



Total Antioxidant Capacity, Catalase Activity and Salivary Oxidative Parameters in Patients with Temporomandibular Disorders

Neda Omidpanah^{1*}, Saba Ebrahimi², Asad Vaisi Raygani^{3,4}, Hadi Mozafari³, Mansour Rezaei⁵

1. Department of Oral Medicine, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Private Practice, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran
3. Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
4. Department of Biochemistry, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
5. Department of Biostatistics, Kermanshah University of Medical Sciences, Kermanshah, Iran

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* Corresponding author:

Department of Oral Medicine, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran

Email: n.omidpanah20000@gmail.com

ABSTRACT

Objectives: Temporomandibular disorders (TMD) are characterized by pain or discomfort in the temporomandibular joint, periauricular region, masticatory muscles, and neck on one or both sides. It may also be associated with joint sounds, restricted mandibular movements and mandibular deviation. Oxidative agents may have a deleterious role in the pathogenesis of joint diseases, and oxidative stress can lead to TMD. The aim of this study was to assess the oxidative stress biomarkers in the saliva of TMD patients and healthy controls.

Materials and Methods: This case-control study was conducted on 30 patients with TMDs (5 males and 25 females) with a mean age of 30.7 ± 13.2 years, and 30 healthy controls (5 males and 25 females) with a mean age of 29.16 ± 11.2 years. Saliva samples were collected according to the standard protocol and the total antioxidant capacity of the saliva (non-enzymatic), catalase activity, and malondialdehyde (MDA) levels were measured using the ferric reducing ability of plasma, Aebi's method, and high-performance liquid chromatography, respectively. Finally, The MDA levels were analyzed by the Mann-Whitney test. Other quantitative parameters were analyzed by independent t-test.

Results: TMD patients had significantly higher salivary levels of MDA compared to the control group ($P=0.001$). But there were no significant differences in catalase ($P=0.49$) and total antioxidant capacity ($P=0.22$) of TMD patients and healthy controls.

Conclusion: It seems that oxidative stress may be involved in the pathogenesis of TMDs.

Keywords: Catalase; Malondialdehyde; Saliva; Temporomandibular Joint Disorders; Free Radicals; Oxidative Stress

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INTRODUCTION

The term temporomandibular disorders (TMDs) as a subgroup of orofacial pain syndrome [1] is a general term that includes a

number of clinical problems, including involvement of the masticatory muscles, temporomandibular joints (TMJs), and associated structures (ligaments, tendons and

nerve). The TMD symptoms include pain or discomfort in the TMJ, headache, pain around the ears, pain and tenderness of the masticatory muscles, unilateral or bilateral neck pain, joint sounds, movement limitation of the jaw, deviation of mandible, and tooth wear. About 20% to 30% of the adult population are affected by TMDs, and approximately 25% of TMD cases are characterized by progressive pain due to inflammatory processes in the TMJ. Inflammation can increase the level of free radicals, which can further aggravate the condition [2]. TMDs have a multifactorial etiology [3]. It has been reported that TMJ pain and dysfunction can occur following inflammation [4]. The resultant inflammatory mediators and products can cause destruction and degeneration of articular disc. Free radicals are also produced in this process [5]. Oxidative stress can occur due to the release of free radicals in concentrations higher than that required for the natural cleansing mechanism of antioxidant defense system, and initiate processes that play a role in the pathogenesis of many inflammatory diseases [6]. Some defects in the antioxidant defense system lead to increased concentrations of oxidative stress biomarkers, such as 8-hydroxydeoxyguanosine and malondialdehyde (MDA), and decrease the total antioxidant capacity (TAC) of the serum or saliva [7,8]. In addition, high levels of salivary 8-hydroxydeoxyguanosine and MDA and low levels of salivary superoxide dismutase and glutathione peroxidase have been reported in patients with periodontitis [8]. Demir et al. [9] reported higher levels of MDA in TMD patients compared with controls; whereas, serum levels of catalase, superoxide dismutase, and glutathione were significantly higher in the control group. Moreover, de Almeida and Amenábar [10] reported significantly higher oxidative stress index in TMD patients suffering from TMJ pain.

Some biomarkers have been studied in individuals with TMD in order to clarify the mechanisms of pain, take initial steps for early detection of pain and degeneration, and find potential targets for treatment or prevention of severe pain and functional impairment [11,12]. The correlation between reactive oxygen

species (ROS) biomarkers in the saliva and joint pain has not been studied; thus, more studies are needed on their efficacy for diagnostic purposes [8,13,14]. Excess amounts of H_2O_2 in the mammalian cells can be detoxified through a powerful system of antioxidants, which contains catalase and glutathione peroxidase [15]. Catalase can break down H_2O_2 to H_2O and O_2 and prevent the production of hydroxyl radical ($OH\cdot$) from H_2O_2 . This molecule is the most harmful and the most reactive molecule for the viable cells [16].

The main functions of the saliva include lubrication, buffering action, anti-bacterial and anti-viral activity, and helping food digestion [16]. For diagnosis, saliva has some advantages over blood and urine. Easy and non-invasive collection of saliva requires simple tools, and it has less protein content and less compositional variability than serum. Several proposals have been suggested for making a diagnosis by the use of saliva [17,18].

This study aimed to measure the TAC of the saliva, and salivary levels of catalase and MDA in patients with TMDs and healthy controls in order to assess the relationship of these biomarkers with TMDs.

MATERIALS AND METHODS

This case-control study evaluated 60 participants. Totally, 30 TMD patients (25 females, 5 males) were selected among those referred to the Dental School of Kermanshah University of Medical Sciences during an 11-month period using convenience sampling. The patients had pain and mouth opening limitation. The inclusion criterion was patients complaining of localized pain in the jaw, preauricular area, ears, and/or masticatory muscles. The perceived intensity of pain by patients was mild, moderate or severe according to a visual analogue scale. For assessment of mouth opening, the distance between the maxillary and mandibular central incisors was measured using a ruler by taking into account the amount of open bite and deep bite. If this distance was less than 40 mm, it was considered as mouth opening limitation. TMJ examination was done by an oral medicine specialist. The participants were asked about

their general health, medication intake, and smoking status. The exclusion criteria were other local diseases and/or systemic disorders, use of anti-inflammatory drugs, analgesics, muscle relaxants, vitamin C and/or vitamin E, and multivitamins, smoking (because it increases the level of oxidants), and individuals who had previously received treatments for TMD. Thirty age- and sex-matched volunteers (25 females, 5 males) were included as the control group. Controls were selected among healthy individuals without any systemic disease or history of taking medications, smoking or alcohol use. Also, pregnant women were excluded from the study. Subjects in both groups did not have periodontal disease.

All participants were recruited among those presenting to the Dental School of Kermanshah University of Medical Sciences in Kermanshah city located in the west of Iran. The diagnosis of TMD was confirmed in patients according to the Research Diagnostic Criteria for TMDs [2]. The present study was approved by the ethics committee of Kermanshah University of Medical Sciences (Ir.Kums.Rec.1394.219). Written informed consent was obtained from all participants. Next, 5 mL of unstimulated saliva was collected from all participants using the spitting method. For this purpose, the patients were asked to refrain from eating, drinking, smoking, chewing gum, and oral hygiene practice or any other oral stimulation for at least 90 min before sampling. They rinsed their mouth for 30 s with water, and the water was then discarded. Saliva samples were centrifuged and stored at -70°C for further analysis [19]. The TAC of the saliva was evaluated by ferric reducing antioxidant power assay to assess the ferric reducing ability of the saliva. This method is based on the ability of the saliva for reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions at low pH in presence of 2, 4, 6-tripyridyl-striazine (Merck, Darmstadt, Germany) [20]. Finally, the intensity of blue-colored (Fe^{2+} -2, 4, 6-tripyridyl-striazine) complex was measured at 593 nm wavelength by a spectrophotometer (6320D; Jenway, China). The results were expressed in millimole per liter (mmol/L). The lipid peroxidation level was evaluated by the MDA assay. Salivary MDA

levels were determined by the thiobarbituric acid method [21] using high-performance liquid chromatography (1200 series; Agilent Technologies, USA). Briefly, in a screw-capped test tube, 50 μL of the saliva sample was mixed with 50 μL of 0.05% butylated hydroxytoluene (Merck, Germany), 400 μL of H_3PO_4 (0.44 M) and 100 μL (42 mM) of thiobarbituric acid (Merck, Germany) and kept in a boiling water bath for 1 h. After rapid chilling on ice, 250 μL of n-butanol was added to the samples, vigorously shaken for 5 min, and centrifuged (14000 rpm for 10 min). Then, 20 μL of the supernatant was injected into the C-18 (5 μm , 4.6 x 150 mm) column. Elution was performed with methanol (40:60, v/v) containing 50 mM KH_2PO_4 buffer (pH 6.8). The flow rate was equal to 1 mL/min. Detection was carried out using the fluorometric method by excitation at 515 nm and emission at 553 nm. The peak of MDA-TBA adduct was calibrated with 1,1,3,3-tetramethoxypropane as standard solution similar to the protocol for the saliva sample. The MDA values were expressed in nanomole per milliliter (nmol/mL).

The catalase activity was determined using the Aebi's method [19]. Briefly, 100 μL of the saliva was diluted with 4.9 mL of 50 mM phosphate buffer at a pH of 7. Then, 2 mL of the diluted saliva was mixed with 1 mL of 30 mM hydrogen peroxide (Merck, Germany). The course of reaction was followed kinetically at 240 nm by a spectrophotometer (CE 7250; Cecil, England) at every 20 s for 1 min. Statistical analysis was performed using SPSS version 19.0. To compare age and sex, independent t-test and chi-square test were used, respectively. Quantitative data distribution was examined by the Kolmogorov-Smirnov test. Based on the obtained data, the distribution of MDA data was not normal, and we used nonparametric Mann-Whitney test. Other quantitative parameters were analyzed by independent t-test.

RESULTS

The mean age of patients was 30.7 ± 13.2 years, and the mean age of healthy controls was 29.16 ± 11.2 years. The catalase activity, TAC, and MDA levels in the patient and control groups are presented in Table 1.

Table 1. Comparison of the mean total antioxidant capacity (TAC), catalase (CAT), and malondialdehyde (MDA) levels in the saliva of TMD patients and control subjects

Biomarkers	TMD subjects (Mean± SD)	Controls (Mean± SD)	P-value
TAC (mmol/L)	751.2± 408.7	939.1±723.8	0.22
CAT	0.535±0.405	0.466±0.37	0.49
MDA (nmol/mL)	30.52(25.85-40.91)	24.13(19.85-29.78)	0.001

SD: Standard deviation

The mean salivary TAC in patients with TMD was lower than that in controls, but not significantly (P=0.22). The mean salivary catalase activity in patients with TMD was higher than that in controls, but not significantly (P=0.49). The mean rank of MDA level in the saliva of patients with TMD was higher than that in controls, and the difference in this respect was statistically significant between the two groups (P=0.001, Fig. 1).

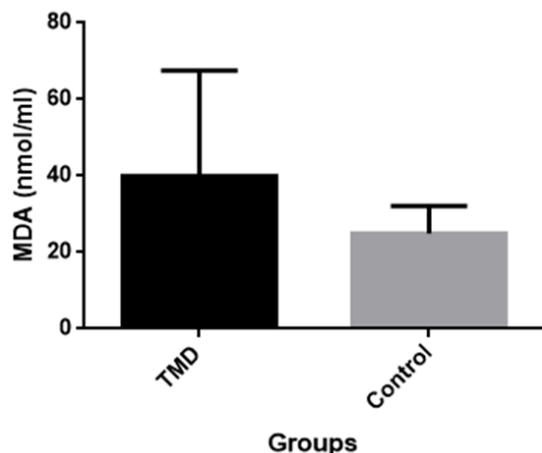


Fig. 1. Comparison of malondialdehyde (MDA) levels between TMD patients and Controls

On the other hand, MDA levels were higher in females with TMD than male patients (Table 2). However, this difference was not significant (P>0.05). The pain intensity of patients was moderate to severe.

Table 2. Comparison of the mean salivary MDA levels between males and females in TMD and control groups

Groups	Gender (n)	MDA (Mean± SD)	P-value
TMD	Males (5)	28.3±8.4	0.22
	Females (25)	42.3±29.5	
Controls	Males (5)	25.2±7.8	0.42
	Females (25)	22.9±7.3	

SD: Standard deviation

DISCUSSION

This case-control study showed that TMD patients had significantly higher levels of salivary MDA as an oxidative biomarker compared with the control group. Dotan et al. [22] stated that MDA can be directly indicative of oxidative stress. Significant difference in salivary MDA levels between patients in this study compared with controls supports the role of oxidative stress in pathophysiology of TMDs. Despite the decreased TAC of the saliva in patients, there was no significant difference between the two groups in this respect. The aim of this study was to assess the possible association of oxidant/antioxidant status and TMDs. The results showed a difference in MDA levels between the two groups.

Nitzan et al. [23] reported that uncontrolled oxidative stress causes collapse of the lubrication system, which is considered a major initiator of TMJ dysfunction. Previous investigations showed decreased TAC and increased levels of MDA in patients with inflammatory diseases and/or acute pain [24-26]. Trauma, mechanical stress and degenerative disc disease in TMD can lead to release of free radicals, oxidative stress, and imbalance in biomarkers [6]. Decreased TAC in TMD patients indicates imbalance of oxidant-antioxidant system due to a number of mechanisms such as pain.

Rodriguez de Sotillo et al, [6] in a pilot study evaluated the correlation of TMD pain and level of ROS biomarkers including 8-hydroxydeoxyguanosine, MDA and TAC in the

saliva and serum of patients with TMD and found significant associations between both saliva and serum levels of biomarkers and TMD pain. They found high levels of MDA and low levels of TAC in serum and saliva of TMD patients, which was in line with our results.

Free radicals can be produced through a number of mechanisms due to the mechanical stress of the TMJ and masticatory muscles that can promote a series of reactions. These reactions can worsen tissue damage and inflammation [4]. The mechanical strain, internal derangements, and injury of the joint tissues can lead to accumulation of free radicals in the joint and disturb local antioxidant defense reactions. In addition, Lawaf and colleagues [27] in a case-control study compared antioxidants in the serum and saliva of patients with TMDs and healthy controls. They found that there was no significant association between the two groups regarding the TAC of the saliva. This result confirms our findings. Lee et al. [28] investigated the role of ROS in the synovial fluid of TMD patients.

They found that OH^\cdot was generated in the synovial fluid of patients with TMDs. They showed ROS-induced oxidative stress in the synovial fluid. According to de Almeida and Amenábar [10], oxidative changes seem to influence the pathogenesis of pain in TMDs because lower TAC and higher oxidative stress index were observed in TMD patients who had pain. However, we evaluated the activity of TAC of the saliva in both groups in our study. We did not find statistically significant differences between patients and controls in this respect. Controversy in the results of studies may be due to the use of different methods for measuring the biomarkers, genetic variations in the study populations, and effect of diet and nutrient deficiencies. Since diagnostic data collection was performed by trained specialists with standardize data and specimen collection procedures, the chances of misclassification of subjects or inaccurate measurement of oxidative stress biomarkers are low. Future studies are required to assess the change in concentration of MDA over time and its correlation with different subtypes of TMDs.

In summary, the salivary MDA level can be used as a marker to assess TMDs. This study had a small sample size, which was a limitation of this study. Future studies with a larger sample size are required to assess the diagnostic role of MDA as a TMD biomarker in the saliva.

CONCLUSION

The results of this study indicated that TAC of the saliva and salivary catalase activity were not different in TMD patients and healthy controls. Other oxidative stress biomarkers should be compared between TMD patients and healthy controls.

CONFLICT OF INTEREST STATEMENT

None declared.

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