Investigation of collagen architecture in diseases of temporomandibular joint

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Abstract

Background: Temporomandibular joint (TMJ) is a stress bearing structure. Its resilience and functions are supported by collagen architecture. Visualization of this collagen networking can be done under polarizing microscope. This network gets modified in a variety of conditions affecting the TMJ like- ankylosis, degenerative joint disorder, neoplastic conditions, trauma and ageing. The objective of this study is to analyze the histomorphological changes in TMJ conditions and correlate them with collagen architecture so as to project scoring criteria for distinguishing between osseous changes.

Methods: Paraffin based H and E sections of TMJ conditions were obtained and observed under polarizing microscopy. Sample distribution was as follows: Degenerative joint disease (n=10), ankylosis (n=5) and osteochondroma (n=5). A scoring criterion was devised and cutoff value was determined.

Results: Immature or formative tissue was found to have a score of less than and equal to five and mature tissue had a score of greater than and equal to six.

Conclusion: Scoring criterion proposed in this study is a reliable technique and can be used for confirming the histological architectural changes within a diseased TMJ.

Keywords: Temporomandibular joint, collagen, polarizing microscope, diarthrodial joint, articular cartilage

Introduction

Collagen fibrillar networking is the basic structure supporting the three-dimensional architecture of tissues. This provides tensile strength and volume maintenance [1]. Condylar pathologies may be developmental, traumatic, vascular, abnormal muscle pulling, nutritional, endocrinal, teratogenic and infectious in origin [2]. These disorders cause a change in structural integrity of TMJ histology and underlying collagen network. Most common etiology of TMJ ankylosis is after trauma or infection [3]. Few cases are congenital in origin [4,5]. Approximately 3% to 4% of population seek treatment for TMJ disorders, and roughly 70% of these patients suffer from disc displacement [6]. In this study, an attempt towards understanding architectural changes in collagen networking in various temporomandibular joint disorders has been made by means of polarizing microscopy.

Materials and methods

Hematoxylin and eosin stained paraffin-based sections were obtained from various temporomandibular joint conditions. Sample was distributed as follows: Degenerative joint disease (n=10), ankylosis (n=5) and osteochondroma (n=5). Polarizing light microscopy was used to delineate collagen fibril architecture in tissue sections. A qualitative PLM (Polarizing light microscopy) scoring system was designed (Table 1). Institutional

ethical committee clearance was obtained according to guidelines provided by Declaration of Helsinki. Radiographs were evaluated for all cases included in the study (Figure 9).

Results and observation

Diseased condylar tissue when observed under polarizing microscope exhibited a variety of patterns (Table 2). Woven bone showed an irregular fishnet-like pattern suggestive of a forming tissue. Mature bone had parallel lamellations corresponding to collagen mineralizing at regular intervals. This was observed as thick parallel lines. Cartilage had no birefringence. Overall scores observed were:

a. Ankylosed condylar tissue (Figures 1 and 2):

3 (mature bone) + 2 (thick) + 1 (orange) + 2 (parallel) = 8.

b. Osteochondroma (Figures 5 and 6):

2 (parallel lamellations) + 1 (orange) + 2 (thick) + 2 (parallel) = 7.

c. Degenerative joint disease (Figures 3 and 4):

2 (parallel lamellations) + 0 (light orange) + 1 (thin) + 2 (immature bone) = 5.

2 (parallel lamellations) + 2 (orange-green) + 2 (thick) + 3 (mature) = 9.

Table 1. Proposed PLM qualitative scoring system.

Microscopic feature	Score
I. Pattern of collagen bundles:	
a. No birefringence:	0
b. Irregular pattern (woven bone):	1
c. Parallel lamellation pattern (mature bone):	2
II. Birefringence color:	
a. Light orange (immature):	0
b. Orange (mature):	1
c. Orange-green (maturing):	2
III. Fibril thickness:	
a. Thin:	1
b. Thick:	2
IV. Tissue type:	
a. Osteoid:	1
b. Woven bone:	2
c. Mature lamellar bone:	3
d. Cartilage:	4

Figure 1. Photomicrograph showing mature bone with Osteocytes in ankylotic mass. (10X, H & E).

Figure 2. Photomicrograph depicting parallel orange collagen lamellations in ankylotic mass (10X, polarizing).

Figure 3. Photomicrographs depicting mature lamellar bone with absence of Osteocytes in degenerative joint disease (10X, H & E).

Figure 4. Haversian systems with concentric and parallel orange-green to light orange lamellations seen in degenerative joint disease (20 X, polarizing).

Figure 5. Mature bone with vital Osteocytes and bone marrow seen in osteochondroma (4X, H & E).

d. Forming condyle (Figures 7 and 8):

1 (Irregular lamellations) + 1 (orange) + 1 (very thin) + 1 (woven) = 4.
It was surmised from scores obtained that a score ≤ 5 was indicative of a condylar tissue constituted by immature tissue

Table 2. Polarizing microscopy patterns and histomorphology in condylar diseases.

Tissue type	Pattern of collagen bundles	Color of birefringence	Fibril diameter	Histomorphology
Young condyle	Irregular (fishnet pattern)	Orange	Very thin	Woven bone
Degenerative joint disease	Parallel lamellations	Light orange	Thin, Nonuniformly spaced	Immature bone
		Orange-green	Thick, uniformly spaced	Bone
Ankylosis	Parallel lamellations	Orange	Thick, uniformly placed	Mature lamellar bone
Osteochondroma	Parallel lamellations	Orange	Thick, uniformly spaced	Mature lamellar bone
	Parallel lamellations	Orange	Thin, Nonuniformly spaced	Osteoid
	No birefringence	--	--	Cartilage

Figure 6. Photomicrograph showing thick orange parallel lamellations (20X, polarizing).

Figure 8. Young condyle showing orange fishnet pattern (20X, polarizing).

Figure 7. Photomicrograph showing woven bone with bone marrow tissue in forming condyle (20X, H & E).

Figure 9. OPG depicting bilateral ankylotic mass.

elements like osteoid, thin collagen fibrils and woven bone whereas a score of ≥ 6 was indicative of a mature condylar tissue consisting of mature lamellar bone and thick, mature collagen fibers. Image analysis software (NIH Image J) was used for validating the color pixels and fiber thickness in each condition. For thick fibers, pixels varied in range of 183, 162, 157 whereas, for thin fibers, pixels varied from 172, 105, 112. For evaluation fiber thickness, each image was

contrasted using Image J software and thickness measured ([Supplementary figures S1 and S2](#)).

Discussion

The normal biomechanical behaviour of a diarthrodial joint is dependent on the composition and ultrastructural organization of the articular cartilage. The collagen network of articular cartilage protects the chondrocytes, resists tensile forces produced by compression as well as interstitial swelling, provides attachment for proteoglycans, and anchors the

cartilage to the subchondral bone. Normally, articular cartilage that is regularly subjected to high levels of shear stress shows a high degree of collagen orientation and a thick superficial zone [1]. Collagen organization is critical factor responsible for load bearing and durability of TMJ. Many scoring systems have been proposed for studying the histomorphology and collagen orientation of the temporomandibular joint. These systems can be divided into a) semi-quantitative methods: OsScore and ICRS (International Cartilage Repair Society) I and II and b) qualitative technique like PLM (polarizing light microscopy) [7,8]. Polarized light microscopy is a traditional method for visualizing the collagen network architecture of condylar cartilage [9]. Polarized light microscopic technique can be used to measure collagen fibril orientation, parallelism, and birefringence [10,12]. To overcome the drawbacks associated with polarizing microscopy as quoted by Rieppo et al., two observers viewed the slides and scoring was done with unanimous decision. Linear polarizing microscopy can be performed on a light microscope with addition of two filters, polarizer and analyzer. The polarizer is placed after the light source and ensures only linear polarized light i.e., light in a single plane that is perpendicular to direction of light propagation, is transmitted to the specimen. Optically anisotropic materials change the direction of light through birefringence. The fibrillar structure of collagen splits the incident polarized light into two orthogonal rays depending on the direction of collagen at each point in the section. The analyzer filter positioned after the specimen is at right angle to the polarizer. The polarized specimen is observed through an eyepiece. The objective of using polarizing light microscopy is to assess the extent to which a sample demonstrates collagen organization. This microscopic technique can be used to visualize fibrillar collagen for describe its network, changes in collagen orientation during ageing, validation of collagen imaging modalities and for observing changes during loading and degradation. Use of polarizing microscope for scoring purposes offers a systematic assessment of collagen organization. In this study, this technique for microscopic visualization has been used to demarcate a cutoff scoring value for immature and mature osseous structures in temporomandibular joint disorders. Less than five score value is suggestive of an ongoing formative process whereas a score of greater than or equal to six is indicator of a stabilized structure (no remodeling process). Here, a new scoring criteria using histomorphology as well as polarizing light microscope has been proposed. Almeida et al., performed histologic and histomorphologic analysis of collagen using picrosirius red. They compared the light intensity between diseased TMJ and normal controls using image analysis software. No statistical difference was observed between both groups. However, this was a qualitative analysis and did not study collagen birefringence in terms of collagen remodeling in immature and mature bone [10]. In a contradictory study studying effects of distraction osteogenesis on temporomandibular

joint, disorganization of collagen fibers was observed (H & E). However, no significant difference was found in histology and fiber thickness between the distracted and contralateral sides [11]. Panula et al., reported reduced birefringence and thinning of fibers in arthritic temporomandibular joints [1]. However, in this study, both thick and thin fibrils were found with varying intensities of birefringence. The authors have added few modifications in the PLM scoring proposed by Changoor et al., by including some additional groups so that the validity of the scoring system can be enhanced. No other study has included other pathologies affecting temporomandibular joint for studying collagen architecture using any of the methodologies available. Hence, further validation of the method proposed in this study is required and perhaps can be verified with a larger sample size as well as by other investigators.

Conclusion

TMJ is a synovial joint that is affected by numerous changes that can be age-related or pathologic in nature. The underlying collagen architecture provides mechanical support and resilience to the joint. The changes in collagen structure are reflected upon the structural changes and defects in the joint itself [12].

Additional files

[Supplementary figure S1](#)
[Supplementary figure S2](#)

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	SC	SJ
Research concept and design	✓	--
Collection and/or assembly of data	✓	✓
Data analysis and interpretation	✓	✓
Writing the article	✓	--
Critical revision of the article	✓	--
Final approval of article	✓	--
Statistical analysis	✓	--

Acknowledgement

Author would like to acknowledge Department of Oral and Maxillofacial Surgery, MMCD SR, Mullana (India).

Publication history

EIC: Markus H. Frank, Harvard Medical School, USA.
Received: 05-Sep-2013 Revised: 17-Sep-2013
Re-Revised: 01-Oct-2013 Accepted: 08-Oct-2013
Published: 18-Oct-2013

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Citation:

Chatterjee S and Jindal S. **Investigation of collagen architecture in diseases of temporomandibular joint.** *Pathol Discov.* 2013; **1**:7.
<http://dx.doi.org/10.7243/2052-7896-1-7>