Comparison of Serum and Salivary Antioxidants in Patients with Temporomandibular Joint Disorders and Healthy Subjects

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Abstract
Objectives: Temporomandibular dysfunction (TMD) is a group of disorders in the facial region and temporomandibular joint (TMJ). Biomarkers are assumed to play a role in pain and early detection of destruction. The aim of this study was to compare the saliva and serum antioxidant levels in patients with TMD and healthy subjects.

Materials and Methods: This case-control study was conducted on 28 TMD patients without pain, 28 TMD patients with pain and 28 healthy controls. The total antioxidant capacity of saliva and serum of patients was measured. Data were analyzed using ANOVA and Tamhane’s test.

Results: The mean (±SD) total antioxidant capacity of serum (plasma TAC) was 0.8900 (±0.11627) mmol/L in TMD patients with pain, 1.2717 (±0.18711) mmol/L in TMD patients without pain and 1.7500 (±0.18711) mmol/L in the control group. Based on ANOVA, the difference in this regard among the three groups was statistically significant (P=0.000). The mean salivary TAC was 1.34 (±0.06721) mmol/L in TMD patients with pain, 1.42 (±0.16677) mmol/L in TMD patients without pain and 1.35 (±0.11627) mmol/L in the control group. The difference in this respect among the three groups was not significant (P>0.05).

Conclusion: The mean plasma TAC in TMD patients with/without pain was significantly lower than that in the control group but no significant difference was detected in salivary TAC among the three groups.

Key words: Antioxidants; Saliva; Serum; Temporomandibular Joint Disorders; Oxidative Stress

INTRODUCTION
Temporomandibular disorders are a group of joint disorders associated with pain, muscular tenderness, articular sounds, jaw deviation and mouth opening limitation [1]. This condition can become chronic and adversely affect the quality of life. The underlying molecular events responsible for the degenerative changes of the TMJ have been poorly understood. Recent evidence suggests that a variety of molecular substances namely cytokines, matrix degrading enzymes, neuropeptides, and catabolites of arachidonic acid may play a role in this process [2]. Levels of biomarkers have been evaluated in patients with TMD to describe the pain mechanism and enable early...
detection of joint pain and destruction with the aim of preventing the progression of pain and disability [3-5].

Oxidative stress (OS) occurs due to the release of free radicals in high concentrations overwhelming the natural scavenging mechanisms of antioxidant defense leading to subsequent initiation of inflammatory processes [4,6]. Physiologically, antioxidants neutralize the toxicity of free radicals and cytokines, and reduction in antioxidant levels leads to OS [7].

Oxidative stress, at least in part, is responsible for development of a wide range of conditions such as the Alzheimer’s disease, rheumatoid arthritis, Parkinson’s disease and premalignant conditions [8-12]. However, it is not clear whether the oxidants initiate the disease or are secondary to tissue damage [12]. But, the role of oxidants (LDL) in initiation of atherogenesis and subsequent atherosclerosis and cardiovascular diseases has been confirmed [13].

Several antioxidant enzymes such as the superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase protect DNA against OS [14]. Oxidative stress biomarkers were found to be significantly higher in serum of rats with TMJ arthritis [15]. In TMD, trauma, mechanical stress, disc disorders and destructive changes can trigger the release of free radicals leading to OS and oxidant/antioxidant imbalance [16]. This reduction in antioxidant defense system leads to an increase in the concentration of OS biomarkers namely 8-hydroxydeoxyguanosine (8-OHDG) and malondialdehyde (MDA) and reduction in TAC of serum and saliva [4,17].

Rodriguez de Sotillo et al, in a pilot study evaluated the correlation of TMD pain and level of OS biomarkers including 8-hydroxydeoxyguanosine, MDA and saliva and serum TAC of patients and found significant associations between both saliva and serum levels of biomarkers and TMD pain [18]. Canakci et al. reported high levels of salivary 8-OHDG and MDA and low levels of salivary SOD and glutathione peroxidase in patients with periodontitis [19].

Ueno et al. proposed a theory regarding the accumulation of free radicals as the result of mechanical stress to the TMJ of susceptible individuals leading to OS [2].

Studies evaluating the serum and saliva TAC in TMD patients are scarce. Considering the existing controversies in this respect in the literature, this study sought to assess the serum and saliva TAC of TMD patients and make a comparison with healthy subjects. The null hypothesis was that the serum and saliva TAC of TMD patients would not be significantly different from those of healthy controls.

MATERIALS AND METHODS

This case-control study was conducted on 28 TMD patients with no systemic disease and no history of drug use or TMJ pain as the case group one and 28 TMD patients with no systemic disease or drug consumption and positive history of TMJ pain as the case group two. Moreover, 28 controls matched with cases in terms of no cigarette smoking, no periodontal disease according to the clinical attachment level (CAL), no systemic condition, no drug use and no alcohol consumption were enrolled. Patients with a history of systemic disease or drug consumption were excluded from the study. The study design was approved by the Ethics Committee of the Islamic Azad University. All participants provided written informed consent. Pain evaluation was done by questioning the patient and clinical examination. For the assessment of the tenderness of the muscles of mastication, masticatory muscles were passively examined. Pain upon palpation of the masseter, temporal, medial pterygoid or lateral pterygoid muscle was considered as tenderness of the respective muscle and was recorded in the patient’s file [20]. Click was determined by clinical examination and use of a stethoscope. For assessment of mouth opening, the distance between the maxillary and mandibular central incisors was...
measured using a caliper by taking into account the amount of open bite and deep bite. We measured maximum opening without pain. If this distance was less than four centimeters it was considered as mouth opening limitation. Jaw deviation was determined by clinical examination and observation of the midline shift.

To assess the reliability of the laboratory, five saliva and blood samples obtained from patients were divided into two parts and sent to the laboratory under different names. The kit for assessment of antioxidant was valid. The laboratory results were exactly the same for all five specimens. To assess the reliability of the examiners, five patients and five healthy individuals were examined by an oral medicine specialist and a prosthodontist during a two-week period. The results showed that the diagnoses made by the examiners during the two-week time period were uniform for both healthy and TMD cases. The selected patients in both groups were dentulous. None of them were edentulous or had lost more than two teeth.

The degree of mouth opening was measured using a caliper. The degree of mouth opening was calculated taking into account the patient’s primary open bite or deep bite. Painless mouth opening was the criterion for calculation of this variable. For assessment of jaw deviation during opening, the alignment of upper and lower midlines in centric occlusion and when opening the mouth was checked. None of the patients had closed lock (anterior disc displacement without reduction). To assess the presence of closed lock, articular MRI was performed. If the examined patients had three of the six following symptoms, the diagnosis of TMD was made. These symptoms included: 1. Articular pain, 2. Cervical or facial muscle pain, 3. Articular sounds, 4. Mouth opening limitation, 5. Jaw deviation during opening, and 6. Masticatory muscle tenderness on palpation [20].

Case group one had articular pain or facial muscle pain along with at least two of the other symptoms. Case group two had at least three of the third to sixth symptoms. The control group had none of the mentioned symptoms on clinical examination [20]. According to a study by Rodríguez de Sotillo et al, [18] and use of Compare Means option to perform multiple comparisons of group means, sample size was calculated using Minitab software. Considering \( \alpha=0.05, \beta=0.1 \), minimum difference of 1.2 and mean standard deviation of 0.62, the sample size for each group was calculated to be 28 subjects. The case group patients were randomly selected among TMD patients presenting to the Oral Medicine Department of Islamic Azad University, Dental Branch. Patients were clinically examined by an oral medicine specialist and a prosthodontist and TMD sign and symptoms such as muscle tenderness, articular sounds, jaw deviation and mouth opening limitation were evaluated. The controls were selected among healthy individuals presenting to the Oral Medicine Department of the university who had no systemic condition or drug use. The case and controls were matched in terms of cigarette smoking and periodontal disease based on CAL.

**Sample collection**

Blood and salivary samples (5mL) were collected. Samples were collected for five minutes during 9-12 am in a stress-free environment. Blood samples were also taken and centrifuged at 3000 rpm and serum and saliva specimens were stored in a freezer at -7°C until the experiment.

**Measurement of saliva and serum TAC:**

Serum and saliva TAC was measured spectrophotometrically using Randox kit (Randox, Antrim, England). To ensure the reliability and validity of the laboratory, blood and salivary samples of two patients were evaluated twice in terms of TAC. By obtaining similar results, the validity and reliability of the laboratory were confirmed.
Statistical analysis
After ensuring the normal distribution of data, one-way ANOVA was used for comparing TAC among the study groups. The linear regression analysis was applied to evaluate the simultaneous effects of TMD and other covariates on TAC and the Spearman’s correlation test was used to compare the crude correlations between outcomes. Data were analyzed using SPSS version 18 (Chicago, IL, USA) considering P=0.05 level of significance.

RESULTS
Twenty-eight patients were evaluated in the group of TMD patients with pain; out of which 14 were females and 14 were males with a mean (±SD) age of 29.50±3.8 yrs. Twenty-eight other patients were evaluated in the group of TMD without pain; out of which 14 were males and 14 were females with a mean (±SD) age of 28.83±4.2 yrs.

The control group consisted of 28 healthy subjects with a mean (±SD) age of 28.56±3.9 yrs.; out of which 14 were males and 14 were females. Subjects in the three groups were matched in terms of smoking status, alcohol consumption and CAL. Table 1 summarizes the demographic characteristics of patients in the three groups. Statistical analyses of data showed that the mean plasma TAC was 0.8900 (±0.11627) mmol/L in TMD patients with pain, 1.2717 (±0.18711) mmol/L in TMD patients without pain and 1.7500 (±0.18711) mmol/L in the control group (Table 1).

The mean saliva TAC was 1.34 (±0.06721) mmol/L in TMD patients with pain, 1.42 (±0.16677) mmol/L in TMD patients without pain and 1.35 (±0.11627) mmol/L in the control group.

Table 1 summarizes the descriptive statistics of serum and saliva TAC in the three groups. ANOVA demonstrated that the difference among the three groups was statistically significant for plasma TAC (P<0.001) and insignificant for salivary TAC (P=0.117). Multiple comparisons by Tamhane’s test revealed that plasma TAC was significantly different among the three groups (P≤0.005). Also, analyses demonstrated that plasma TAC was not significantly correlated with age (P=0.830), sex (P=0.327), cigarette smoking (P=0.520), alcohol consumption (P=0.129) or CAL (P=0.901) but had a significant association with TMD (P<0.001). Salivary TAC had no significant correlation with any of the mentioned factors (P>0.05).

Moreover, among TMD patients with pain, 19 had clicking, 28 had tenderness of the muscles of mastication, 15 had mouth opening limitation and six had deviation. Of TMD patients without pain, 17 had clicking, 10 had tenderness of the masticatory muscles, eight had deviation and four had mouth opening limitation. The results showed an inverse relationship between the plasma TAC and the severity of pain, and the lower the plasma concentration of TAC, the more severe the pain. This association for salivary TAC was not significant.

The Spearman’s correlation test found no significant association between the plasma and salivary TAC. The mean (±SD) pain score in TMD patients with pain was 6.5±2.1.

DISCUSSION
This study showed that TMD patients with/without pain had significantly lower plasma TAC compared to the control group. But, salivary TAC was not significantly different among the three groups.

Table 1. Descriptive statistics (mean±SD) of plasma and salivary total antioxidant capacity (TAC)

| Groups                | Plasma TAC       | Saliva TAC     |
|-----------------------|------------------|----------------|
| Normal                | 1.750±0.187      | 1.350±0.116    |
| TMD without pain      | 1.271±0.169      | 1.420±0.167    |
| TMD with pain         | 0.890±0.116      | 1.340±0.067    |
| P value               | <0.001           | 0.117          |

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Lower TAC levels were associated with higher pain intensity. Decreased TAC in TMD patients with and without pain indicates oxidant/antioxidant imbalance due to the pain mechanisms. Although the pathological processes involved in TMJ dysfunction have yet to be fully understood, TAC may play a role in this respect. Previous studies have confirmed reduction of TAC in patients with acute pain or inflammation [6,21-23].

Cai et al, in their study on oxidation of free radicals and activity of SOD in synovial fluid of patients with TMJ disorders concluded that free oxygen radicals and antioxidant enzymes may be involved in the pathogenesis of TMD, which is in accord with our findings [24]. Guven et al. evaluated the activity of SOD in synovial fluid of patients with internal disorders of the TMJ. They reported that SOD activity progressively decreased by the aggravation of disease and concluded that decreased activity of SOD may be due to the insufficient elimination of free radicals, which is in agreement with our results [25].

Tomida et al. investigated the correlation of intra-articular oxidative state with the pathogenesis of TMD and showed that TMJ was influenced by the intra-articular OS. Moreover, they indicated a high correlation between TMD and number of oxidative factors and suggested that OS is responsible for development of TMD. These results confirm our findings. They also emphasized the role of inflammatory and molecular mediators present in synovial fluid in the pathogenesis and etiology of TMD [26]. Etoz et al. believed that a correlation may exist between the serum TAC and facial pain disorder and stated that decreased serum TAC may be responsible for pain in such patients [27].

The current study showed that TAC in the TMD group with pain was lower than that in the other two groups. This finding indicates that presence of inflammation and pain in the muscles or the TMJ results in higher production of free radicals in the site that initiate a cascade of inflammatory reactions in the joint or muscle. Previous studies have also confirmed that serum or salivary TAC decrease in patients with muscular or joint pains. Studies on the mechanism of chronic muscular pain suggest that regional progressive increase in oxidative metabolism especially in type I muscle fibers increases the byproducts of oxidative metabolism by draining the energy sources and subsequently stimulates peripheral pain receptors [16,7,28,29].

Rodriguez de Sotillo et al [18], in their pilot study showed significantly different OS biomarkers in TMD patients compared to the controls and stated that salivary biomarkers were reliable diagnostic predictors for pain intensity and were also correlated with higher serum levels of antioxidants. Higher levels of antioxidant markers were associated with higher pain scores. Decreased salivary and serum TAC were noted in TMD patients with pain. Their results regarding decreased serum TAC are in accord with ours. However, in contrast to them, we could not find any difference in saliva TAC of the three groups. Small sample size in their study may be responsible for lower accuracy of results and the mentioned difference.

Moreover, they did not match patients in terms of periodontal status, smoking status and alcohol consumption while these factors can affect saliva TAC. The role of inflammation and OS in TMD is being increasingly elucidated. In most cases of TMD, inflammation is present. The inflammatory process is a complex of biochemical pathways with the final goal of pain generation, movement limitation and initiation of a compensatory process. Evidence supports the association of serum levels of some mediators and OS with pain, chronicity, severity of degenerative changes and response to treatment [30]. Just like chemical antioxidants, cells are protected from OS by the interactions of a network of antioxidant enzymes [31,32]. Acute inflammatory processes include a cascade of mediators.
Active macrophages produce numerous cytokines mainly IL1 and TNF-alpha. Cytokines derived from macrophages further induce the vascular adhesion of endothelial cells through intercellular adhesion molecule-1 and E-Selectin.

This phenomenon enhances the migration of polymorphonuclear leukocytes to the inflamed tissue. Eventual tissue destruction depends on the production of free radicals and reactive oxygen species (ROS) by phagocytic cells namely neutrophils and macrophages. Free radicals and ROS have been indirectly detected in patients with symptomatic joints; OS pursues as the result of an imbalance between the production of ROS and the ability of the biologic system to detoxify them. Inability to quickly manage the situation leads to destruction mainly due to lipid peroxidation [33].

Mechanical stresses to the TMJ and muscles of mastication may lead to production of free radicals through several mechanisms and initiate a cascade of responses aggravating tissue damage, inflammation and pain. Factors neutralizing the free radicals may prevent this pathological process. In other words, antioxidants may have the potential for use in prevention of TMD.

CONCLUSION
Within the limitations of this study, we found that TMD patients with/without pain had significantly lower serum TAC compared to controls. But, no significant difference was detected in salivary TAC. Lower TAC was associated with higher intensity of pain. Limitation of this study: One limitation of our study was finding an accredited laboratory to obtain reliable saliva and serum test results. Three different labs and several kits were evaluated and the reliability and validity of the one, used in our study, were confirmed.

REFERENCES
1- Al-Belasy F, Dolwick M. Arthrocentesis for the treatment of temporomandibular joint closed lock: a review article. Int J Oral Maxillofac Surg. 2007 Sep;36(9):773-82.
2- Ueno T, Yamada M, Sugita Y, Ogawa T. Acetyl cysteine protects TMJ chondrocytes from oxidative stress. J Dent Res 2011 Mar;90(3): 353-9.
3- Richards RS, Roberts TK, McGregor NR, Dustan RH, Butt HL. Blood parameters indicative stress are associated with symptom expression in chronic fatigue syndrome. Redox Rep 2000;5(1):35-41.
4- Halliwell B, Gutteridge JM. Free radicals in biology and medicine; 1st Ed.; Oxford University. 2007:112-118.
5- Slavish DC, Graham-Engeland JE, Smyth JM, Engeland CG. Salivary markers of inflammation in response to acute stress. Brain Behav Immun 2015 Feb;44:253-69.
6- Sheets DW Jr, Okamoto T, Dijkgraaf LC, Milam SB, Schmitz JP, Zardeneta G. Free radical damage in facsimile synovium: correlation with adhesion formation in osteoarthritic TMJs. J Prosthodont 2006 Jan-Feb;15(1):9-19.
7- Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol.1990;186:421-31.
8- Christen Y. Oxidative stress and Alzheimer disease. Am J Clin Nutr. 2000 Feb;71(2): 621S-629S.
9- Nunomura A, Castellani RJ, Zhu X, Moreira PI, Perry G, Smith MA. Involvement of oxidative stress in Alzheimer disease. J Neuropathol Exp Neurol. 2006 Jul;65(7):631-41.
10- Hitchon CA, El-Gabalawy HS. Oxidation in rheumatoid arthritis. Arthritis Res Ther. 2004;6(6):265-78.
11- Azizi A, Farshchi F. Comparison of salivary and plasma antioxidant between lichen planus patients and healthy subjects. J Oral Pathol Med. 2012 Aug;41(7):524-6.
12- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39(1):44-84.
13- Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature. 2006 Dec 14;444 (7121): 875-80.
14- Khan MA, Chen HC, Wan XX, Tania M, Xu AH, Chen FZ, et al. Regulatory effects of resveratrol on antioxidant enzymes: A mechanism of growth inhibition and apoptosis induction in cancer cells. Mol Cells. 2013 Mar;35 (3): 219-25.
15- Kaneyama K, Segami N, Sato J, Yoshimura H, Nishiura R. Expression of receptor activator of nuclear factor-kappaB ligand in synovial tissue: comparison with degradation of articular cartilage in temporomandibular joint disorders. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Aug;104(2): e12-7.
16- Wang XD, Kou XX, Mao JJ, Gan YH, Zhou YH. Sustained inflammation induces degeneration of the temporomandibular joint. J Dent Res 2012 May;91(5):499-505.
17- Ozgocmen S, Ozurt H, Sogut S, Akyol O, Ardicoglu O, Yildizhan H. Antioxidant status, lipid peroxidation and nitric oxide in fibromyalgia: etiologic and therapeutic concerns. Rheumatol Int. 2006 May;26(7):598-603.
18- Rodriguez de Sotillo D, Velly AM, Hadley M, Fricton JR. Evidence of oxidative stress in temporomandibular disorders: a pilot study. J Oral Rehabil. 2011 Oct;38(10):722-8.
19- Canakci CF, Cicek Y, Yildirim A, Sezer U, Canakci V. Increased levels of 8-hydroxydeoxyguanosine and malondialdehyde and its relationship with antioxidant enzymes in saliva of periodontitis patients. Eur J Dent. 2009 Apr;3(2):100-6.
20- Shillinburg HT, Sather DA, Wilson EL. Fundamentals of fixed prosthodontics, Chicago, IL, Quintessence publishing, 2012: 6-7.
21- Abu-Hilal M, McPhail MJ, Marchand L, Johnson CD. Malondialdehyde and superoxide dismutase as potential markers of severity in acute pancreatitis. JOP. 2006 Mar 9;7(2):185-92.
22- Krupa W, Rozwodowska M, Sielski S, Czarnecka-Zaba E, Fabiszak T, Drewa G, et al. Influence of cardiac resynchronization therapy on oxidative stress markers in patients with chronic heart failure. Cardiol J. 2014;21 (5):576-82.
23- Chi CH, Shiesh SC, Lin XZ. Total antioxidant capacity and malondialdehyde in acute abdominal pain. Am J Emerg Med. 2002Mar; 20(2):79-82.
24- Cai HX, Luo JM, Long X, Li XD, Cheng Y. Free-radical oxidation and superoxide dismutase activity in synovial fluid of patients with temporomandibular disorders. J Orofac Pain. 2006 Winter;20(1):53-8.
25- Güven O, Tekin US, Durak I, Keller EE, Hatipoglu M. Superoxide dismutase activity in synovial fluids in patients with temporomandibular joint internal derangement. J Oral Maxillofac Surg. 2007 Oct;65(10):1940-3.
26- Tomida M, Ishimaru JI, Murayama K, Kajimoto T, Kurachi M, Era S, et al. Intrarticular oxidative state correlated with the pathogenesis of disorders of the temporomandibular joint. Br J Oral Maxillofac Surg. 2004 Oct;42(5):405-9.
27- Etoz OA, Ataoglu H, Erel O, Celik H, Herken EN, Bayazit YA. Association of serum total antioxidant capacity and total oxidant status with pain perception in patients with myofacial pain dysfunction. Int J Neurosci. 2009; 119(9):1282-91.
28- Stark TR, Perez CV, Okeson JP. Recurrent TMJ Dislocation managed with Botulinum Toxin Type A infections in a pediatric patient. Pediatr Dent 2015;37(1):65-9.
29- Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, et al. 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. Proc Natl Acad Sci U S A. 2007 Aug 14;104(33):13519-24.
30- Bouloux GF. Temporomandibular joint pain and synovial fluid analysis: a review of the literature. J Oral Maxillofac Surg. 2009
31- Lee MC, Kawai Y, Shoji H, Yoshino F, Miyazaki H, Kato H, et al. Evidence of reactive oxygen species generation in synovial fluid from patients with temporomandibular disease by electron spin resonance spectroscopy. Redox Rep 2004;9(6):331-6.

32- Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Herbal antioxidant in Clinical practice: a review. Asian Pac J Trop Biomed 2014 Jan;4(1):78-84.

33- Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med. 2001 Jun;30(11):1191-212.