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Association Of Estrogen Receptor Alpha 1 And TMJ Dysfunction: A Pilot Study

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Abstract

Objective

Temporomandibular disorder (TMD) is a multifactorial condition and the most common cause of orofacial pain, affecting mostly women, which points to a female hormone predilection. Therefore, the aim of this study was to analyze the association between TMD and estrogen receptor alpha 1 expression in disks of patients with TMD and condyle fracture (CFx).

Study Design

Forty specimens (from 27 patients) included *n* = 8 CFx, *n* = 21 anterior disk displacement with reduction (ADDwR), and *n* = 11 anterior disk displacement without reduction (ADDwoR). Age, area, and intensity of immunostaining were statistically compared between CFx, ADDwR, and ADDwoR groups using analysis of variance and Kruskal-Wallis analysis ($P < .05$).

Results

No significant difference between CFx, ADDwR, and ADDwoR groups with respect to age and expression of estrogen receptor alpha 1 was observed on immunohistochemical examination.

Conclusion

No association of estrogen receptor alpha 1 expression and age was found in the CFx, ADDwR, and ADDwoR groups.

Statement of Clinical Relevance

Although ESR1 have been associated in the TMJ retrodiscal tissue and synovial membrane, in this study there was low association of ESR1 in human discs of patients with TMD.

Temporomandibular disorder (TMD) is a multifactorial condition and is the most common cause of facial pain, affecting 25% of the population, mostly women.[1] According to the National Institutes of Health,[2] TMD management in the United States costs approximately $4 billion per year. Some factors have been linked to predisposing temporomandibular joint (TMJ) dysfunction, including parafunction, trauma, stress, joint hypermobility, long dental treatments with open mouth, genetics, and hormone.[3,4] However, the TMD etiopathogenesis cascade still needs to be elucidated in order to promote an assertive treatment to TMD patients.

Studies have been focusing on TMD in women, because of the contribution of female hormones to its derangement.[5] Estrogen is a female hormone known to be involved in TMJ tissue remodeling processes that, when upregulated, contributes to the inflammatory process inducing the matrix metalloprotease cascade, and its upregulation is associated with TMJ inflammation and hyperalgesia.[6,7] Beta-estradiol is the most potent form of estrogen in the human female, having biological effects through binding the estrogen receptor alpha 1 (ESR1) present in TMJ fibrocartilage, which increases the inflammatory cascade through MMP-9 and MMP-13 activation in TMJ.[8] Moreover, estrogen knockout studies have revealed its major role in immune response.[9] These studies show the importance of ESR1 in the development and progression of TMD, and ESR1 expression was found mainly in some female human TMJ disks; however, this study analyzed a very small sample of male and female disk samples.[5] In addition, in animal synovial lining cells, articular disks and chondrocytes in the TMJ showed expression of ESR1.[10] Because ESR1 may act differently in human and animal models,[11] a study investigating the presence and association of ESR1 in human disks with TMD is necessary to elucidate this matter.

Although there are a few studies associating ESR1 in animal disks with TMD, studies with human disks are scarce. Therefore, knowledge regarding the expression of ESR1 in TMJ human disks needs to be elucidated to improve the understanding of the role of estrogen in the progression and severity of TMD. Thus, the aim of this study is to investigate the association between ESR1 expression in human disks of patients with anterior disk displacement with and without reduction and with condyle fracture as a control.

Materials and Methods

Sample selection

A sample of 40 temporomandibular disks was collected at the Evangelico School Hospital, Curitiba, Brazil, after approval from 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. Samples were obtained from 27 patients with a mean age of 37.9 years (range, 17-57). Patients were not included if they were using orthodontic appliances or anti-inflammatory drugs; had a history of hepatitis, diabetes, immunosuppressive chemotherapy, HIV infection, any disease known to compromise immune function, dentofacial deformity, major jaw trauma, previous open TMJ surgery, or previous steroid injection in the TMJ; or were currently pregnant or lactating.

Patients were informed of the nature of the study, signed consent forms, and completed personal medical history questionnaires. All patients were clinically analyzed according to *Clinical Practice Guidelines for TMJ Surgery* of the American Association of Oral Maxillofacial Surgeons. The patients were treated surgically when presenting painful clinical signs of disk displacement and/or severe mouth opening limitation after unsuccessful noninvasive treatment for at least 6 months.

Patients presenting disk displacement with and without reduction were grouped according to its clinical diagnosis for analysis. Patients with extra-articular CFx with 90° displacement and rupture of the internal structures, confirmed by computed tomography scan, were used as controls. They were clinically analyzed and it was confirmed that they did not have chronic TMD before trauma. The 8 individuals with CFx required operation for fracture reduction and disk reposition.

The samples were as follows:

- Patients without any signs of disk displacement (control: *N* = 7; 8 specimens).
- Patients with anterior disk displacement with reduction (ADDwR: *N* = 14; 21 specimens).
- Patients with anterior disk displacement without reduction (ADDwoR: *N* = 6; 11 specimens).

Patients were included in clinical categories according to the presence or absence of disk displacement using Wilkes classification.[12] Table I shows the baseline characteristics of the sample.

Table I. Baseline characteristics

					Affected side		
Patient	**Race**	**Gender**	**Age (y)**	**Diagnosis**	**Right**	**Left**	**Wilkes stage**
1	Caucasian	F	33	ADDwR	X		III
2	Caucasian	F	27	CFx		X	
3	Caucasian	F	33	CFx	X		
4	Caucasian	F	26	ADDwR	X		III
4	Caucasian	F	26	ADDwR		X	III
5	Caucasian	F	43	CFx		X	

6	Caucasian	F	17	CFx		X	
7	Caucasian	F	30	ADDwR		X	III
8	Caucasian	F	25	ADDwR		X	III
8	Caucasian	F	25	ADDwR	X		III
9	Caucasian	F	37	ADDwR	X		III
9	Caucasian	F	37	ADDwR		X	III
10	Caucasian	F	42	CFx	X		
11	Caucasian	F	20	ADDwR		X	III
12	Caucasian	F	23	ADDwoR		X	V
12	Caucasian	F	23	ADDwoR	X		V
13	Caucasian	F	36	ADDwR	X		III
13	Caucasian	F	36	ADDwR		X	III
14	Caucasian	F	38	ADDwR	X		III
14	Caucasian	F	38	ADDwR		X	III
15	Caucasian	F	22	ADDwR	X		III
15	Caucasian	F	22	ADDwR		X	III
16	Caucasian	F	26	ADDwoR	X		IV
16	Caucasian	F	26	ADDwoR		X	IV
17	Caucasian	F	32	ADDwoR		X	IV
17	Caucasian	F	32	ADDwoR	X		V
18	Caucasian	F	45	ADDwoR	X		V
19	Caucasian	F	35	ADDwoR		X	IV
19	Caucasian	F	35	ADDwoR	X		IV
20	Caucasian	F	24	ADDwoR		X	V
21	Caucasian	F	34	ADDwR		X	III
22	Caucasian	F	57	ADDwR		X	III
23	Caucasian	F	18	CFx		X	
23	Caucasian	F	18	CFx	X		
24	Caucasian	F	46	ADDwR		X	III
24	Caucasian	F	46	ADDwR	X		III
25	Caucasian	F	40	CFx		X	
26	Caucasian	F	56	ADDwR	X		III
27	Caucasian	F	42	ADDwR	X		III
27	Caucasian	F	42	ADDwoR		X	v

ADDwoR, anterior disk displacement without reduction; *ADDwR*, anterior disk displacement with reduction; *CFx*, condylar fracture.

Surgical technique

TMJ surgery was performed according to the technique described by Mehra and Wolford.[13] First the displaced disc is freed by the surgeon, repositioned, and tied down to the latero-posterior side of the condyle with a Mitek bone-cleat and a nonresorbable 2-0 or 3-0 suture. The suture is placed at the junction of the posterior and intermediate bands, and the deformity of the disc precludes repositioning it into a more normal position; thus, recontouring the thickened disc with a scalpel is necessary (this material constitutes the sample). All samples were collected from the posterior band of the disk.

This procedure was conducted for all patients with disk displacement and in the group with CFx. In patients with CFx, the disk displaced by fracture was repositioned.

Histologic sections obtained by removal of excess disk tissue were prepared for observation of the in situ expression of ESR1 by immunohistochemistry.

Immunohistochemistry

After deparaffinization and rehydration of 4-µm-thick sections, endogenous peroxidase was inactivated with 0.5% Hydrogen Peroxide Blocking buffer (Sigma Darmstadt, Germany) for 10 min at room temperature. Antigen recovery was carried out by leaving the samples in Immuno Retriever buffer (Thermo Fisher Massachusetts, USA) in a bath at 99°C for 25 min. After cooling and washing, samples were incubated with ESR1 antibody (Thermo Fisher), diluted 1:62, and held overnight at 4°C in a moist chamber. Spring REVEAL Polyvalent HRP (Spring Bioscience California, USA) was used as a secondary antibody. All staining procedures included positive (breast cancer) and negative controls (e.g., no primary antibody). The slides were then washed and incubated in Spring REVEAL complement buffer for 10 min at room temperature, after which the slides were washed and incubated with Spring REVEAL Conjugate for 15 min at room temperature. The immunoreactions were visualized by incubating the sections using 3,3'-diaminobenzidine chromogen (REVEAL kit). The sections were lightly counterstained with Harris hematoxylin Sigma as well for 5 min and finally mounted. Immunostaining was considered to be specific to ESR1 because immunoreactivity was not observed in the negative controls.

Statistical analysis

The Allred method was used to analyze the ESR1 immunostained area in the TMJ disk tissue, focusing on fibrochondrocyte and endothelial tissue. Allred analysis focused on positive area distribution and intensity: 1 = 1%, 2 = 10%, 3 = 33%, 4 =66%, and 5 = 67% to 100%. The results of the intensity and area distribution, as well as the results for the addition of both, were analyzed statistically. This procedure was performed by a single examiner in a blind manner.

To analyze the association of age with the control, ADDwR, and ADDwoR groups, analysis of variance with 1 factor was employed, and nonparametric Kruskal-Wallis analysis was used to compare intensity and area distribution between groups. *P* values <.05 were considered statistically significant. Data were analyzed using IBM SPSS Statistics version 20.0 (IBM Corporation, Armonk, NY).

Results

No association of age was found between the CFx, ADDwoR, and ADDwR groups (Table II). In addition, there was no statistically difference between groups for immunostaining of the intensity and area distribution of fibrochondrocyte and endothelial tissue by Allred analysis (Table III). Table IV shows the percentage of the control, ADDwR, and ADDwoR groups for ESR1 immunostaining in fibrochondrocyte and endothelium quantity, intensity, and quantity and intensity combined.

Table II. Analysis of the association of TMD with age

			Age (years)				
Group	**n**	**Mean**	**Median**	**Minimum**	**Maximum**	**SD**	**P value***
Control	8	29.8	30	17	43	11.3	
ADDwR	21	34.6	36	20	57	10.8	.439
ADDwoR	11	31.2	32	23	45	7.6	

ADDwoR, anterior disk displacement without reduction; *ADDwR*, anterior disk displacement with reduction; *TMD*, temporomandibular disorder.

∗Analysis of variance with 1 factor, $P < .05$.

Table III. Analysis of association between control, ADDwR, and ADDwoR groups in relation to ESR1 immunostaining in fibrochondrocyte and endothelium quantity, intensity, and quantity and intensity combined

Variable	Group	n	Median	Minimum	Maximum	P value*
Fibrochondrocyte quantity	Control	8	1	0	2	
	ADDwR	21	1	0	2	.388
	ADDwoR	11	1	1	2	
Fibrochondrocyte intensity	Control	8	1	0	2	
	ADDwR	21	1	0	2	.451
	ADDwoR	11	1	1	1	
Fibrochondrocyte quantity + intensity	Control	8	2	0	3	
	ADDwR	21	2	0	4	.691
	ADDwoR	11	2	2	3	
Endothelium quantity	Control	8	1	1	3	
	ADDwR	21	1	1	3	.955
	ADDwoR	11	1	1	2	
Endothelium intensity	Control	8	1	1	2	
	ADDwR	21	1	1	2	.435
	ADDwoR	11	1	1	1	
Endothelium quantity + intensity	Control	8	2	2	5	
	ADDwR	21	2	2	4	.938
	ADDwoR	11	2	2	3	

ADDwoR, anterior disk displacement without reduction; *ADDwR*, anterior disk displacement with reduction; *ESR1*, estrogen receptor alpha 1.

∗Nonparametric Kruskal-Wallis test, $P < .05$.

Table IV. Percentage of the control, ADDwR, and ADDwoR groups for ESR1 immunostaining in fibrochondrocyte and endothelium quantity, intensity, and quantity and intensity combined

Variable	Group	n	Allred 0 (%)	Allred 1 (%)	Allred 2 (%)	Allred 3 (%)	Allred 4 (%)	Allred 5 (%)
Fibrochondrocyte quantity	Control	8	25	62.5	12.5	0	0	0
	ADDwR	21	4.76	76.19	19.04	0	0	0
	ADDwoR	11	0	81.81	18.18	0	0	0
Fibrochondrocyte intensity	Control	8	25	62.5	12.5	0	0	0
	ADDwR	21	4.76	80.95	14.28	0	0	0
	ADDwoR	11	0	100	0	0	0	0
Fibrochondrocyte quantity + intensity	Control	8	25	50	0	25	0	0
	ADDwR	21	4.76	0	71.42	14.28	9.52	0
	ADDwoR	11	0	0	81.81	18.18	0	0
Endothelium quantity	Control	8	87.5	0	0	12.5	0	0
	ADDwR	21	0	80.95	14.28	4.76	0	0
	ADDwoR	11	0	81.81	18.18	0	0	0
Endothelium intensity	Control	8	0	87.5	12.5	0	0	0
	ADDwR	21	0	85.71	14.28	0	0	0
	ADDwoR	11	0	100	0	0	0	0
Endothelium quantity + intensity	Control	8	0	0	87.5	0	0	12.5

	ADDwR	21	0	0	80.95	0	19.04	0
	ADDwoR	11	0	0	81.81	18.18	0	0

ADDwoR, anterior disk displacement without reduction; *ADDwR*, anterior disk displacement with reduction; *ESR1*, estrogen receptor alpha 1.

Discussion

TMD has been extensively studied because its notable incidence and economic implications of its treatment. Although many presumptive conditions have been associated with TMD, there are still many factors that need to be understood with regard to the etiology and progression of TMD, in order to treat patients properly and avoid TMJ degeneration, pain, and functional impairment.

In this study, age was not associated with TMD, perhaps because the patients in this study were in the age range in which TMD may be expected to develop.[14]

Many studies focused on the expression of synovial fluids to evaluate which cytokines might be involved in the internal derangement of the joint, showing mainly the presence of inflammatory cascade.[15,16] Ibi[16] found an association of inflammatory markers such as interleukin-1 and tumor necrosis factor-α with pain and TMD, which could be modulated by the higher expression of estrogen. Another study found an elevation of endocrine gland–derived vascular endothelial growth factor in the synovial fluid of patients without TMD, suggesting an important role of hormones in the development of TMD.[17]

Pain and inflammatory cascade in TMD originate in the synovial tissue, whose physiologic composition includes estrogen[18]; however, it is not present in the synovial fluid of healthy patients.[15] A study in rats found a reduction in TMJ cartilage thickness when biomechanical stress and higher levels of estrogen were applied,[19] and a blockage of ESR1 in an animal model suppressed MMP-9 and MMP-13 levels induced by 17 β-estradiol, which could be a therapeutic intervention target for TMJ disorders.[8]

Nevertheless, all of these studies were conducted in animal models, and TMD could progress differently in humans. In the sample in this study, patients with CFx were used as controls, because it is not possible to collect disk samples from healthy patients. Thus, TMD due to previous trauma was ruled out in all control patients through the combination of examination and questionnaire.

Estrogen is involved in the initiation of the inflammatory cascade,[7] and its expression in the TMJ is likely a chronic dysfunction, not present in the TMJ of these patients with acute trauma. Despite the difficulty in collecting a sample size of 40 specimens because the small number of patients who require TMJ surgery, both samples with and without TMD were analyzed. In addition, samples from both TMJs of the same patient were collected from a few patients to increase the sample size, which could have influenced the negative association.

ESR1 was found in some female human disks with TMD,[5] yet the sample of this study was limited and included both males and females, which could bias the estrogen analysis. This is the only study of ESR1 in human disks with, analyzing men and women, in addition to the few that employed animal models; hence the need to elucidate the role of ESR1 in human TMJ disks in TMD and how it could affect its progression and severity.[5,10,11] In our study, the presence of ESR1 was analyzed in fibrochondrocyte and endothelium of female patients' disks with and without TMD. There was no statistically difference between the groups for expression of ERS1 in TMJ disks, although ERS1 was present in the disks of patients with and without TMD. Although there are animal studies associating the presence ERS1 in TMJ disks with TMD, in humans the contribution of ERS1 to the modulation of the inflammatory cascade may be due to its greater expression in the synovial membrane and retrodiskal tissue.[6,10] There are several questions that remain, such as whether the contribution of the retrodiskal tissue to TMJ degeneration is initiated by a hormone alteration or whether the disk displacement

contributes to progression of the inflammatory cascade. However, a study with a larger sample of human TMJ disks may confirm whether the expression of ERS1 in TMJ disks contributes to TMD.

Conclusion

A low level of expression of ERS1 was observed in human disks of patients with CFx, ADDwoR, and ADDwR and there was no association with TMJ dysfunction, and no association with age was observed. Thus, future studies with a large sample should be performed to confirm the results of this study.

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