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Immunohistochemical Analysis of IL-1 Receptor 1 In the Discs of Patients with Temporomandibular Joint Dysfunction

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Abstract

Objective

Temporomandibular joint dysfunction (TMD) may affect a patient's quality of life, and one of the etiologies can be anterior disc displacement with reduction (ADDwR) and anterior disc displacement without reduction (ADDWoR). Interleukin 1 Receptor 1 (IL-1R1) is a membrane receptor that plays an important role on initiating immune and inflammatory response by binding the agonists ligands of IL-1 alpha and IL-1 beta. Therefore, the aim of this study was to evaluate, through immunohistochemical analysis, the association of IL-1R1 with TMD.

Methods

Thirty-nine human disc samples were collected and composed three different groups: ADDwR (n = 19), ADDwoR (n = 12), and control group (n = 8). The samples were immunostained with IL-1R1 antibody and evaluated on both quantity and intensity of staining.

Results

There was a statistically significant difference ($p < 0.05$) between the control and test groups for both quantity and intensity of staining.

Conclusion

IL1-R1 was associated with ADDwR and ADDwoR in TMD discs of humans.

Keywords:

TMJ, interleukin 1, inflammation

Introduction

Temporomandibular joints are considered the main support for jaw stability, occlusion, and function. Temporomandibular joint dysfunction (TMD) may affect a patient's quality of life and could lead to dentofacial deformity and even compromise orthodontic treatment [1 Wolford LM. Can orthodontic relapse be blamed on the temporomandibular joint? J Orthod Sci. 2014 Oct–Dec;3(4):95–105.10.4103/2278-0203.143227[Crossref], [PubMed], [Google Scholar]]. Women between the ages of

19 and 40 years old are more likely to develop TMD [2 Ferreira CL, Silva MA, Felício CM. Signs and symptoms of temporomandibular disorders in women and men. CoDAS. 2016 Jan–Feb;28(1):17–21.10.1590/2317-1782/20162014218[Crossref], [PubMed], [Google Scholar]], and according to a Korean study, the prevalence of TMD is 24.9% [3 Piao Y, Kim SJ, Yu HS, et al. Five-year investigation of a large orthodontic patient population at a dental hospital in South Korea. Korean J Orthod. 2016 May;46(3):137–145.10.4041/kjod.2016.46.3.137[Crossref], [PubMed], [Web of Science ®], [Google Scholar]].

Among the several types of TMD, the most common is muscle disorder, followed by disc displacement with reduction [4 Blanco-Hungría A, Blanco-Aguilera A, Blanco-Aguilera E, et al. Prevalence of the different Axis I clinical subtypes in a sample of patients with orofacial pain and temporomandibular disorders in the Andalusian Healthcare Service. Med Oral Patol Oral Cir Bucal. 2016 Mar 1;21(2):e169–e177.10.4317/medoral.20854[Crossref], [PubMed], [Web of Science ®], [Google Scholar]]. Anterior disc displacement with reduction (ADDwR) is characterized by an anterior position of the disc in relation to the condyle head when teeth are in occlusion, which slides into its physiological position during mouth opening, and during mouth closing it becomes anteriorly displaced again. On the other hand, anterior disc displacement without reduction (ADDWoR) is associated with an altered disc position when the mouth is closed or opened, and it may lead to pathologic and painful conditions. Some patients do not present any pathological clinical condition with these types of disc displacement; however, others present severe pain, mouth opening limitations, dysphagia, and hearing alterations.

Interleukin-1 (IL-1) is an important physiologic pro-inflammatory cytokine, which is also involved in the amplification of the immune response. An imbalance on its expression may lead to tissue and organ damage, and excessive production of this cytokine was associated with severe autoimmune and inflammatory diseases [4,5 Blanco-Hungría A, Blanco-Aguilera A, Blanco-Aguilera E, et al. Prevalence of the different Axis I clinical subtypes in a sample of patients with orofacial pain and temporomandibular disorders in the Andalusian Healthcare Service. Med Oral Patol Oral Cir Bucal. 2016 Mar 1;21(2):e169–e177.10.4317/medoral.20854
Almeida LE, Pierce S, Zacharias J, et al. Immunohistochemical analysis of IL-1 beta in the discs of patients with temporomandibular joint dysfunction. CRANIO®. 2016 Jul;13:1–5.]. The activity of the IL-1 is mediated by its family receptor, which encompasses 10 different molecules involved in the activation of early non-specific defense response, triggering a more specific adaptive response and innate immunity. The family of the receptor shares an intracellular common sequence, but a variant Ig ligand-binding extracellular domain [6 Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol. 2009;27:519–550.10.1146/annurev.immunol.021908.132612[Crossref], [PubMed], [Web of Science ®], [Google Scholar]]. The Interleukin 1 Receptor 1 (IL-1R1) was the first identified family receptor, with an extracellular portion of three Ig-like domains. It is a membrane receptor that plays an important role on initiating immune and inflammatory response by binding the agonists ligands of IL-1 alpha and IL-1 beta (expressed during initial defense response and has major effects on inflammation), and it is inhibited by the binding of antagonist ligand IL1-Ra [7 Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood. 2011 Apr 7;117(14):3720–3732.10.1182/blood-2010-07-273417[Crossref], [PubMed], [Web of Science ®], [Google Scholar]]. It is expressed by all cell types responsive to IL-1 and is the predominant receptor on T cells, fibroblasts, endothelial and epithelial cells [8 Boraschi D, Tagliabue A. The interleukin-1 receptor family. Semin Immunol. 2013 Dec

15;25(6):394–407.10.1016/j.smim.2013.10.023[Crossref], [PubMed], [Web of Science ®], [Google Scholar]]. IL-1 binds to IL1-R1, activating its signaling cascade, recruiting IL1-R3.

Studies have pointed out that sustained inflammation induces degeneration of the TMJ [9 Wang XD, Kou XX, Mao JJ, et al. Sustained inflammation induces degeneration of the temporomandibular joint. J Dent Res. 2012 May;91(5):499–505.10.1177/0022034512441946[Crossref], [PubMed], [Web of Science ®], [Google Scholar]], and one of the most prevalent cytokines in TMD synovial fluid is IL-1 [10 Kim YK, Kim SG, Kim BS, et al. Analysis of the cytokine profiles of the synovial fluid in a normal temporomandibular joint: preliminary study. J Craniomaxillofac Surg. 2012 Dec;40(8):e337–e341.10.1016/j.jcms.2012.02.002[Crossref], [PubMed], [Web of Science ®], [Google Scholar]]. Previous studies showed the association of IL1 in synovial fluid and TMD [10–12 Kim YK, Kim SG, Kim BS, et al. Analysis of the cytokine profiles of the synovial fluid in a normal temporomandibular joint: preliminary study. J Craniomaxillofac Surg. 2012 Dec;40(8):e337–e341.10.1016/j.jcms.2012.02.002
Asakawa-Tanne Y, Su S, Kunimatsu R, et al. Effects of enzymatic degradation after loading in temporomandibular joint. J Dent Res. 2015 Feb;94(2):337–343.10.1177/0022034514560588
Matsumoto K, Honda K, Ohshima M, et al. Cytokine profile in synovial fluid from patients with internal derangement of the temporomandibular joint: a preliminary study. Dentomaxillofacial Radiol. 2006 Nov;35(6):432–441.10.1259/dmfr/77288976] and lack of its presence in a healthy TMJ [13 Kristensen KD, Alstergren P, Stoustrup P, et al. Cytokines in healthy temporomandibular joint synovial fluid. J Oral Rehabil. 2014 Apr;41(4):250–256.10.1111/joor.12146[Crossref], [PubMed], [Web of Science ®], [Google Scholar]]. One study with human TMJ discs showed an increased expression of IL-1 being associated with the inflammatory process of the TMJ and its degradation [5 Almeida LE, Pierce S, Zacharias J, et al. Immunohistochemical analysis of IL-1 beta in the discs of patients with temporomandibular joint dysfunction. CRANIO®. 2016 Jul;13:1–5. [Google Scholar]]. Regarding IL-1R1, no study analyzing its association with the TMD disc has been published to date.

Therefore, the aim of this study is to analyze the association of the presence of IL-1R1 in TMD discs through immunohistochemistry.

Materials and methods

A total of 39 disc samples were collected from patients treated between 2002 and 2009 at the HOSPITAL UNIVERSITARIO EVANGELICO DE CURITIBA – PARANA – BRAZIL. Eight virtually unaffected human discs were in the control group for the study. Exclusion criteria for subjects included: use of orthodontic appliances; chronic usage of anti-inflammatory drugs; 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; previous major jaw trauma, TMJ surgery; or previous steroid injection in the TMJ. The 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 an experienced oral and maxillofacial surgeon performed a clinical examination. The clinical examination included palpation of the TMJ region, noting the occurrence of painful opening/closing of the mouth, and listening for crepitation. The patients considered to be affected were treated surgically when they presented painful clinical signs of disc displacement after unsuccessful non-surgical treatment for at least six months. Patients presenting with pain related only to muscular spasms

were not included in this study. For the complementary exams, all patients received a panoramic radiograph and MRI imaging studies. The subjects were included in clinical categories, according to the presence or absence of disc displacement. The test group included a total of 31 samples of discs that were diagnosed with TMJ dysfunctions. This group was separated into ADDwR (n = 19) and ADDwoR (n = 12). The control group included a total of eight samples. The discs of these patients were removed for reasons other than TMJ dysfunction. Four of the patients presented with condyle fractures that were confirmed by radiographs and CT scans. Four other control samples were obtained from patients who displayed active condyle hyperplasia. Wedges of the control discs were removed from the posterior regions so that the disc was able to physiologically adapt to the head of the condyle during function.

Interleukin-1 Receptor 1 immunohistochemical analysis

The discs were fixed overnight in 10% neutral-buffered formalin. After fixation and overnight washing, each disc was sectioned through its center along a parasagittal plane, perpendicular to its long axis. Each tissue block was dehydrated in graded ethanol and embedded in paraffin, preserving the longitudinal anatomical orientation. Specimens were sectioned at a thickness of 5 μm using a microtome, placed on silanized glass slides, and warmed at 60 °C for a minimum of 2 h to ensure proper tissue adhesion to the slides prior to immunohistochemical staining.

The TMJ disc slides were deparaffinized with 100% xylene (3 min) and a 50:50 mix of xylene and ethanol (3 min). The discs were then rehydrated with absolute ethanol (2 × 3 min), 90% ethanol (3 min), 70% ethanol (3 min), and distilled water (3 min). The discs were cold incubated for 24 h with monoclonal CD121a antibodies (Thermo Fisher Scientific, Rockford, IL, USA) diluted 1:2 000 in a solution of 0.1 M NaPO4 buffer, 0.3% Triton, and 0.25% carrageenan. Biotinylated secondary anti-goat/anti-rabbit was diluted 1:100 in the same solution as the primary antibodies and then added to the discs for two hours. Avidin-biotin peroxidase complex was then added to the slides and allowed to incubate for two hours. The immunoreactions were visualized by incubating the slides with 3.3′ diaminobenzidine chromogen. The slides were once again dehydrated and mounted.

Evaluation of immunohistochemistry

Interleukin-1 Receptor1 staining was identified as either positive or negative for each disc. Positive staining was defined as the presence of brown chromogen detection, distributed within the cytoplasm or in the immediate lacunar/pericellular space. Two blinded observers evaluated the percentage of immunopositive cells. The observers scored the percentages based on a predetermined scale ($0 \leq 5\%$; 1 = 6–30%; 2 = 31–50%, 3 = 51–75%; 4 = 76–100%). Counting was performed at 200 × magnification. The intensity of the staining was also categorized on a four-point predetermined scale (0 = no detectable staining, 1 = light staining, 2 = moderate staining, 3 = strong staining, and 4 = very strong staining). If the observers' grading disagreed by more than one point, the field of view that was being graded was thrown out, and a new field of view was chosen. For each disc, five areas were analyzed for both percentage and intensity of staining.

Statistical analysis

SPSS version 22.0 (SPSS Institute, Inc., Chicago, IL, USA) was used for the statistical analyses. To analyze the area and density, the average of the results from all available samples of each patient was considered. Means were compared using one-way ANOVA and least significant difference test for multiple comparisons of groups. A value of $p < 0.05$ indicated a statistical difference.

Results

Immunoreactivity for IL-1R1 expression was confirmed, following immunohistochemistry (IHC). Staining was localized to fibroblasts expressing IL-1R1. All experimental samples were identified as positively stained. The samples were first analyzed for the percentage of positively stained cells. The experimental groups, ADDwR (Figure 1) and ADDwoR (Figure 2), were both statistically significant when compared to the control group (Figure 3). As shown in Table 1, IL-1R1 receptor expression was statistically significant for both ADDwR and ADDwoR ($p < 0.001$). However, there was no significant difference of percentage of positively stained cells between each of the experimental groups (ADDwR × ADDwoR) ($p = 0.720$) (Table 1). The intensity of fibroblast staining for each sample was also analyzed. ADDwR fibroblast staining intensity was statistically significant compared to the control tissue ($p < 0.007$). Additionally, fibroblast ADDwoR staining intensity was also statistically significant compared to the control samples ($p < 0.014$). There was no statistical difference between the two experimental groups (ADDwR × ADDwoR) ($p = 0.917$). The results are shown in Table 2.

Figure 1. Image showing Anterior Disc Displacement with Reduction.

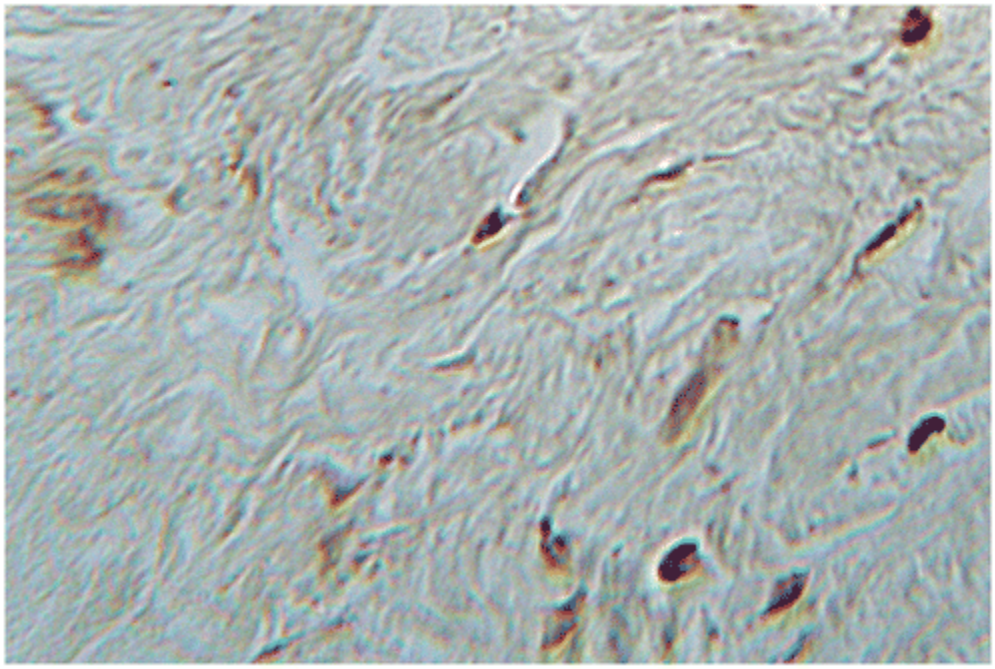

Figure 2. Image showing Anterior Disc Displacement without Reduction.

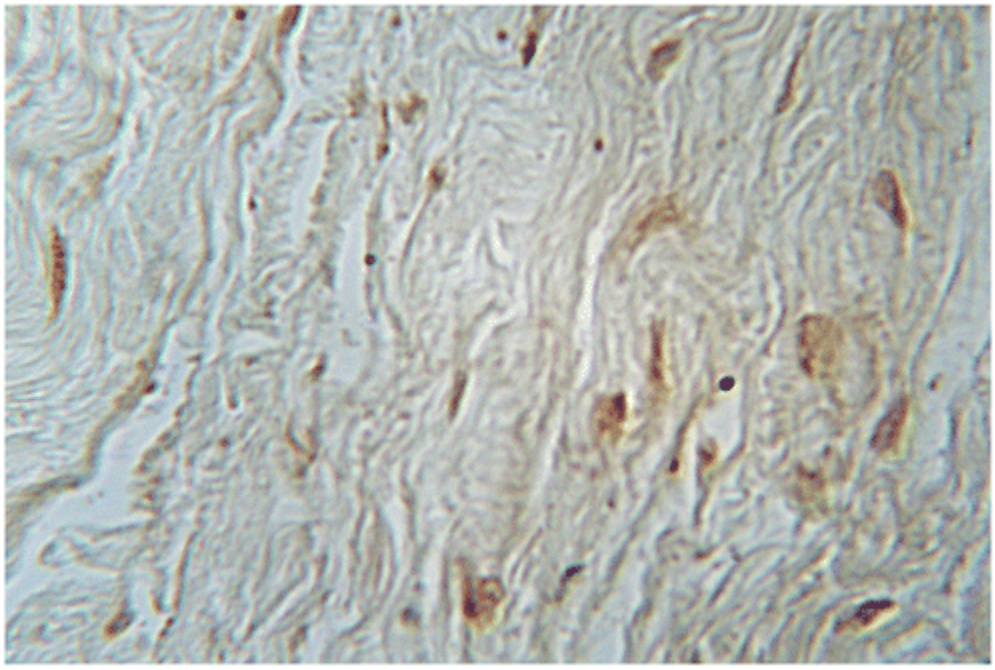

Figure 3. Image showing Control.

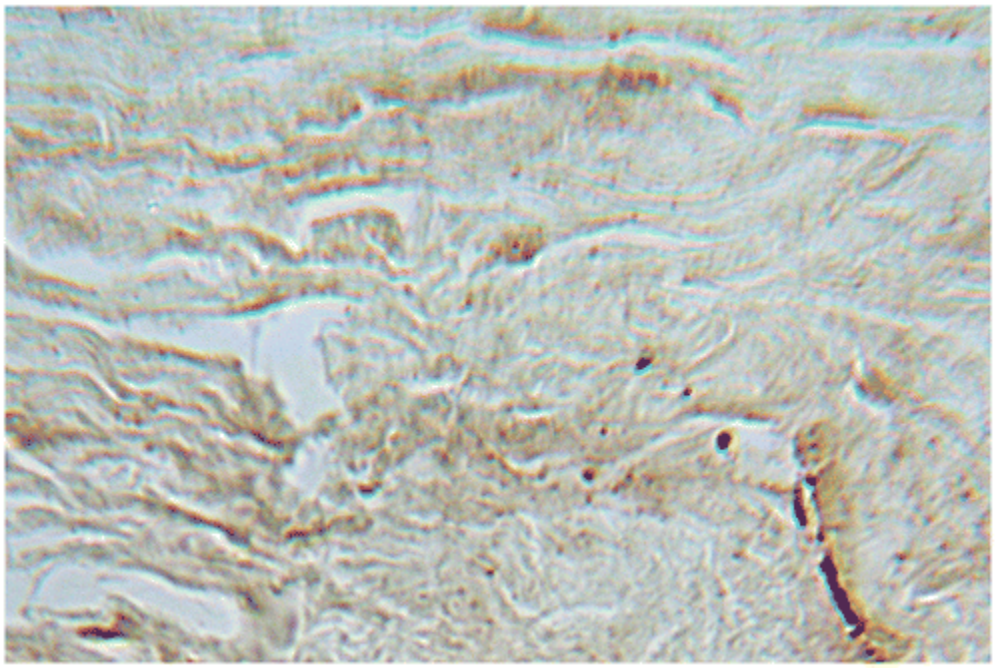

Table 1. Immunoreaction IL1-R1.

Number of positively stained fibroblasts	Group	*n* (discs)	Mean	Median	Minimum	Maximum	Standard deviation
	Control	8	0.513	0.500	0.000	1.200	0.445
	With red	19	2.337	2.600	0.200	4.000	1.193
	Without red	12	2.483	2.800	0.600	4.000	1.225
p Value		*p* Value		*p* Value		*p* Value	
Control × With red × Without red		Control × With red		Control × Without red		With red × Without red	
0.001		<0.001		<0.001		0.720	

Note:

n = number; red = reduction.

Table 2. Intensity of fibroblast staining.

Intensity of fibroblast staining	Group	*n* (discs)	Mean	Median	Minimum	Maximum	Standard deviation
	Control	8	0.900	0.800	0.000	2.400	0.733
	With red	19	2.174	2.400	0.000	4.000	1.111
	Without red	12	2.133	2.300	0.600	4.000	1.106
p Value		*p* Value		*p* Value		*p* Value	
Control × With red × Without red		Control × With red		Control × Without red		With red × Without red	
0.017		0.007		0.014		0.917	

Note:

n = number; red = reduction.

Discussion

TMJ dysfunction has been affecting people's quality of life worldwide, especially women's. It has been estimated that almost one quarter of the population presents TMD, which probably has an impact on the health treatment budget [3]. The identification of the predisposing causes and being able to prevent or diminish its hazard will benefit greatly those patients who must have had to adapt their lifestyle with distressing chronic TMD pain.

TMD development is multifactorial, with host factors such as hormones, occlusion changes, parafunction, and stress being associated with the disorder [14].

According to Ernberg [15] some internal derangements are symptom-free, but the degree of inflammation in the TMJ plays a role on TMD pain behavior. Several studies aimed to identify the TMJ degenerating cascade, and many of them researched synovial fluid and the TMJ disc through immunohistochemistry. Matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) have been elevated in the synovial fluid of TMD patients, as well as hyaluronic acid synthase, disintegrin and metalloproteinase with thrombospondin motifs (ADAMTs), aggrecan, fibromodulin, biglycan, and lumican [15]. Studies focused on apoptosis, immune, and inflammatory pathways, being the inflammatory disc degeneration cascade of great interest, in order to better comprehend how TMD take place.

Regarding TMJ discs, ADAMTS-4 and ADAMTS-5 are metalloproteinases that degrade proteoglycan and were found highly expressed in TMD discs, contributing to its degeneration [16,17]. Hyaluronan

synthase 3, involved with TMJ lubrication, was also found associated with TMD [18]. Beta-defensin 4 acts on the immune system but has been associated with TMJ inflammation, as well [19]. The MMP family has been widely studied in TMD, due to its main role on tissue disruption, and MMP-2 [20,21], MMP-7, and MMP-9 [22] have been associated with TMD.

The Interleukin family is composed of inflammatory cytokines, and the main types present in the inflamed TMJ were IL-1β and IL-6 [23]. IL-1β is absent in healthy TMJs [20], and a study showed that it impedes the chondrogenic differentiation of synovial fluid mesenchymal stem cells in the TMJ, affecting its balance [24]. It was associated with arthritic TMJ hyperalgesia, altered mouth mobility, anterior open bite [25], and also with the deformation of the TMJ disc [26]. In addition, IL-1 regulates the expression of hyaluronan, a major glycosaminoglycan of synovial fluid [27], responsible for its lubrication and homeostasis.

The IL1-R1 is the main receptor of IL-1β, and its function is fundamental for the IL-1β inflammatory cascade. IL1-R1 presence in synovial fluid has been associated with TMJ degeneration [28,29]. Most studies concerning IL-1 and IL1-R1 were in synovial fluid, and little is known on how these cytokines act on human TMJ discs. The IL1-R1 is the receptor of IL-1β, and it has been the target of many therapies aiming to block its actions, diminishing the TMJ hazard [30].

IL-1 was associated with temporomandibular dysfunction (TMD) [5] and with the deformation of the TMJ disc [26]. However, this is the first study to analyze the association of IL1-R1 and TMD in human TMJ discs. The results of the present study indicate the association of IL1-R1 with ADDwR and ADDwoR, coinciding with the association of IL-1 with TMD. This result will be important for therapies designed to interfere with the TMD degeneration cascade. The control group of this sample, composed of disc samples affected by trauma and condylar hyperplasia, is not ideal. However, there was a strong association of IL1-R1 with disc displacement, eliminating a false positive association. Patients with condylar fracture exhibit an inflammatory process, and the control group was expected to exhibit expression of IL1-R1. More significantly, this study showed patients with TMD presented an even higher expression. This demonstrates an association of ADDwR and ADDwoR with IL1-R1. To the authors' knowledge, this is the greatest sample size extracted from human patients and not cadavers. It must be noted that most patients with TMD do not require surgery, but rather are treated clinically; hence this sample was difficult to collect. In conclusion, IL1-R1 was associated with ADDwR and ADDwoR in TMD discs of humans.

Conclusion

According to the authors' findings, IL1-R1 was associated with ADDwR and ADDwoR in TMD discs of humans.

Future analyses of other interleukins, combined with the results of this study, may contribute to a better understanding of the role of the inflammation process on tissue remodeling and destruction in the temporomandibular joint.

Disclosure statement

No potential conflict of interest was reported by the authors.

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