-day cases showed significant differences (p=0.043 for 1-week and p=0.000



Figure 3 Variables abundance in one week and 45 day specimens



Figure 4 Variable abundance, time and position details

for 45-day cases). Variations were generally greater in 45-day group than 1-week group, especially in the disk fibrosis (p = 0.011).

Discussion

The effect of traumatic occlusion on the temporomandibular joint has always been a very controversial issue in dentistry. About a century ago, most of the dentists considered occlusal interferences as an obvious factor in TMD. The recent epidemiologic and clinical studies, however, do not present such a significant relationship between the occlusal interferences and TMD [2].

Due to lack of easy access to similar laboratory conditions, most studies on the effects of traumatic occlusion on TMJ are only conducted by observing patients' TMD symptoms. Few studies have really dealt with TMJ histopathologic alterations that are caused by traumatic occlusion. In this study, TMJ histopathologic examinations were chosen instead of TMD symptoms examinations to omit the interfering factors related to the patients. It seems impossible, of course, to ignore the differences between dogs used as specimen in this study, and human beings. Counseling veterinarians, we have did our best to choose the anatomically nearest animal to humans. There are some limitations in the animal studies. For example, in this study if the traumatic occlusion causes pain in the animal, it is possible that the animal does not eat and swallow food without truly chewing it, or changes its chewing behavior to avoid pain. So, the results of this study cannot be reliable scientifically.

The specimens were chosen based on similar age, race, and gender to control the possible effects of these factors. Zhang [9] and Chaves [11] used the occlusal splint in order to cause traumatic occlusion in their studies. To make similar conditions, we used the composites only on one posterior tooth.

To create a better composite bond to the intact enamel, it was etched by 37% phosphoric acid. A micro-hybrid posterior composite was used to avoid wearing through time, and to keep the amount of traumatic occlusion intact.

Based on the aim of this study for examining the type and presence of inflammation, one week and 45 day periods were chosen in order to set enough time for swelling and inflammation and other tissue alterations.

The survey of 1-week and 45-day cases showed significant differences with the control group in the condyle and disk fibrosis increase. Zhang showed that traumatic occlusion causes damages in the joint

disk and acute fibrillation in the pit and fissures [9]. Chaves mentioned an increase in the thickness of the fibro-cartilage tissue on the surface of the condyle. This increase may cause an adaptation to the unwanted loads on TMJ [11].

The comparison of the 1-week and 45-day specimens showed an increase in all the factors as time passed. The significant increase, however, was only observed in the amount of disk fibrosis after 45 days. Zhang also mentioned acute disk fibrosis after 1 month, 2 month, 3 month, and 5 month periods. Thus, we can conclude that the amount of fibrosis increases as time passes [9]. Generally, the comparison of the right and left sides shows no significant difference, neither in the 1-week nor the 45-day specimen. The vascularization, however, proved to be significantly higher on the right than the left side. As time passes, increase of these two alterations is more probable. It is possible that adaptation of the animal to the new occlusion condition causes more force to the working condyle at the time of chewing on the right side. Thus, it increases the amount of fibrosis and vascularization on the right side.

Chaves observed histopathological alterations such as bleeding and thickening of the fibro cartilage tissue in TMJ on the opposite side of the occlusal interferences. This difference between the two studies on the account of the involved side might be due to the difference in the species and neuromuscular replies to trauma. Nishide showed the presence of inflammation in the masseter after traumatic occlusion. This inflammation may cause variations in masticatory functions or influence TMJ indirectly, leading to the previously mentioned alterations [10]. In this study, we did not observe any inflammatory alterations in TMJ, synovial membrane and joint eminence, subsequent to similar studies.

Conclusion

Considering the limitations in this study, it is concluded that:

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1. Traumatic occlusion caused histopathological alterations including an increase in the fibrosis tissue and vascularization in the condyle and disk.

2. As the traumatic occlusion continued, the disk fibrosis tissue also increased.

3. On the right joint (the one adjacent to the traumatic occlusion), the probability of vascularization on the surface of the condyle increased.

4. Traumatic occlusion did not cause any inflammation process in TMJ during the 1-week and 45day time periods.

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