Histopathological features of hypertrophic bone mass of temporomandibular joint ankylosis (TMJA): An explanation of pathogenesis of TMJA

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A B S T R A C T

Temporomandibular joint ankylosis (TMJA) is a severe organic disease with progressive limitation of the mouth opening. Histopathologically, a residual joint space is reported to consist of fibrous tissue and/or cartilage, indicating two types of interface (osteo-fibrous and osteo-chondral) of residual joint space. It is well known that adverse mechanical stress results in pathological changes of osteoarthrisis and enthesopathy in these interfaces. What would happen pathologically in these interfaces of TMJA under repeated mandible movement has not been elucidated. Fourteen tissue samples of residual joint space and temporal and condylar bone were stained with hematoxylin and eosin and evaluated by collagen I and II immunohistochemistry. A pathological study of 14 TMJA patients showed that the residual joint space presented a fibrocartilage entheses structure and an articular cartilage structure. Moreover, these two structures were associated with pathological alterations of both osteoarthrisis and enthesopathy, including degenerated and necrotized tissue, chondrocyte cloning, crack and fissure, various bone scleroses, and inflammatory granulation tissue. It is suggested that the pathological alterations of both osteoarthrisis and enthesopathy occurred in TMJA, which hints at mechanical stress on TMJA development.

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1. Introduction

Temporomandibular joint ankylosis (TMJA) is a severe organic disease with progressive limitation of the mouth opening, seriously impairing the patient’s stomatognathic functions. The pathogenesis of TMJA has not been demonstrated well so far. Adverse mechanical stress in TMJ generated from repeated mandible movement has been hypothesized as a key factor affecting TMJA development (Yan et al., 2012). Histopathologically, a residual joint space characterized by an indistinct radiolucent zone on computed tomograms was reported to consist of fibrous tissue and/or cartilage (Blackwood, 1957; Miyamoto et al., 2000; Wu et al., 1994). There were thus two types of interface of residual joint space, namely, osteo-fibrous and osteo-chondral. However, histopathologic changes related to both types of interfaces in TMJA have not been elucidated.

The osteo-fibrous interface form, which is common in the junction between the tendon or ligament and the bone, is often termed the enthesis. It has long been known that there are two fundamentally different types of entheses: fibrocartilaginous and fibrous (Benjamin M, 1995). In a fibrous enthesis, the collagenous tendon or ligament is directly attached to the bone via Sharpey’s fibers (Benjamin et al., 2002), indicating the presence of traction stimulus, whereas in a fibrocartilaginous enthesis, the collagenous tendon or ligament is indirectly attached to the bone via uncalcified and calcified fibrocartilage. The presence of fibrocartilage is considered an adaptation to compression and shear (Benjamin and Ralphs, 1998). Sporting activities and other repeated strenuous physical activities are the leading cause of enthesopathy, e.g., tennis elbow,
golfer’s elbow and jumper’s knee. Their pathologic changes include bony spurs (enthesophytes), fissures and cracks, subchondral sclerosis, hyaline or mucoid degeneration, and bone cysts (Benjamin and McGonagle, 2001; Benjamin and Ralrh, 1998; Benjamin et al., 2009; Rufai et al., 1995; Shaibani and Rothschild, 1993).

The junction between cartilage and bone in particular is a common form of osteo-chondral interface. Subjected to adverse mechanical stress, the junction would generate the pathological changes of osteoarthritis (OA) (Brandt et al., 2009; Fujisawa et al., 2003; Wu et al., 1994; Yao and Hu, 1999), including degeneration of cartilage, subchondral cysts, subchondral sclerosis, and osteophyte formation (Burr and Gant, 2012; Cascone et al., 2003; Han et al., 2007; Hunter et al., 2009; Kaspiris et al., 2013; Wu et al., 1994; Yao and Hu, 1999).

It has been well accepted that adverse mechanical stress could generate such pathological changes as OA and enthesopathy in the joint and tendon (or ligament), respectively; thus we wondered whether the osteo-fibrous and osteo-chondral interfaces in TMJA subjected to adverse mechanical stress would generate both OA-like and enthesopathy-like pathological changes. The aim of this study was to investigate pathological alteration of the residual joint space interface in TMJA and to present pathological features of TMJA similar to these of OA and enthesopathy, thereby enabling interpretation of the mechanical stress to which the tissue is subjected.

2. Material and methods

The subjects were 14 TMJA patients who had undergone surgery in the Peking University School and Hospital of Stomatology (PKUSS) between 2005 and 2012. All patients had experienced mandibular condyle injury before TMJA developed, but the details of the injuries were not reported in their medical records. Of the 14 patients treated (10 men and 4 women, 10 unilateral, and 4 bilateral), the mean age was 27.1 years (range 7–57 years), and the mean age at injury was 17.0 years (range 4–53 years). The mean maximal mouth opening (MMO) was 6.9 mm (range 0–15 mm) (Table 1). In addition, the institutional review board of PKUSS approved the study, and informed consent was obtained from all study participants (Table 2).

2.1. Hematoxylin and eosin staining and immunohistochemistry staining

Fourteen tissue samples comprising residual joint space, temporal, and condylar bone were harvested from the lateral side of TMJA bone mass during surgery. For the TMJA bone mass located near the skull, surgeons tried their best to avoid injuring the skull bottom during operation. There were 11 cases with small temporal bone and large condylar bone. In the remaining 3 cases, temporal and condylar bone could not be discriminated, as the two elements did not show their respective characteristics. All tissue samples were fixed in 10% neutral buffered formalin solution for 3 days and decalcified with 0.5 M ethylenediaminetetraacetic acid (EDTA). Each sample was dehydrated and embedded in paraffin by the standard method. Serial sections (5-μm thickness) were prepared from the central region of samples in the coronal plane and alternately underwent hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining. IHC staining against collagen I and collagen II was performed in serial sections. Sections were deparaffinized in xylene and rehydrated in grade alcohol, and incubated for 25 min in a 3% H2O2 solution for saturating endogenous peroxidases. To retrieve epitopes, enzyme digestion (collagen I: 0.2% trypsin Z LI-9010; collagen II: pepsin ZLI-9013, ZSGB-BIO, Beijing, China) was performed for 15 min at 37 °C followed by incubation overnight in a humidified chamber at 4 °C in primary antibody (collagen I: 1/500, ab21285, Abcam, UK; collagen II: 1/500, ZM-0390, ZSGB-BIO). On the second day, the sections were incubated in horseradish peroxidase–conjugated broad-spectrum secondary antibody (PV-9000, ZSGB-BIO, CHN) and visualized by reaction with 3,3-diaminobenzidine (DAB) substrate (ZLI-9018, ZSGB-BIO, CHN). Images were captured with an Olympus BX51 microscope (Tokyo, Japan).

2.2. Morphological analysis

For the various sizes of bone tissue beneath the interface, only bone tissue larger than 3.5 × 2.6 mm2 (a 4× field) was captured with the microscope to perform the following measurements using Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA) and Photoshop 7.0 software (Adobe, Inc., San Jose, CA, USA).

2.2.1. Residual joint space width (RJSW)

RJSW was defined as the mean distance of two curves, which indicated the interface of residual joint space with the condyle and temporal bone.

2.2.2. Condyle bone sclerosis index (CBSI)

In order to describe the bone sclerosis of condyle, three fields (4×) of 3.5 × 2.6 mm2 were captured randomly just beneath the interface for each case. CBSI was defined as the mean ratio of bone area to box area for the three images.

2.3. Statistical analysis

Correlation analyses and t-tests were performed using SPSS version 17.0 (SPSS, Chicago, IL, USA), and the level of statistical significance was established at p < 0.05.

3. Results

In all 14 cases, there was a residual joint space filled with fibrous tissue and/or cartilage between the temporal bone and the condyle, and the patterns of its tissue distribution were classified into 5 types. The histopathological alterations are described in detail below.

3.1. Alteration of residual joint space

In the osteo-fibrous interface, the residual joint space was dominantly filled with fibrous tissue. Three patterns of fibrous tissue distribution were observed. In type I, the alignment of collagen bands was irregular in the joint space (Fig. 1A–C). In type II, in
addition to the fibrous tissue with irregular alignment of collagen bands in the middle of the residual joint space, cartilage was also located at the superior and inferior sides of the fibrous tissue (Fig. 1D–F). In type III, the tissue in the residual joint space showed the appearance of fibrocartilage entheses with four zones at both sides (Fig. 2 and Fig. 3). The first zone was fibrous connective tissue, comprised of longitudinally aligned collagen bundles and containing a variable number of elongated fibroblastic cells. IHC staining showed the presence of collagen I. The fibrous connective tissue changed gradually into uncalcified fibrocartilage. The cells took on the chondrocyte phenotype, becoming rounded and arranged in pairs or rows in the extracellular matrix with weakly positive or negative IHC staining for collagen II. The third zone is composed of calcified fibrocartilage of various thicknesses with positive IHC staining for collagen II, and separated from uncalcified fibrocartilage by a basophilic (blue) line, termed the tidemark. Finally, the fourth zone is composed of trabecular bone. In all three types, there were no signs of cracks or fissures, degenerated or necrotized tissue, or crack and fissure (perhaps a residual joint space) separated the cartilage into condylar and temporal parts (Fig. 4). According to the presence or absence of distinct tissue layers, the cartilage in the residual joint space was classified into two types: type IV and type V. In type IV, the cartilage appeared without distinct tissue layers (Fig. 6). In type V, the cartilage filling in the residual joint space showed the appearance of articular cartilage with three zones at both sides (Figs. 7 and 8). The first zone was uncalcified cartilage with round chondrocytes. The second zone was calcified cartilage with round calcified chondrocytes, which was stained with hematoxylin. Between the uncalcified and calcified cartilage was one or more undulating hematoxylin lines, namely, the tidemark, which sometimes were penetrated with angiogenesis. The third zone was bone tissue. The cartilage showed positive IHC staining for collagen II and weakly positive or negative IHC staining for collagen I.

Table 2
Pathologic changes in temporomandibular joint ankylosis.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Tissue distribution type</th>
<th>Dominant Interface type</th>
<th>RJSW</th>
<th>CBSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>II+IV</td>
<td>OFI</td>
<td>2.4</td>
<td>0.94</td>
</tr>
<tr>
<td>2</td>
<td>I+IⅡ</td>
<td>OFI</td>
<td>2.3</td>
<td>0.60</td>
</tr>
<tr>
<td>3</td>
<td>II+IV</td>
<td>OFI</td>
<td>2.6</td>
<td>0.74</td>
</tr>
<tr>
<td>4</td>
<td>II+IV</td>
<td>OCI</td>
<td>1.3</td>
<td>0.72</td>
</tr>
<tr>
<td>5</td>
<td>II+IV</td>
<td>OCI</td>
<td>0.7</td>
<td>0.77</td>
</tr>
<tr>
<td>6</td>
<td>III+Ⅳ</td>
<td>OCI</td>
<td>1.4</td>
<td>0.78</td>
</tr>
<tr>
<td>7</td>
<td>V</td>
<td>OCI</td>
<td>1.1</td>
<td>0.82</td>
</tr>
<tr>
<td>8</td>
<td>II+IV</td>
<td>OFI</td>
<td>2.4</td>
<td>0.65</td>
</tr>
<tr>
<td>9</td>
<td>III+Ⅴ</td>
<td>OCI</td>
<td>2.1</td>
<td>0.59</td>
</tr>
<tr>
<td>10</td>
<td>II+IV</td>
<td>OFI</td>
<td>3.2</td>
<td>0.85</td>
</tr>
<tr>
<td>11</td>
<td>II</td>
<td>OFI</td>
<td>2.1</td>
<td>0.60</td>
</tr>
<tr>
<td>12</td>
<td>V</td>
<td>OCI</td>
<td>2.3</td>
<td>0.94</td>
</tr>
<tr>
<td>13</td>
<td>III+Ⅴ</td>
<td>OFI</td>
<td>1.6</td>
<td>0.85</td>
</tr>
<tr>
<td>14</td>
<td>II+Ⅳ</td>
<td>OCI</td>
<td>1.0</td>
<td>0.65</td>
</tr>
</tbody>
</table>

CBSI, condyle bone sclerosis index; CC, chondrocyte cloning; CF, crack and fissure; DNT, degenerated and necrotized tissue; IGT, inflammatory granulation tissue; OCI, osteochondral interface; OFI, osteo-fibrous interface; RJSW, residual joint space width; TM, tidemark.
3.2. Condyle marrow alteration

Beneath the osteo-chondral (fibrous) interface, there was an inflammatory reaction generating an isolated granulation tissue with multinuclear macrophages degrading the cartilage (Fig. 5) or osteoclasts degrading the bone (Fig. 9) and angiopoiesis scattered in the granulation tissue. Various bone sclerosis were clearly observed beneath the osteo-fibrous and osteo-chondral interfaces, with CBSI ranging from 0.59 to 0.94 (Fig. 10).

3.3. Correlation analysis of pathologic features

Statistical analysis showed that RJSW was related to the interface type ($r = -0.73$, $p = 0.00$) and was wider in the osteo-fibrous
**Fig. 4.** Cracks and fissures (white arrow), chondrocyte clone (black arrow). Tem, temporal bone; RJS, residual joint space; Con, condyle bone.

**Fig. 5.** Degenerated and necrotized tissue with amorphous, metachromatic and eosinophilic staining (A, black open arrow). Inflammatory reaction generating an isolated granulation tissue beneath osteo-chondral interface (B) with a multinuclear macrophage (C, black open arrow) and angiopoiesis (D, black open arrow) scattered in the granulation tissue.

**Fig. 6.** Tissue distribution type. Type IV, the cartilage without distinct tissue layers (white asterisk) located in residual joint space (RJS). Tem, temporal bone; Con, condyle bone.
interface than in the osteo-chondral interface (2.4 mm and 1.4 mm, respectively; \( p = 0.01 \)). However, the correlation between the interface type and CBSI was not significant (\( r = 0.02, p = 0.95 \)).

4. Discussion

During the operation, it was difficult to obtain sandwich-like TMJA bone mass consisting of temporal bone, residual joint space, and condylar bone for the surgeons’ tendency to avoid skull base injury, which resulted in the sample usually consisting of residual joint space and condylar bone. This study collected only 14 sandwich-like tissue samples from the lateral side of TMJA bone mass in the past 8 years and provided illuminating histopathologic findings.

Osteoarthritis (OA) is a degenerative joint disease that is characterized by articular cartilage deterioration (fibrillation, necrosis, fissure, chondrocyte cloning, multiple tidemarks) and sub-chondral alteration (bone sclerosis, bone cyst, osteophyte).
Adverse mechanical stress, subsequent to condylar fracture (Wu, 1992; Wu et al., 1994), disc injury (discectomy) (Bjornland and Haanaes, 1999), disc displacement (Ali and Sharawy, 1994), disc perforation (Sato et al., 1998), or repetitive mouth opening (Fujisawa et al., 2003), has been recognized as one of the major implicating factors in TMJ OA. If so, the patient with TMJA secondary to condyle fracture would present with the pathologic alterations of OA as a matter of course. In this study, the joint spaces with an osteo-chondral interface were dominantly filled with cartilage and were inclined to formation of OA-like features, such as necrosis, crack and fissure, chondrocyte cloning, tidemark, and bone sclerosis.

Compared to the osteo-chondral interface, the histopathology in the osteo-fibrous interface was less OA-like and more complex. What surprised us was that the fibrous tissue of type III shared characteristics of fibrocartilage entheses with longitudinally aligned collagen bundles and gradual transition from connective tissue, uncalcified fibrocartilage, and calcified fibrocartilage to bone tissue. The articular disc in the TMJ is made of fibrocartilage; however, the fibro-cartilaginous material in the joint space is not an articular disc, based on the following observations. First, the direction of collagen fiber of articular disc is parallel to the joint surface, but the direction of collagen fiber of fibrocartilage entheses in TMJ (type III) is vertical to the soft/hard tissue interface. Second, the fibrocartilage entheses presents a four distinct tissue layers, that is, fibrous connective tissue, uncalcified fibrocartilage, calcified fibrocartilage, and trabecular bone; however, the articular disc does not present such kind of tissue layer. Except tendon, ligament, and TMJA, fibrocartilage at a bony interface also presents among spondylolytic spondylolisthesis (Boszczyk et al., 2006) and pseudoarthroses (Heggeness et al., 1993) and is widely believed to provide a two-tier system of protection against stress concentration (Benjamin and McGonagle, 2001). The zone of calcified fibrocartilage anchors the connective tissue to the bone and enables it to withstand shearing forces occurring during changes of insertional angle, whereas the uncalcified fibrocartilage is believed to dissipate bending forces away from the hard/soft tissue boundary (Boszczyk et al., 2006).

In addition, a recent medical hypothesis was proposed by our team that the development of traumatic TMJA was a process similar to hypertrophic nonunion (one type of pseudoarthroses) of long bone fracture (Yan et al., 2012). In this study, we presented a pathologic evidence for this hypothesis that both TMJA and pseudoarthroses shared the features of fibrocartilaginous entheses.
It was surprised that tissue distribution pattern of type I showed no remnants of temporal bone and articular cartilage superiorly and of mandibular bone and cartilage inferiorly. What happened on the cartilage during TMJA development? In our opinion, the remnants of bone and cartilage mentioned above could be there during the very early stage of TMJA development; however, the remnants would experience active bone/cartilage remodeling, and the structure of bone-cartilage in joint surface would change during the following stage of its development. This assumption needs to be investigated further.

Clinically, OA and enthesopathies are two different diseases involved in joint and tendon (or ligament), respectively. However, the pathological changes of these two diseases coexisted in TMJA. Its potential mechanism probably involves direction of differentiation of mesenchymal progenitor cells by mechanical stresses. In this study, there were five tissue distribution types in the residual joint space. From the correlation analysis that RJSW was related to the interface type and the residual joint space with fibrous tissue was wider than that with cartilage, we could deduce that there was a transformation from fibrous tissue to cartilage in the residual joint space with TMJA development. In our opinion, type I was at early stage, type II and type IV were at transition stage and type III was at end stage. In some situations, type I could transform into type II, and then type III in the direction of fibro-cartilage; but in other situations, type I could transform into type II, type IV and then type V in the direction of cartilage. However, this assumption needs to be investigated further.

5. Conclusion

This study demonstrated that the pathologic changes of TMJA involved not only the joint space but also the bone marrow showing osteoarthritic- and enthesopathy-like histopathological changes. This knowledge deepens our understanding of TMJA and may be conducive to explore hints of mechanical stress on TMJA development.

Conflict of interests

The authors declare that they have no conflict of interests.

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References


Hunter DJ, Gerstenfeld L, Bishop G, Davis AD, Mason ZD, Einhorn TA, et al: Bone marrow lesions from osteoarthritic knees are characterized by sclerotic bone that is less well mineralized. Arthritis Res Ther 11: R11, 2009


