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Original Article

Changes in the salivary oxidative status in individuals with temporomandibular disorders and pain

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ABSTRACT

Temporomandibular joint disorders are quite common among the general public. Free radicals may play a role in the pathogenesis of joint diseases, and the oxidative stress is an important aspect in the mechanism of TMDs. The use of new biotechnologies has enabled the use of saliva as a diagnostic method. This is the first paper that aims to investigate changes in the oxidative status, through saliva analysis, in individuals who suffer from temporomandibular disorder and pain. Sixty individuals, 54 women and 06 men, aged between 10 and 60, participated in this research. The questionnaire 'Research Diagnostic Criteria for Temporomandibular Disorders' (RDC/TMD) was filled out in order to determine the presence and the type of TMD, and the Visual Analogue Scale (VAS) was conducted in order to measure the pain perception caused by TMD. In addition, the total oxidant status (TOS), and the total antioxidant capacity (TAC) were measured, and the oxidative stress index (OSI) was calculated. The *t*-test and the Pearson Correlation Test were used with the significance level of $p < 0.05$. The TAC in individuals with TMD and pain was significantly reduced ($p < 0.001$). There was no difference regarding the levels of TOS ($p = 0.765$) between both groups. As a result, the OSI was significantly higher in the group TMD and pain ($p = 0.011$). There was no correlation between VAS, TAC, and TOS values. Within the limits of this study, oxidative changes seem to influence the pathogenesis of pain in TMDs.

1. Introduction

Temporomandibular disorders (TMDs) are characterized by changes in the mandibular kinesiology, joint sounds, and pain in the structures of the stomatognathic system.[1] It is a common condition and, in approximately 25% of cases, TMDs are characterized by floating and progressive pain due to inflammation.[2]

Inflammatory processes in the temporomandibular joint (TMJ) increase the amount of free radicals, which can be either the source or the perpetuating factor in such processes.[3] When the free radicals' cleansing capacity is insufficient, there is oxidative stress, which can initiate processes that take part in the pathogenesis of many inflammatory diseases.[4]

Blood and synovial fluid from the TMJ have been used to detect free radicals and joint disease markers.[5–7] The salivary composition may change due to patients' physiological, nutritional, and emotional states. Several proposals for diagnoses through saliva have been suggested[8] since its collection is simple and inexpensive. Moreover, it is a non-invasive procedure. The possibility of measuring oxidative changes in the TMJ, through salivary examination, constitutes an alternative to invasive methods. However, the literature reports just one pilot study so far. Thus, this was the first study that aimed to evaluate changes in the salivary oxidative status in individuals with TMD and pain.

2. Method

2.1. Ethics committee approval

This study was approved by the Ethics Committee of the Federal University of Paraná. All participants or legal guardians signed a free, prior informed consent.

2.2. Sample

Sixty individuals, aged between 10 and 60, took part in this study. They were divided into two groups: group with TDM/pain was made up of 30 individuals and group without TMD/pain was

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made up of 30 individuals as well. The sample was paired, equally divided in terms of gender and age.

The group with TMD/pain consisted of 30 individuals selected from the Centre of Diagnostic and Treatment for Temporomandibular Joint and Functional Dentofacial Changes (CDTMJ) from Tuiuti University of Paraná, in Curitiba. The group without TMD/pain consisted of 30 students and staff from the Federal University of Paraná. The inclusion criteria determined that the participants should have complaints about localized pain in the TMJ and/or in the masticatory muscles; the intensity perceived by the patients was mild, moderate or severe, according to the visual analogue scale. The participants were asked about general health conditions, medication intake and smoking addiction.

The exclusion criteria of some individuals from the study sample were the following: other local diseases and/or systemic disorders; the use of anti-inflammatory drugs, analgesics and/or muscle relaxants, vitamin C and/or vitamin E; smoking, which increases the levels of oxidants; and individuals who had already been under treatment for TMD.

2.3. Clinical examination

A single examiner, a specialist in TMD and orofacial pain, performed the clinical examination. All participants were previously evaluated by means of a questionnaire, the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD), validated for research and diagnosis of temporomandibular disorder in order to establish the occurrence and standards of TMD.

To register the participants' general perception of pain caused by the TMD, the visual analogue scale (VAS) was used with values from 0 mm (no pain) to 100 mm (the worst imaginable pain suffered by an individual).

2.4. Collection of saliva

The participants' saliva was collected between 03:00 P.M. and 05:00 P.M. These individuals were previously instructed to be fasting for 2 h beforehand. They also had to brush their teeth 2 h before collection, which was obtained with the use of paraffin film in order to perform 5-min stimulation. Then, they spit their saliva into a sterile container (Sterile Universal Collector – J.PROLAB 80 ml) and after the collection, the samples were frozen at −20 °C (−4 °F) in a freezer until further analysis.

2.5. Biochemical analysis

The total antioxidant capacity (TAC) was determined through Erel's TAC method.[9,10] Briefly, the reactions of free radicals initiated with the production of hydroxyl radical (OH) through the Fenton's reaction, and the reaction speed was monitored through the observance of dianisidine radicals. The results were expressed in mmol Trolox Eq/L. The total oxidative status (TOS) in saliva was determined by Erel's TOS method,[11] which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidative species in acidic medium as well as on the determination of ferric ion by xylenol orange. The results were expressed in μmol H_2O_2/L. Erel's methods, TAC and TOS, are colorimetric and automated and the precision of this assay is excellent – lower than 3%.[12,13] The ratio between TOS and TAC was regarded as oxidative stress index (OSI).[9,14,15] To perform the calculation, the TAC unit was changed from mmol Trolox Eq/L to μmol Trolox Eq/L, and the OSI was calculated as follows: OSI = [(TOS, μmol/L)/(TAC, μmol Trolox Eq/L)/100].

The o-dianisidine reagents, ammonium and iron (II) sulfate and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from the company Sigma–Aldrich® (São Paulo, Brazil). The xylenol orange PA (tetrasodium) was purchased from the company *Cloroquímica* (Curitiba, Brazil).

Analyses were performed on a spectrophotometer (S-2000 UV–VIS, São Paulo, Brazil) in duplicate and the results were compared to those from the control group.

2.6. Statistical analysis

Data were tabulated and submitted to *t*-test and Pearson correlation test in order to compare both groups. The software '*Statistical Package for Social Science*' – SPSS 17.0 (IBM, Chicago, IL) was used and the significance level was 5%.

3. Results

Fifty-four women and six men, aged between 10 and 60, participated in this study. According to the RDC/TMD questionnaire, all patients from the group TDM/pain had arthralgia and myofascial pain or myofascial pain with opening limitation. Furthermore, 15 individuals also had articular disk displacement with reduction.

TAC levels were significantly reduced in the group TMD with pain while OSI levels increased. There was no difference in TOS levels between both groups. Table 1 shows the comparison between the values found in the group with TMD and pain and the group without TMD and without pain (Table 1).

With regard to pain, the intensity reported by the participants ranged from moderate to severe. There was no correlation between the TAC and the SOS with the VAS.

4. Discussion

In recent years, studies have been describing the imbalance between oxidants and antioxidants in TMD pathogenesis and other diseases.[16–18] The mechanical stress on the TMJ as well as on masticatory muscles can generate free radicals through some mechanisms, triggering a cascade of reactions that can exacerbate tissue damage, inflammation and pain.[16] The purpose of this study was to determine whether there were changes in the oxidative status in individuals with TMD and pain. As a result, there were differences in TAC and OSI levels between both groups.

Most of the selected sample consisted of women. The literature reports that women[19] suffer more from TMDs than men do – the ratio of women to men is 4:1.[20] During the reproductive years, women experience a higher frequency of TMJ disorders in relation to men. The onset of pain usually happens after puberty.[21] Ovarian hormones modulate pain in women with TMD and changes in oestrogen levels lead to TMJ disorders for affecting the processes of bone remodelling, changing the extracellular matrix and bone volume.[22]

All individuals from group TMD/pain were concurrently diagnosed with myofascial pain and arthralgia in accordance with the RDC/TMD. The aetiologies of joint and muscle pain are

Table 1
Comparison of TAC, TOS, OSI, and VAS results between patients with TMD/pain and patients without TMD/pain.

	TDM and pain ($n = 30$) mean ± SD	No TDM and pain ($n = 30$) mean ± SD	*p*
TAC	0.130 (±0.043)	0.264 (±0.047)	0.000
TOS	4.402 (±0.418)	4.671 (±0.234)	0.765
OSI (%)	4.2 (±0.4)	1.5 (±0.2)	0.011
VAS (mm)	6.677 (±1.536)	0	–

TAC, total antioxidant capacity (the results were expressed in mmol Trolox Eq/L); TOS, total oxidant status (results were expressed in μmol H_2O_2/L); OSI, oxidative stress index; VAS, visual analogue scale.

different and the biological processes that lead to its development are different as well. However, some changes, such as disk displacement or arthralgia, which are not associated with the chewing muscles, are rare.[23] For this reason, joint and muscle aches and pains coexist in most patients with TMD who look for treatment.

This is the first study that aims to compare the total levels of oxidants and antioxidants, found in such patients' saliva, which contains biomarkers of biological parameters derived from the bloodstream. It is an inexpensive, stress-free, and non-invasive method.[8,24,25] A pilot study was able to associate pain with SOD, 8-OHdG, and TAC, both in blood and in saliva.[25] Another study found the same amount of a biomarker of oxidative process, both in synovial fluid and in blood.[5]

Different oxidizing species' concentrations can be measured separately in laboratories, but it is an expensive, laborious, and time-consuming task that requires complicated techniques and also presents additional oxidizing effects. For this reason, the dosage of the total oxidant status (TOS) of a sample was performed instead. Erel's TOS and TAC methods were used for being reliable, sensitive, and inexpensive. Moreover, these methods use durable reagents. The method used by Sotillo et al.[25] in a pilot study to evaluate the TAC in saliva was the TEAC (Trolox equivalent antioxidant capacity), developed by Miller et al.,[26] which was developed into a commercial kit by Randox Laboratories Ltd. (UK). However, when uric acid is produced, it strongly influences the TAC of the sample. In order to solve this problem, Erel[10] reported a new method, TAC, in which the influence caused by this acid is far smaller. Erel's TAC and TOS methods are colorimetric, automated, and accurate – lower than 3%.[27]

TAC levels in individuals with TMD and pain were reduced, while TOS values were similar to the group without TMD. As a result, the OSI was significantly higher in the group TMD and pain. The literature shows that the activity of the superoxide dismutase (SOD), an antioxidant enzyme, is progressively reduced with the advancement of TMDs. This reduction may be related to an insufficient cleansing capacity of free radicals.[28] However, another study showed levels of SOD in synovial fluid and increased lipid peroxidation, suggesting that the increased production of free radicals should cause an increase in the generation of antioxidant enzymes.[6]

A research that compared TMDs with pain showed lower TAC values, but an increase in 8-hydroxydeoxyguanosine and malondialdehyde, associating the results with a worsening of the disease stage as well as greater intensity of pain.[25] In this study, the intensity of pain reported by the participants ranged from moderate to severe; however, there was no correlation between VAS with TAC and TOS values.

According to Etoz et al.,[7] this disease is strongly related to the antioxidant capacity of the TMJ since a lower TAC was observed in individuals who, besides pain, had articular disk displacement without reduction.[7] Basi et al. associated the concentration of F2-isoprostane with the intensity of joint and muscle pain, suggesting that oxidative stress contributes towards pain in patients with painful TMD.[5]

On the other hand, Nitzan et al.[29] stated that increased oxidative stress caused by free radicals in the TMJ could cause the imbalance of local antioxidant defences, deteriorating joint lubrication. Richards et al.[30] evaluated blood oxidative stress in individuals with temporomandibular dysfunction who also suffer from chronic fatigue syndrome, and observed an association between increased levels of oxidative stress and total free radicals. These patients felt a great deal of pain in the masticatory muscles.[30]

A lower TAC suggests that the imbalance in antioxidant mechanisms can influence pain mechanisms in TMDs, characterized by an insufficient response against oxidative stress caused by free radicals. TOS values were similar in both groups suggesting that changes related to the TAC in TMDs with pain can be considered a major determinant factor when compared to oxidative stress.

The use saliva instead of synovial fluid or blood represents a less invasive alternative to investigate oxidative changes in individuals with TMD and pain since saliva can be used for diagnostic and therapeutic purposes.

Within the limits of this study, oxidative changes seem to influence the pathogenesis of pain in TMDs. Thus, further studies should be conducted in order to investigate the influence of the TAC in various TMD subgroups, along with longitudinal studies, in order to observe changes in oxidative mechanisms during the progression of TMDs.

Conflicts of interest

The authors have none to declare.

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