Comparative Histomorphometric Evaluation of Healthy and Ankylosed Mandibular Condylar Process

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Abstract

Background Temporomandibular joint (TMJ) ankylosis is one of the most disruptive maladies afflicting the masticatory system. The characteristic feature is the formation of bony mass bridging condyle with glenoid fossa. The exact pathogenesis is, however, not completely understood.

Purpose To investigate and compare histomorphometric features of ankylosed condylar specimen with normal condylar process.

Materials and Methods Group I included 17 post-traumatic unilateral TMJ ankylosis patients managed by excision of ankylosed mass and interpositional arthroplasty. Group II included 13 condylar head fracture patients managed by surgical debridement. The bony specimens of both the groups were subjected to histomorphometric

examination for assessment of percentage of bone in trabeculae area (%BONE), osteocyte cell density (OSTCD), the presence of inflammation and fibrosis.

Results The mean %BONE, OSTCD, %inflammation, %fibrosis was 60.4%, 340.9 mm², 52.9 and 58.8% in group I and 29.6%, 202.6 mm², 31 and 0% in group II. %BONE, OSTCD and fibrosis in cases of TMJ ankylosis were significantly higher than the controls while no significant difference was observed in the presence of inflammation.

Conclusion The persistence of joint inflammation following condylar head fracture causes aggressive reparative process leading to ankylosis.

Keywords Temporomandibular joint · Ankylosis · Histomorphometry

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Introduction

Mandibular condylar process is an oblong-shaped structure which contributes to the formation of bilaterally symmetrical temporomandibular joint (TMJ), thereby optimizing the mandibular growth and function. Histologically it consists of four distinct zones: articular, proliferative, fibrocartilaginous and calcified cartilage zones [1]. The condylar process undergoes adaptive changes throughout the life without any clinical significance. In TMJ ankylosis, there is structural derangement of the joint leading to fusion of the articulating surfaces causing severe deformity and functional disability. Condylar fractures have been documented to be the most common cause of TMJ ankylosis [2, 3]. Trauma and persistent inadequate jaw movement lead to gradual reduction of mouth opening followed by fusion of the condylar head with articular surface of temporal bone [4].

TMJ ankylosis is a chronic clinico-pathological condition characterized by the formation of bony mass that replaces the joint space resulting in the restriction of mandibular movements. There are numerous studies on TMJ ankylosis related to pathophysiology, imaging, classification and treatment modalities [4–7]. However, there is a paucity of the literature involving microscopic and histomorphometric evaluation of ankylosed temporomandibular joints. Quantitative histomorphometry is a tool that allows tissue level assessment of bone turnover and bone mineralization. Histomorphometry has widely been utilized to study the pathogenesis of metabolic bone diseases as well as for defining mechanisms by which drugs affect the bone [8]. Remarkable advances in bone histomorphometry in the last few decades have widened the scope of this investigative approach [9].

The purpose of this study was to investigate the histomorphometric features of ankylosed condylar specimen and to compare the findings with normal human condylar process. As per authors' knowledge, such a comparative objective evaluation has not been done previously. The authors conducted an objective evaluation of the percentage of bone (%BONE) in trabeculae area, osteocyte cell density in bone (OSTCD) and the presence of inflammation and fibrosis in the bony tissue of TMJ ankylosis cases and compared it with the controls using morphometric technique on hematoxylin and eosin (H&E)-stained sections. Further the correlation between %BONE with OSTCD was also investigated.

Materials and Methods

The prospective study was conducted in the Department of Maxillofacial Surgery from January 2011 to December 2014 after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from patients before their inclusion in the study, and Helsinki guidelines were followed. The study group (group I) comprised 17 patients (8 males and 9 females) in between 6 to 60 years age of post-traumatic unilateral TMJ ankylosis. Patients with recurrent TMJ ankylosis, history of any previous surgical intervention of TMJ and systemic illness were excluded. The control group (group II) comprised 13 patients (9 males and 4 females) in between 18 to 55 years age of unilateral dicapitular fracture of condylar head for more than 8 weeks and not responding to conservative treatment with no clinico-radiological evidence of remodeling. These patients continued to have pain, discomfort and limitation in the mouth opening even after 8 weeks that included period of maxillomandibular immobilization for 2–3 weeks followed by physiotherapy. Patients with

history of previous surgical intervention, medically compromised and unwilling were excluded.

Surgical Technique

The affected joint was exposed by preauricular approach. In group I, gleno-condylar fusion was separated with an osteotome along the line of demarcation. The mass was excised to create a gap of 1–1.5 cm. The ankylosed interface of mandibular condylar head was marked. If necessary, contralateral coronoidectomy is done intraorally so that at least 35-mm mouth opening is achieved. An inferiorly based temporalis muscle-pericranium (TMP) flap was utilized as an interpositional disk replacement material. In group 2, the inferior joint space was explored and the fragments of the mandibular condylar head were debrided. Remaining surgery and postoperative care in both the groups continued as per the institutional protocol. Aggressive jaw movements and physiotherapy was started from second postoperative day in both the groups. The specimen were preserved in 10% formalin and sent to laboratory.

Processing of Specimens and Morphometric Evaluation

The bony tissue was grossed around 5-mm-thickness cut sagittally using electric saw and fixed with 10% formalin. The tissue was fully decalcified with 10% nitric acid aquatic solution for 3–7 days, processed and embedded in paraffin using standard procedure. Five micron section was taken on glass slide and stained by H&E stain, and the representative section was used for morphometric evaluation. The morphometric parameters were evaluated using the Dewinter Research Microscope having CCD camera and image analyzing software (Biowizard 4.2) [10].

For the estimation of %BONE in trabeculae area, four images at 40X magnification for each sample were captured. The boundary of the bone in the captured image was marked using cursor of the computer, the software was used to select the marked bony area, and %BONE was measured for each image. The mean value of the four images was determined for each sample. OSTCD is defined as mean value of osteocyte count per field or area of each field. To estimate OSTCD, four images at 500X magnification for each sample in bony region were recorded where a maximum number of osteocytes were seen. Then, osteocytes were counted for each image and their mean value was determined. The original area of an image captured at 400X magnification in authors' system was 0.0577 mm². Captured images used for the estimation of %BONE and OSTCD for both the groups are shown in Figs. 1 and 2. Inflammation and fibrosis were also

Fig. 1 H&E-stained sections used for morphometric assessment of area of bony trabeculae (original magnification = 40X) in study group (a) and in control group (b)

Fig. 2 H&E-stained sections used for morphometric assessment of osteocytes in bone (original magnification = 400X) in study group (a) and in control group (b)

Fig. 3 H&E-stained section showing inflammatory infiltration (original magnification = 400X) (a); and fibrosis in cartilage tissue (original magnification = 200X) (b)

examined in both the groups. The cases where it was present were considered as positive and otherwise negative. Images depicting inflammatory infiltration and fibrosis are shown in Fig. 3.

Statistical Analysis

SPSS version 17 (SPSS Inc, Chicago, Illinois, USA) was used to perform the statistical analysis. Mean value with standard deviation and range of the studied parameters except for inflammation and fibrosis were calculated for both the groups. For inflammation and fibrosis, percentage of positive cases was determined for the two groups. The difference between the measurements of %BONE and OSTCD was analyzed with unpaired *t* test and of

inflammation and fibrosis was analyzed with Mann–Whitney test. Pearson correlation coefficient (*r*) and regression line drawn were used to observe correlation between OSTCD and %BONE. *p* values ≤ 0.05 were considered as statistically significant.

Results

Group I comprised 17 cases, 8 males and 9 females of unilateral TMJ ankylosis (9 right and 8 left side). The age of patients ranged from 6 to 60 years with an average of 26.5 years. The average age of onset was 9.9 years with a range of 1–45 years. The preoperative maximum interincisal opening (MIO) was 0–15 mm with an average of

6.4 mm. The average %BONE was 60.4% with a range of 49–83%, and the average OSTCD was 340.9 mm² with a range of 211–514 mm². Inflammation was present in 9 cases (52.9%), and fibrosis was present in 10 cases (58.8%). The mean follow-up period was 3 years 2 months with a range of 5 years 1 month–2 years 2 months. The mean postoperative MIO was 40.1 mm with a range of 34–50 mm. There was no clinical and radiological evidence of re-ankylosis in all the cases.

In group II, there were 13 cases (9 males and 4 females) of unilateral condylar head fracture (6 right and 7 left side). The age of these patients was between 18 to 55 years with an average of 30.1 years. The average %BONE was 29.6% with a range of 19–40%, and the average OSTCD was 202.6 mm² with a range of 166–256 mm². Inflammation was present in 31% of cases, and fibrosis was not present in any case. The follow-up period ranged from 4 years 10 months to 2 years 4 months with a mean of 3 years 4 months. Jaw motions with a full range were present postoperatively, and the mean postoperative MIO was 43.5 mm with a range of 39–54 mm. There was no clinical and radiological evidence of ankylosis following surgical debridement in all the cases. The mean and standard deviation of various parameters for both the groups are summarized in Table 1.

There was not any significant difference in age ($p = 0.551$) and the presence of inflammation ($p = 0.232$) between the two groups. However, %BONE, OSTCD and the presence of fibrosis in cases of TMJ ankylosis were significantly higher ($p < 0.001$) than the controls. The comparison of the various parameters between the two groups is presented in Table 2. Scatter plot of %BONE and OSTCD for all samples with linear regression is presented in Fig. 4. %BONE exhibited strong positive correlation with OSTCD ($r = 0.725$).

Table 1 Measurements of various parameters of group I (study group, $n = 17$) and group II (control group, $n = 13$)

Parameters	Groups	Min	Max	Mean	SD	%
%BONE (area)	I	49	83	60.4	9.70	–
	II	19	40	29.6	7.14	–
OSTCD (per mm ²)	I	211	514	340.9	87.51	–
	II	166	256	202.6	28.16	–
Inflammation (%)	I	–	–	–	–	52.9
	II	–	–	–	–	31.0
Fibrosis (%)	I	–	–	–	–	58.8
	II	–	–	–	–	0

SD standard deviation, OSTCD: osteocyte cell density in bone

Discussion

TMJ ankylosis is one of the most common pathologies afflicting the facial skeleton. It is the most over looked and under managed problem in which all the components of the joint are affected due to deranged structural integrity. TMJ forms the very cornerstone of craniofacial integrity, and hence, ankylosis in growing children adversely affects the growth and development of the jaws and occlusion. Increase in size of condylar head adversely affects its functional relationship with glenoid fossa. Trauma is the primary etiological factor of TMJ ankylosis [2, 3]. Therefore, the present study included only post-traumatic TMJ ankylosis cases.

The glenoid fossa is composed of dense cortical bone, whereas the mandibular condylar head is composed of spongy bone covered by a thin layer of cartilaginous tissue and has a rich vascular supply. The articular disk is a fibrocollagenous band lined with synovial membrane, and it divides the joint space into upper and lower compartment. The articular disk and capsular ligament have poor vascularity and reparative ability [1]. The exact mechanism for the pathogenesis of TMJ ankylosis was unclear. It was simply described as the organization or ossification of an intra-capsular hematoma following an intra-capsular fracture in the presence of immobility and an injured disk. The hematoma that resulted after condylar head fracture acts as a scaffold for inflammatory cells to perform their reparative function and limited jaw movement hastens the progress of ankylosis. Though the hematoma organizes, the condyle never returns to its pretraumatic form and rather manifests as a mass of bony tissue adherent cranially to the skull base and caudally to the remaining condylar head.

The results of the present study have revealed that inflammation was present in 52.9% TMJ ankylosis cases and statistically no significant difference was observed in the presence of inflammation between cases of TMJ ankylosis and condylar head fracture. The inflammatory condition of the joint capsule and disk never resolved due to poor reparative ability. The presence of significant number of chronic inflammatory cells indicates an active healing process. The authors viewed that persistence of joint inflammation is due to the presence of regular stress in these cases. Once the ankylosis gets established, even limited mandibular movement due to the elastic nature of tissue along with the suprahyoid group of muscles produced an electric field for most of the time in that region due to piezoelectric effect [11]. It has been documented that the action of the piezoelectric effect may modify the chemistry of collagen and influence cellular activity directly [12]. The transportation of the ions in that region was enhanced due to electromechanical signals that further

Table 2 Comparison of measurements of various parameters between the two groups by unpaired *t* test and Mann–Whitney test

Parameters	Mean difference	SE of difference	<i>T</i> value	<i>Z</i> value	<i>p</i> value
%BONE area	30.79	3.20	9.617	–	< 0.001
OSTCD (per mm ²)	138.27	25.30	5.465	–	< 0.001
Inflammation (%)	–	–	–	– 1.194	0.232
Fibrosis (%)	–	–	–	– 3.330	< 0.001

SE standard error, *OSTCD* osteocyte cell density in bone

Fig. 4 Scatter plot and linear regression of %BONE and OSTCD for all the samples

increased the activity of fibroblasts and osteoblasts. The increased migration and activity of fibroblasts and osteoblasts lead to increased collagen synthesis and callus bone formation. The increased density of osteoblasts, later on get transformed to osteocytes, contributes to the increase in percentage of bone in the trabeculae region of TMJ ankylosis cases. Therefore, an orderly progression of tissue differentiation and maturation eventually leads to organization into dense lamellar bone and formation of bony block bridging the condyle and glenoid fossa. The %BONE, OSTCD and the presence of fibrosis in cases of TMJ ankylosis were significantly higher than the controls. The study also revealed strong positive correlation of %BONE with OSTCD. Hence, it can be concluded that the inflammatory condition of joint space following condylar fracture did not get resolved in majority of the patients and the persistence of joint inflammation eventually leads to ankylosis of the joint.

Active jaw motion is of paramount importance in combating ankylosis in highly vascularized and osteogenic environment [4]. Repeated movement at the condyle fracture site causes repeated disruption of the developing microvasculature and failure of osteogenesis. The measurement of the maximum interincisal distance is a very good indicator of TMJ function. With reduced maximum interincisal distance, the disability is greater. In the present study, there were no cases of limited mouth opening

following surgical intervention in both the groups. Aggressive physiotherapy was started from second post-operative day in both the groups. Therefore, active jaw motion halts the progress of ankylosis, but once it is established, even limited degree of mandibular movement is deleterious to the joint as it stimulates the healing process and bone formation due to piezoelectric effect.

Histomorphometry of bone is a powerful investigative tool to study the metabolism of bone. It provides valuable information that is not available by any other investigative approach. Means and standard deviations are best used as a method of comparison. The results of this study have provided vital information that pathogenesis of TMJ ankylosis is due to the presence of chronic inflammation. It also formed the basis of surgical intervention or only short period of immobilization in cases of condylar head fracture. Quantitative histomorphometry of bone can also be extrapolated to animal models where assessments can be done at periodic intervals to gain further vital information.

The role of articular disk in preventing ankylosis is a debatable issue. Some authors have shown that damage to the articular disk is necessary for the formation of TMJ ankylosis in an animal model [4–6, 13]. Commonly, the model involves surgical removal of the condylar head, while maintaining the medial capsule and reconstituting the lateral capsular boundaries, thus containing the post-injury hematoma within the intra-capsular space. It also formed the basis of interpositional arthroplasty to construct a barrier and block the formation of a bony bridge between the condylar stump and the articular surface. The opponents have pointed out that extrapolation of these observations to human beings is tenuous since accurate reproduction of the human condylar fracture milieu in an animal model is technically difficult and ethically questionable. The disk hardly provides any mechanical separation against an enlarged and deformed condylar head. Radiologically many a times a thin radiolucent jag jag line is well appreciated within the ankylotic mass which may be the remnant of articular disk. Ankylosis follows wherever the hematoma and the base of skull converge, and since this is most often extra-capsular, ankylosis occurs despite the presence of an intact disk [14]. The authors intra-operatively have observed a definite cleavage through which the ankylotic mass can be detached from skull base in the present study,

but there was no evidence of articular disk. Interpositioning is a good means of limiting resection and preventing recurrence. Temporalis muscle-pericranium flap, an axial pedicled flap, was used to provide soft tissue lining for TMJ reconstruction [15]. The most important aspect of the TMP flap is its role in maintaining the functional movements of the TMJ. As the flap is attached to the mandible anteriorly via the coronoid process, when the condyle translates, the movement of the coronoid process pulls the muscle-pericranium flap, simulating the natural dynamic function of the disk.

The present article emphasizes that TMJ ankylosis is a progressive chronic inflammatory condition of the joint. Based on the outcomes of this study, we are in opinion that dicapitular fracture of condylar head more than 8 weeks old and not responding to conservative treatment may be considered for surgical debridement. Undoubtedly, vigorous post-trauma mobilization is very important in preventing the complication of ankylosis following condylar fracture as once the ankylosis sets up, even limited mobility of the joint leads to further osteogenesis. Continued studies could further enlighten the pathogenesis of TMJ, thereby enabling the clinician to understand disease process better and render effective care.

Compliance with Ethical Standards

Conflict of interest All authors have no conflict of interest.

Ethical Approval All procedures involved in the present study involving human participant were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed Consent Informed consent was obtained from the individual included in this study.

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