Article

Comparison between two different fixation techniques on experimentally induced unilateral subcondylar fracture in TMJ of the dogs (Histopathological and histomorphometrical study)

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Abstract: Objectives: The present study was performed to compare between two different fixation techniques and evaluate the histopathological changes which occur in temporomandibular joint (TMJ) of the dogs after surgically induced unilateral subcondylar fracture. Methods: Twenty- One healthy beagle male dogs with a mean age of 30 months were used in the present study. One of these animals was sacrificed to study the normal histology of the dog TMJ. The rest of the animals (20 animals) were operated on to induce a displaced unilateral subcondylar fracture then, the animals were randomly equally divided into 2 groups. After surgery, 5 dogs of each group were sacrificed at 2 months after surgery (Short-Term), while the remaining 5 were sacrificed at 4 months after surgery (Long-Term). The heads were separated and fixed, oscillating saw was used to articulate the entire TMJ. Specimens were decalcified, then the specimens were washed, grossed and sectioned in a sagittal plane. Tissue sections of 4 um thick were cut and stained with Hematoxylin and Eosin to be studied microscopically. Results: In group I animals, at 2 months after surgery, there was a marked hypertrophy of the articular fibrous covering of the condylar head, while at 4 months after surgery, the articular fibrous covering appeared thinner than normal, with marked decrease in thickness of the articular cartilage layer. In group II, at 2 months after plate insertion, the articular fibrous covering became hypertrophic, and the articular cartilage was markedly reduced but it was of a uniform thickness, while at 4 months after subcondylar fracture and plate fixation, no significant changes could be detected either in thickness or structure of the articular fibrous covering. Conclusion: Based on this study, it is obvious that the plate fixation group gave the most favorable results, when compared with maxillomandibular fixation (MMF) group.

Keywords: Subcondylar fracture; Histopathologic study; TMJ; MMF; Miniplate fixation

1. Introduction

Treatment of condylar fractures has been a controversial topic. Four main treatment modalities have been described in the literature: Mandibular physical therapy consisting of ranging exercises without MMF, a short period of MMF followed by mandibular physical therapy, open reduction followed by MMF and open reduction and internal fixation of the condylar fracture [1]. Condylar fractures are common fractures that accounting for

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29-52%of mandibular fracture and 11-16 % of facial fractures [2]. In most cases, condylar fractures can be treated conservatively with the best results, even in cases of fracture with dislocation. The opinion has been expressed that surgery should normally be reserved for cases of bony ankylosis, for example, following intracapsular fractures, unreduced major condylar dislocation, especially if bilateral that can lead to an open bite, severe dysfunction of the jaws and pain [3].

Open reduction and plating of subcondylar fractures allow patients to have immediate mobilization, better oral hygiene, improved nutrition, normal speech, occlusion, masticatory function, mouth opening, bone morphology and better functional reconstruction of mandibular condyle compared to non-surgical closed reduction methods [4, 5]. Open reduction and fixation also overcomes the complications of closed reduction as malocclusion, diminished posterior facial height, and decreased mandibular motion [6].

Open reduction and fixation can be done either intra-orally or extraorally, in spite of the easier intra-oral approach with preservation of facial nerve, and better esthetics without facial scarring, but it is indicated in special cases of very low subcondylar fracture that are laterally displaced, otherwise it is technically difficult, inaccessible, and need for endoscopic assistance [7].

A previous study results of 40 miniplates osteosynthesis, performed on displaced low subcondylar fractures of the mandible in single form are presented with better results [8], with restoration of the anatomical form shown on x-ray, no functional disorder restricting mandibular motion. The results indicated that miniplate osteosynthesis is practically feasible and recommended as a therapy depending on the particular indication. Other clinical study [9] has shown that, single straight miniplate is inadequate in fixation of subcondylar fracture, with possibility of mechanical failure due to plate fracture, instability, and screw loosening.

Ellis and Dean [10], advocated using mini compression plates in subcondylar fracture as it’s stronger than the adaptation miniplate. Also Single dynamic compression is thick and can reduce the fracture in subcondylar area using the mechanical advantages of the compression plate that can better neutralize function stress at condylar neck, as the highest level of compression occurs on the posterior surface of condylar neck [10, 11].

So, the question still remain regarding the actual effect of plate fixation displaced subcondylar fracture. Thus, the aim of the present study was to compare between two different fixation techniques and evaluate the histopathologic changes which occur in the TMJ of the dogs after surgically induced unilateral subcondylar fracture

2. Materials and Methods

2.1. Animals

Twenty- One healthy beagle male dogs with a mean age of 30 months (weight 15-20 kg), were used in the present study, all animals were placed on a soft diet of bread, cooked food and milk 2 weeks prior to surgery. The animals were kept in a separate and labelled cages and allowed to live in optimal conditions under the supervision of the staff members of military veterinary hospital according to the protocol by the local ethical committee (AE-LU-001/11/PRODMED 03/3-11).

One of these animals was sacrificed to study the normal histology of the dog TMJ. The rest of the animals (20 animals) were operated on to induce a displaced unilateral subcondylar fracture through a pre-auricular approach on the left side of the mandible. According to the method of fixation of the induced fracture, the animals were randomly equally divided into 2 groups:

Group I (10 animals): The displaced condylar fragment was surgically reduced and MMF was in place for 7 days [1], followed by active jaws movement.

Group II (10 animals): The condylar process was surgically reduced and fixed in place with a titanium miniplate and 2 screws.

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2.2. Pre-Operative preparations

Atropine sulphate (Chemical Industries Development "CID", Giza- A.R.E.) was injected 10-30 minutes before the operation in a dose of 0.02-0.04 mg/kg body weight. Acepromazine maleate (Covetrus North America) was injected I.M. as pre-operative tranquilizer in a dose of 0.25-0.5 mg/lb of body weight. Intacef PET – Ceftriaxone Injection (Intas Pharmaceuticals LTD- India) was injected I.M. in a dose of 15-25 mg/kg, every 24 hours for 2 days before and 3 days after the surgery to avoid secondary infection.

Sterilization of the screws and plates was done by autoclaving for 30 minutes at 160^{o} C. Propofol (Flagship Biotech International Pvt. Ltd.) was injected I.V. in a dose of 0.2–0.4 mg/kg/min, as a general anaesthesia.

The pre-auricular area was shaved and clipped bilaterally between the external auditory meatus and the eye. The surgical site was scrubbed with 2% alcoholic iodine solution. The animal was draped with sterile towel to isolate the surgical field.

2.3. Surgical operation

The neck of the mandibular condyle was surgically exposed through a pre-auricular approach. Three to four holes were done by round bur No 502, on horizontal line that was drawn in the narrowest area of the condylar neck, perpendicular to the long axis of the neck, the holes were connected by a fissure bur under saline cooling system. The fracture line was completed outside the capsular attachment by a mallet and chisel about 20 mm from the condylar head. The direction and position of the osteotomies were kept consistent for all animals. The proximal segment was gently grasped by a hemostat and then displaced to an anterior position 45o to the ramus without disc displacement. At this stage the animals were equally divided into two groups (I & II).

After surgery, animals of the two groups were put on a soft diet for 7 to 10 days, then returned to a regular diet. 5 dogs of each group were sacrificed at 2 months after surgery (Short-Term), while the remaining 5 dogs were sacrificed at 4 months after surgery (Long-Term).

2.4. Histologic specimen preparation

The jaws were secured with the teeth in the intercuspal position by wire ligature, the mandible was stripped to the angle and the tongue and the orbital contents were removed, then the head was placed into a large 1- gallon jug of 10% neutral buffered formalin under constant agitation and periodic change of fluids every 24 hours. The head was removed from the fixative and the soft tissue elements were debrided. The oscillating saw was used to articulate the entire TMJ. Specimens were decalcified utilizing formic acid-sodium citrate solution for 48 hours, then the specimens were washed, grossed and sectioned in a sagittal plane. Tissue sections of 4 um thick were cut and stained with Hematoxylin and Eosin to be studied microscopically.

2.5. Histomorphometric analysis

The total thickness of the articular fibrous covering of condylar head, and the width of condylar cartilage in normal animal and in the two groups (at the two periods, 2 and 4 months after surgery) were measured. The image analysis was performed using a computer system (software Leica Quin 500). The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer (μm) units.

2.6. Statistical analysis

The data obtained from computer image analysis were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The Non-Paired

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"T" test was used to compare between the two groups. The p-value was considered significant as the following: Non-significant when p-value > 0.05, significant when p-value ≤ 0.05, and highly significant when p-value ≤ 0.01.

3. Results

3.1. Histopathologic Results

In normal animal, the TMJ was compose of; the articular fibrous covering of the condylar head appeared as a continuous uniform thickness of fibrous connective tissue covering composed of a densely packed collagen bundles running parallel to each other interspersed with flattened deeply stained nuclei. The articular cartilage band in the condylar head lies immediately below the fibrous covering, which composed of hyaline cartilage and showed features of endochondral ossification. The disc is composed of tightly packed interlacing collagen bundles, interspersed by several fibroblasts and spare amount of ground substance. The articulating surface of temporal bone is composed a superficial layer of thin fibrous tissue covering, and cartilage of the temporal bone forming the roof of the mandibular fossa which appeared of variable thickness (Figures 1A & 1B).

A B

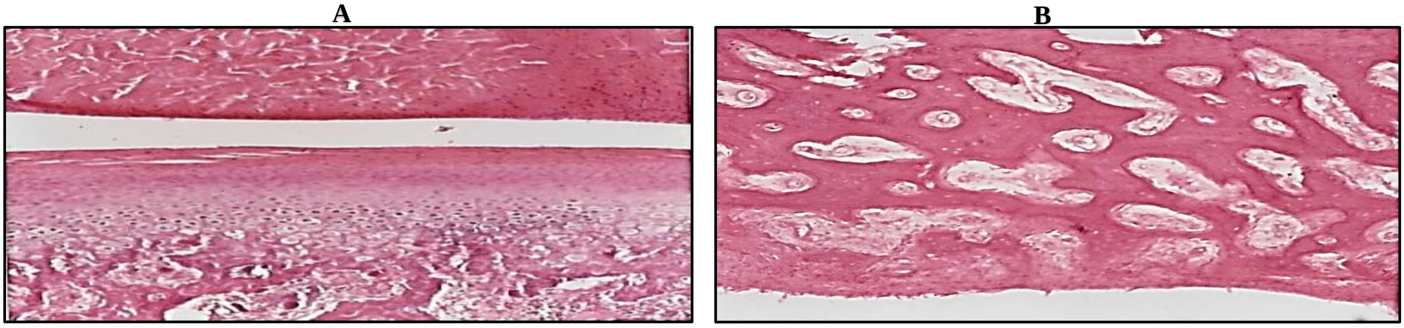

Figure 1. (A) Photomicrograph of normal animal, showing articular disc, condylo-discal space, and condylar head; **(B)** Photomicrograph showing roof of mandibular fossa which composed of osseous tissue enclosing marrow spaces and covered by a thin fibrous tissue covering.

In group I animals, at the first follow up period (2 months after surgery), there was a marked hypertrophy of the articular fibrous covering of the condylar head due to thickening and proliferation of its fibrillar components, presence of interfibrillar vacuolations, occasional detachment and homogenization of collagen fibers. The articular cartilage band in the condylar head was markedly decreased and the bone formed subsequent to condyle cartilage was very dense. The disc showed homogenization of the collagen bundles and irregular distribution of its fibrillar component. The temporal bone showed no histological changes at that period (figure 2A). In group I animals, at the second follow up period (4 months after surgery), the articular fibrous covering appeared thinner than normal, with marked decrease in thickness of the articular cartilage layer and the same changes which occurred in the bony part of the condylar head at the first interval period were noted but in an exaggerated pattern. The disc and the temporal bone showed no changes from what is noted in the first scarification date (figure 2B).

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A B

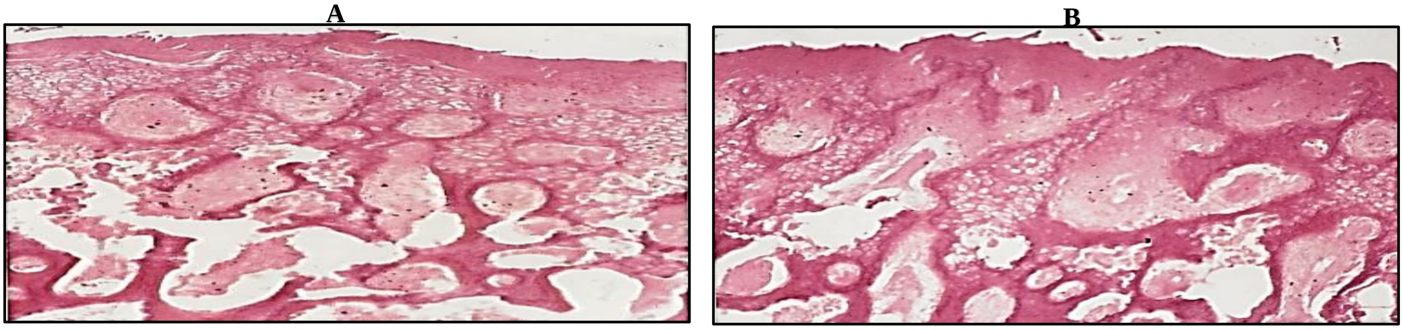

Figure 2. (**A**) Photomicrograph of group I at 2 months showing condylar head with hypertrophic fibrous covering, and the band of articular cartilage is interrupted by invading fibrous bone marrow;(B) Photomicrograph of group I at 4 months showing condylar head with alveoli of bone marrow invades the condylar cartilage, marrow tissue is in contact with the fibrous covering leaves small islands of hyaline cartilage.

In group II, at the first scarification date (2 months after plate insertion), the following histological changes were detected, the articular fibrous covering became hypertrophic when compared with normal one, the articular cartilage was markedly reduced in width but it was of a uniform thickness and the condylar spongiosa showed extensive thickening of the bone trabeculae and decreased amount of bone marrow tissue (figure 3A). In group II, at 4 months after subcondylar fracture and plate fixation, no significant structural changes could be detected either in thickness or structure of the articular fibrous covering. The articular cartilage became thin, the condylar spongiosa showed rapid mineralization and ossification thus forming a delicate irregular bone trabeculae arranged vertically. The temporal bone and the articular disc showed no detectable histological changes (figure 3B).

A B

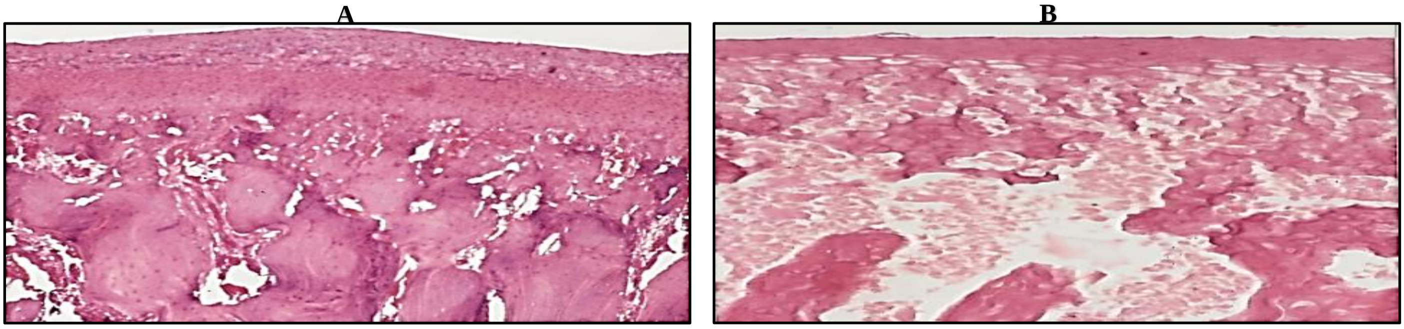

Figure 3. (A) Photomicrograph of group II at 2 months showing condylar head with hypertrophic fibrous covering, thinning of articular cartilage, and condylar spongiosa appeared densely thickened enclosing a small amount of bone marrow tissue; **(B)** Photomicrograph of group II at 4 months showing condylar head with no apparent changes in the articular fibrous covering, thin condylar cartilage, and thin mineralized and ossifying strands of ground substance.

3.2. Histomorphometric analysis and Statistical analysis results

There was a highly significant difference in the total thickness of the articular fibrous covering of condylar head between the studied groups at the different periods, where p-value was <0.01. Meanwhile, there was no significant difference in the thickness of condylar cartilage between the studied groups where p-value was 0.2 (Table 1).

Table 1: Comparison between different studied groups in relation total thickness of fibrous covering and width of condylar cartilage

Thickness/ μm	Normal	Group I		Group II		*P*-value
		2Months	4Months	2Months	4Months	
Fibrous covering	150±22.4	340± 62	128± 37.2	220± 33.3	148± 27.4	<0.01*
Condylar cartilage	163±43.9	134± 23	146± 19.9	144± 41.6	152± 42.8	0.2

4. Discussion

Odontogenic tumors are a diverse group of lesions with a variety of biologic behaviors. Mandibular subcondylar fractures are among the commonest facial bone fractures; however its management remained controversial. Both surgical and non-surgical treatment have been used. Although the general policy is as conservative as possible regarding surgery in management of fractures of the condylar process, an exception is made in cases with displacement of the condyle. Once the condyle is displaced, replacement and repositioning usually cannot be achieved by nonsurgical means. The luxation of the condyle may lead to various long - term complications such as open bite on the contralateral side, deviation in opening and closing movements, dysfunction, and aberrative changes of the contralateral joint, as well as bone apposition leading to ankylosis. The incidence of these complications is considered to be great. The majority of the cases of ankylosis were caused by unreplaced nonsurgically treated condyles [12].

Most of previous studies focused on evaluation of clinical and radiographic changes, following condylar or subcondylar fracture, so, the present study is an attempt to study the histological changes which occurred in the articulating surfaces of the TMJ of dogs after plate fixation of displaced subcondylar fracture. The dog as animal model had been used previously to investigate joint responses as a result of its close similarity to the human TMJ [13, 14]. In order to elaborate the relation between the time factor and the applied surgery on the articulating surfaces of the dog TMJ. The animals in this study were classified into two groups, group I and II, according to their modality of treatment and each group was subdivided into two subgroups according to their scarification dates 2 and 4 months following the operation. This classification was designed to observe the short - term changes (2 months) and the long - term changes (4 months) and to determine the degree of joint response in relation to the type of applied management.

In the present study, the normal histologic features of TMJ of the dog was described, including the articular fibrous covering of the condylar head, the articular cartilage band in the condylar head, the disc, and the articulating surface of temporal bone, in addition to the thickness of the articular fibrous covering of condylar head (150±22.4 μm) and the thickness of condylar cartilage (163±43.9 μm) were measured. This features are in agreement with other previous investigators [15].

In group I, at the first scarification date (2 months after surgery), the results of the present study showed different histological changes including hypertrophy of the articular fibrous covering of the condylar head, the articular cartilage band in the condylar head was markedly decreased and the bone formed subsequent to condyle cartilage was very dense. The disc showed homogenization of the collagen bundles and irregular distribution of its fibrillar component, also, the thickness of the articular fibrous covering of condylar head (340± 62.8 μm) and the thickness of condylar cartilage (134± 23.6 μm). While, at the second follow up period (4 months after surgery), the articular fibrous covering appeared more thinner than normal, with marked decrease in thickness in the articular cartilage layer and the same changes which occurred in the bony part of the condylar head at the first interval period were noted but in an exaggerated pattern. The disc and the temporal bone showed no changes from what is noted in the first scarification date, the thickness of the articular fibrous covering of condylar head (128± 37.2 μm) and the thickness of condylar cartilage (146± 19.9 μm). The previous observations are in agreement

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with the findings of a previous study [16], who found almost similar changes in the TMJ after subcondylar osteotomy. They explained the hypertrophy of articular fibrous covering which appeared at 2 months postoperatively to be a reservoir of cells that aid in the repair of the condylar surface in the case of injury. The histologic changes of the articular cartilage could be considered as degenerative changes. Furthermore, these alterations are in agreement with other investigators [5, 17]. The majority of experimental studies, indicated that the TMJ is young or growing animals, especially the mandibular condyle, is a structure with considerable response to changes in the biomechanical environment. Apparently, this ability to adapt to local or regional changes decreases gradually in mature individuals. Therefore, the histologic findings of the present study may suggest that the condylar cartilage in mature animals shows less ability to recover from functional alterations in articular loading and may be more vulnerable to initial degenerative changes [18]. In the present study, the articular disc showed homogenization of the collagen fibers without adherence to the temporal bone or to the condylar surface. This observation is in agreement with what was noted by a previous investigation [16]. Contradictory results by other previous studies [19, 20], were suggested. These disagreements might be explained due to different surgical techniques used during the operation, the use of different fixation or different parameters measured at different times after surgery. The present study did not observe disc adherence, and this finding is in agreement with other studies [16, 18]. Heamarthrosis, the most important factor in jaw ankylosis, was not observed in any of the specimens. This finding is in agreement with many investigators [1, 18].

In group II, at the first scarification date (2 months after plate insertion), the following histological changes were detected, the articular fibrous covering became hypertrophic when compared with normal one, the articular cartilage was markedly reduced in width but it was of a uniform thickness and the condylar spongiosa showed extensive thickening of the bone trabeculae and decreased amount of bone marrow tissue, also, the thickness of the articular fibrous covering of condylar head (220± 33.3 µm) and the thickness of condylar cartilage (144± 41.6 µm). Meanwhile, 4 months after subcondylar fracture and plate fixation, it was very interesting to denote that no significant structural changes could be detected either in thickness or structure of the articular fibrous covering. The articular cartilage became thin, the condylar spongiosa showed rapid mineralization and ossification thus forming a delicate irregular bone trabeculae arranged vertically. The temporal bone and the articular disc showed no detectable histological changes in the plate fixation group, and the thickness of the articular fibrous covering of condylar head (148± 27.4 µm) and the thickness of condylar cartilage (152± 42.8 µm). These observations are in accordance with other investigations [1, 14]. The explanation of these findings may be the presence of adequate blood supply during open reduction and internal rigid fixation of the fractured and displaced condylar process as the condyle is still attached to its pedicle during the operation. Also, the jaw function after surgery may help

Although, the findings of the present study have indicated that open reduction and internal rigid fixation is the most favorable method for management of displaced subcondylar fractures, these findings come in contrary to other investigators [21, 22]. These inconsistencies might be attributed to different surgical techniques used during the operation, the use of different fixation or different parameters measured at different times after surgery.

5. Conclusion

Based on this study, it is obvious that the plate fixation group gave the most favorable results when compared with MMF group, from the histological point of view. Despite these results, the clinical variables of unilateral versus bilateral fractures, degree of dislocation and displacement, extra or intra articular involvement with the threat of ankylosis, patient age and expected acceptable outcome all rename variables requiring individual case-by-case evaluation.

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