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Histologic and histomorphometric analysis of posterior region of the human temporomandibular disc

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Objective
The aim of this study was to analyze histologic and histomorphometric features of the articular disc in groups with and without disc displacement.

Study design
A sample of 39 temporomandibular joints TMJs (31 case specimens, 8 control specimens) from 28 patients (mean age 31.2 years) were recruited for this study. The patients were considered to be affected and treated surgically with disc repositioning when presenting painful clinical signs of disc displacement after unsuccessful nonsurgical treatment for at least 6 months. Of the control patients, 4 presented condyle fracture which required opening to be reduced for treatment, and 4 displayed active condyle hyperplasia. The posterior region of the disc was removed and sent for histologic and histomorphometric analysis. Histologic (hematoxylin-eosin) and histomorphometric (picro-Sirius red) analyses were performed. Statistically significant differences between the analyzed groups were accessed through the chi-squared test ($P \leq .05$). The Mann-Whitney *U* test was used to observe the differences between mean values when variables did not present normal distribution [Kolmogorov-Smirnov(a) test].

Results
There were no significant differences between the groups in relation to the parameters studied by histologic and histomorphometric analysis (using or not using polarization).

Conclusions
To the limits of this study, there were no significant histologic and histomorphometric differences in the articular disc between groups with and without TMJ dysfunction.

Approximately 3% to 4% of the population seek treatment for temporomandibular joint (TMJ) disorders,[1] and roughly 70% of these patients suffer from disc displacement.[2]

Disc displacement is the morphologic alteration of the TMJ most commonly encountered when patients with signs and symptoms of temporomandibular disorders are examined.[3] Most frequently the disc is displaced anteriorly, but there is also high incidence of medial or lateral displacement, as well as a combination of displacement.[4], [5] There are 2 types of disc displacement, with and without disc reduction, the latter being considered a more severe clinical manifestation.[5]

In internal derangements of the TMJ, the disc is often malpositioned. Initially, it was believed that disc displacement preceded the onset of degeneration of the mandibular condyle; however, the high association of

disc malposition with osteoarthritic changes of the joint has led many to suggest that osteoarthritic degeneration predisposes the disc to displacement.[6]

One of the problems with TMJ research in patients is the difficulty of obtaining adequate control groups. This is particularly evident in patients for which surgery is involved. For ethical reasons, it is not possible to obtain control biopsy specimens from healthy living persons, and therefore we must rely on autopsy material. During the past few decades,[7], [8], [9] several histologic studies have been performed primarily using autopsy specimens from older persons. Consequently, it has been difficult to distinguish age-related changes from those due to true joint disease. In a few studies, this problem was addressed by using age-matched control subjects[10], [11] or control subjects of a young age.[12] However, most earlier studies have focused on structural features related to inflammation, and little attention has been paid to a more systematic characterization of the various tissue components in the normal state and in degenerative diseases. Moreover, the histologic aspects have been limited to basic approaches, with hematoxylin-eosin stain, and have considered anatomic structures other than the articular disc.

The knowledge of histologic aspects of TMJ anatomic components may help surgeons diagnose, plan the surgical technique, and better understand the clinical prognosis of tissue alterations related to disc displacement.

The aim of the present study was to analyze histologic and histomorphometric features of the posterior region of the articular disc in groups with and without disc displacement.

Materials and Methods

Study sample

A convenience sample of 39 temporomandibular discs from 28 patients, (mean age 31.24 years, range 17 to 57 years), were recruited for study from the patient pool at the Evangelico School Hospital, Curitiba, PR, Brazil, as approved by the Ethical Committee on Research at Pontifical Catholic University of Paraná, according to Resolution 196/96 of the National Health Council and approved under registration number 104. The patients were from the southern region of Brazil. Subjects did not present any of the following exclusion criteria: use of orthodontic appliances; chronic usage of antiinflammatory drugs; a history of diabetes, hepatitis, or HIV infection; immunosuppressive chemotherapy; history of any disease known to severely compromise immune function; current pregnancy or lactation; dentofacial deformity; major jaw trauma; previous TMJ surgery; or previous steroid injection in the TMJ.

Subjects completed personal medical history questionnaires and, within a protocol approved by an Institutional Review Board, signed a consent form after being advised of the nature of the study.

All patients were asked to complete a pain questionnaire, and a clinical examination was performed by the operating oral and maxillofacial surgeon. The clinical examination consisted of palpation of the TMJ region, the occurrence of painful opening/closing mouth, and crepitation. The patients were considered to be affected and treated surgically when presenting painful clinical signs of disc displacement after unsuccessful nonsurgical treatment for at least 6 months. Regarding complementary exams, all patients had a panorex. These patients were from the Brazilian public health systeml therefore, a few of them had financial conditions to afford other exams such as computerized tomography (CT) scan or a TMJ magnetic resonance imaging. Accordingly, the diagnoses were primarily clinical.

Subjects were included in clinical categories according to the presence or absence of disc displacement:

1) Patients presenting anterior disc displacement with reduction (ADDWR) (n = 15; 23 specimens);
2) Patients presenting anterior disc displacement without reduction (ADDWOR) (n = 5; 8 specimens);
3) Subjects without any signs of disc displacement (control group; n = 8; 8 specimens).

Patients presenting disc displacement with and without reduction were grouped together for analysis. Out of the control patients, 4 individuals presented condyle fracture (CFx), confirmed by radiographs and CT scan, which needed to be operated for the fracture reduction, and 4 subjects displayed active condyle hyperplasia (CH), diagnosed by radiographs, CT scan, and scintillography. These subjects also attended the exclusion criteria and did not present any clinical signs of disc displacement.

Table I shows the baseline characteristics of the groups.

Table I. Baseline clinical characteristics of the study group with and without TMJ dysfunction

Patient	Race	Gender	Age (yrs)	Diagnosis	Affected side	
					Right	Left
1	Caucasian	Female	35	ADDWOR	X	X
2	Caucasian	Female	24	ADDWOR		X
3	Caucasian	Female	23	ADDWOR	X	X
4	Caucasian	Female	45	ADDWOR	X	
5	Caucasian	Female	32	ADDWOR	X	X
6	Caucasian	Female	30	ADDWR		X
7	African American	Female	20	ADDWR		X
8	African American	Female	57	ADDWR		X
9	Caucasian	Female	33	ADDWR	X	
10	Caucasian	Female	25	ADDWR	X	X
11	Caucasian	Female	22	ADDWR	X	X
12	Caucasian	Female	42	ADDWR	X	
13	Caucasian	Female	56	ADDWR	X	
4	Caucasian	Female	26	ADDWR	X	X
15	Caucasian	Female	38	ADDWR	X	X
16	Caucasian	Female	37	ADDWR	X	X
17	Caucasian	Female	36	ADDWR	X	X
18	Caucasian	Female	34	ADDWR		X
19	Caucasian	Female	46	ADDWR	X	X
20	Caucasian	Female	26	ADDWR	X	X
21	Caucasian	Female	17	CH		X
22	Caucasian	Female	43	CH		X
23	Caucasian	Female	40	CH		X
24	Caucasian	Female	18	CH		X
25	Caucasian	Male	26	CFx		X
26	Caucasian	Female	30	CFx		X
27	Caucasian	Male	27	CFx	X	
28	Caucasian	Male	18	CFx		X

ADDWOR, Anterior disc displacement without reduction; ADDWR, anterior disc displacement with reduction; CH, condylar hyperplasia; CFx, condylar fracture.

Surgical technique

General anesthesia was administered via nasotracheal intubation in all patients. An endaural incision was done to achieve access to the TMJ area. The superior joint space was entered and the capsular attachments were incised anteriorly beyond the articular eminence and posteriorly along the posterior wall of the fossa. The anterior, lateral, and medial ligamentous attachments were freed when necessary to allow passive repositioning

of the disc over the condylar head. The excessive bilaminar tissue was resected, and a small portion of the disc posterior band was removed. At this time any abnormal form of the posterior part of the disc, if present, could be visualized. The disc was then sutured as in Mehra and Wolford's technique.[13] This procedure was conducted for all patients with disc displacement and the control group. In the CFx patients, the disc displaced by fracture was repositioned, and in the CH patients the disc was sutured to prevent disc displacement caused by the gap that was created after the high condylectomy. Postsurgical physical therapy was indicated at the discretion of the surgeon.

Histologic and histomorphometric analysis

The specimens were obtained from the posterior segment of the disc. They were fixed by immersion in 10% neutral-buffered formaldehyde solution. Subsequently they were embedded in paraffin wax, and 4-µm thin sections were cut and stained with hematoxylin-eosin (HE) for the histologic analysis and picro-Sirius red for the histomorphometric analysis. The slides were examined by light microscopy (American Optical BX50) for the histologic analysis and by Image Pro Plus software (Media Cybernetics, Silver Spring, MD) for the morphometric analysis.

Histologic analysis. One coded HE slide (the most representative of 5) per sample was analyzed without knowledge of group identification (blind). The following parameters were analyzed: degree of vascularization (absence, mild, and moderate/severe), presence of blood vessels at the disc periphery, hyperemia, synovial hyperplasia, synovial vascularity, presence of condrocytes/fibrocondrocytes, hyalinization/fibrosis (absence, mild, and moderate/severe), inflammatory infiltrate, and presence of adipocytes (Table II). All parameters of interest were estimated 3 times, and the mean value was then calculated.

Table II. Histologic parameters analyzed for the groups

Group*	Degree of vascularization†	Blood vessels at disc periphery	Vascular hyperemia	Synovial hyperplasia	Synovial velocity	Condrocytes	Degree of hyalinization	Inflammatory infiltrate	Adipocytes
1	2	Y	N	Y	Y	Y	1	N	N
1	3	N	N	Y	Y	Y	1	N	N
1	2	N	N	N	N	N	1	N	N
1	2	N	N	N	N	Y	2	N	N
1	2	N	N	N	N	Y	2	N	N
1	2	Y	N	N	N	Y	2	N	N
1	2	Y	N	N	N	N	0	N	N
1	3	Y	N	Y	Y	Y	2	Y	N
1	2	Y	N	N	Y	N	2	N	N
1	3	Y	Y	Y	Y	Y	1	N	N
1	2	Y	N	N	Y	Y	2	N	N
1	2	N	N	N	N	Y	2	N	Y
1	3	Y	N	N	Y	Y	2	N	N
1	2	Y	N	Y	Y	Y	2	N	N
1	3	Y	N	Y	Y	Y	1	N	N
1	3	N	N	N	N	N	0	N	Y
1	2	N	N	N	N	Y	2	N	N
1	2	Y	N	N	Y	Y	2	N	N
1	2	N	N	N	N	Y	1	N	Y
1	2	N	N	N	N	N	0	N	N

1	2	N	N	N	N	N	2	N	N
1	2	Y	N	Y	Y	Y	1	Y	Y
1	2	N	N	N	N	Y	2	N	N
2	2	N	N	N	N	Y	2	N	N
2	3	Y	N	Y	Y	N	1	Y	N
2	3	N	N	N	N	Y	2	N	N
2	3	N	N	N	N	Y	0	N	N
2	2	N	N	N	N	Y	2	N	N
2	3	Y	Y	N	Y	N	1	Y	N
2	2	Y	N	N	Y	Y	2	N	N
2	2	N	N	N	N	Y	1	N	N
3	3	Y	N	N	N	N	1	N	N
3	3	Y	N	N	N	N	2	N	Y
3	2	N	N	N	N	N	2	N	N
3	3	N	N	N	N	Y	1	N	Y
3	3	Y	N	Y	Y	N	2	Y	N
3	2	Y	N	N	Y	Y	1	N	N
3	2	Y	N	N	Y	Y	2	N	N
3	2	N	N	Y	N	Y	2	N	N

Y, Yes; N, no.

∗ Group 1: n = 8; specimens presenting with anterior disc displacement without reduction. Group 2: n = 23; specimens presenting with anterior disc displacement with reduction. Group 3: n = 8; specimens presenting with condylar hyperplasia or condylar fracture.

† 0 = absent; 1 = mild; 2 = moderate/severe.

Histomorphometric analysis. One picro-Sirius red slide per sample was analyzed without knowledge of group identification (blind). The slides were examined: 1) without polarization, giving the total amount of collagen; and 2) under polarized light, using a binocular microscope attached in a computerized image analysis system (Image Pro Plus software) to quantify types I and III collagen fibers. The image software was calibrated in micrometers for a 20× objective, and the density of the tissue sample was calibrated by the range 0 to 2, where 0 = total light passing through the tissue (low density) and 2 = no light passing through the tissue (high density). After the calibration, a sample of collagen from the picro-Sirius red–staining slide (magenta) was taken without polarization. The software calculated the sum of mean density at each point of the collagen and the sum of the total area of the collagen by color histogram function. The same protocol was used for picro-Sirius red–staining slides using polarized light, but with 2 color samples: one for collagen type I (yellow) and another for collagen type III (red). The color of the birefringence was determined by the collagen type; the color varied from green to yellow through orange to red depending upon the cross-linking of the collagen, which reflected the age and fiber bundle size of the collagen, because, in general, it is first deposited as fine fibrils that later become cross-linked into larger fibers and bundles.[16], [17]

Statistical analysis

Statistically significant differences among the analyzed groups were accessed through the chi-squared test (χ^2) and Fisher test at the level of probability $P \leq .05$. When expected frequencies were smaller than 5, in 2×2 tables, the Fisher test was also used. The Kruskal-Wallis test was used for nonparametric multiple comparisons for independent variables among the 3 groups: collagen area and density. The Mann-Whitney *U* test was used to observe the differences between the mean values of the 2 groups (test and control) for the variables if the variables did not present normal distribution in relation to the groups by the Kolmogorov-Smirnov(a) test.

Results

There were no significant differences among the groups in relation to all of the parameters studied by HE. Regarding histometrical analysis, no differences among the groups were observed for picro-Sirius red staining, whether or not polarization was used. In addition, no differences were noted when groups 1 (ADDWR) and 2 (ADDWOR) were considered together versus the control group. Vascularization was associated with inflammatory infiltrate (P = .029). Figure 1 shows common histologic aspects of a control disc and an affected posterior region articular disc, and Fig. 2 presents aspects of control and diseased discs under polarization.

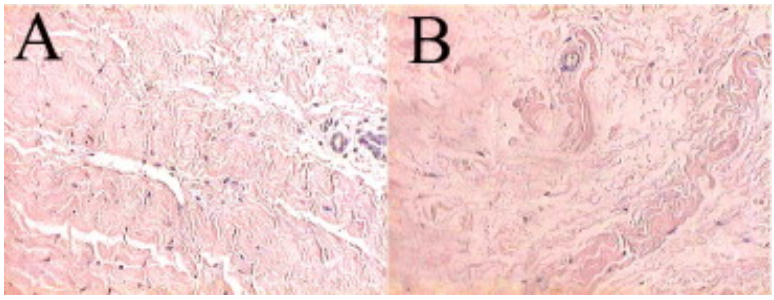

Fig. 1. Histologic aspects (HE) of articular discs from a control (A) and an affected (B) individual.

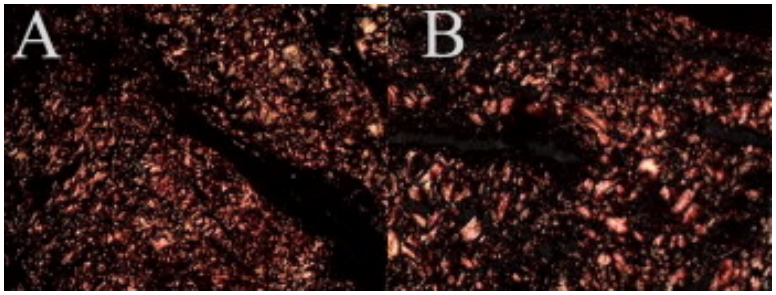

Fig. 2. Aspects of the articular disc of control (A) and diseased (B) patients (picro-Sirius Red under polarization).

Discussion

Few histologic studies have been performed on the TMJ disc in disease.[7], [8], [9] However, the interpretation of these has been hindered by the lack of control specimens[13], [14], [15] or availability of control specimens only from elderly persons.[7], [8], [9] In the present study, individuals without disc displacement of any kind or clinical signs of TMJ dysfunction were considered for the control group. Although not the ideal control group, those presenting trauma or condyle hyperplasia could also be considered, because the discs may lack degenerative alterations. In addition, the mean age was 27.4 years (range 17 to 43 years), thus avoiding findings in asymptomatic joints that may be attributed to aging rather than pain.[9]

Although the present study considered a limited number of individuals presenting condyle hyperplasia and condyle fracture as the control group, a lack of painful clinical signs together with low age could be an alternative control to cadaveric specimens. Cadaveric specimens, even if the sample is collected immediately after death, are generally from older and systemically impaired patients, masking specific degenerative aspects of disease, such as those caused by disc displacement.

With respect to gender, Paegle et al.[16] reported no significant differences between female and male joint specimens regarding morphologic structures. Thus, it appears to be unnecessary to match control specimens by gender. However, our results should be interpreted with caution, because all members of the test groups (100%) and only 5 (62.5%) of the control group were female.

A strong aspect of this study is that the disc itself was considered. Most studies have been conducted not in the disc but rather in adjacent tissues such as retrodiscal and posterior attachments.14, 15, 16
This study compared histologic and histomorphometric features of painful and asymptomatic TMJ discs. No differences among the groups were observed concerning degree of vascularization, presence of blood vessels at the disc periphery, hyperemia, synovial hyperplasia, synovial vascularity, presence of condrocytes/fibrocondrocytes, hyalinization/fibrosis, inflammatory infiltrate, and presence of adipocytes. An inflammatory cellular infiltrate was seldom observed in our material, which is in accordance with several other earlier studies7, 8, 12 and associated with degree of vascularization.

Perhaps the lack of histologic differences among the study groups was due to the fact that the disc is a continually remodeling anatomic structure in a progressive process of healing in response to trauma. Further studies should be carried out, particularly with a greater number of samples, to corroborate the present findings.

In conclusion, there were no significant histologic or histomorphometric differences in the articular disc between the groups with and without TMJ dysfunction.

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