

Total antioxidant capacity and total oxidant status of synovial fluids in patients with temporomandibular joint pain and dysfunction

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Abstract

Objectives The objective of this study was to investigate whether a relationship exists between total antioxidant capacity (TAC) and total oxidant status (TOS) of synovial fluids (SFs) of temporomandibular joint (TMJ) pain patients with pain and dysfunction.

Materials and methods Forty-two patients with TMJ pain were included in this study. TAC and TOS values of SFs were measured with a novel colorimetric method. Independent *t* test and correlations were used to analyze the data.

Results TAC of SFs in patients with TMJ pain and limited mouth opening (LMO; $n=21$) were significantly lower ($P=0.03$) than patients without LMO ($n=21$). TOS of SF was negatively correlated with duration of the disease. There was no correlation between TAC, TOS, and VAS scores of the patients as well as age and maximum mouth opening values.

Conclusions Antioxidant response to oxidative changes (TAC and TOS) in SF decreased as the stage of dysfunction increased.

Clinical relevance Local administration of antioxidant agents might be considered in management of TMJ pain and dysfunction to prevent possible increased oxidative stress.

Keywords Temporomandibular joint pain · Temporomandibular dysfunction · Total antioxidant capacity · Total oxidant status · Free radical

Introduction

Temporomandibular joint (TMJ) pain is a common condition which may be attributed to multiple factors including synovitis, capsulitis, anchored disk phenomenon, osteoarthritis, and internal derangements [1–3]. Osteoarthritic changes which lead to TMJ pain and dysfunction are characterized by hard tissue destruction which may also develop subsequent to untreated internal derangement of TMJ. In osteoarthritis, proteolytic enzymes cause degradation of cartilage which usually occurred subsequent to abnormal mechanical stresses. Inflammatory mediators and waste products are also possible causes of degeneration. In addition, aforementioned waste products can subsequently increase free radical expression [4]. Internal derangements of TMJ is defined as disturbance in the normal anatomic relationship between the disk and condyle which may cause pain and dysfunction [5]. Clinically, internal derangement is described by interference of joint function during mandibular movement. Joint noises, especially clicking may be present at the early stage of internal derangement. Anchored disk phenomenon has been described individually as the condition of sudden onset of persistent closed lock and pain which may occur without any sign of internal derangement [6]. Although the aforementioned entities are thought to be unique diseases, clinical challenge that mutually needs to be treated is pain and/or dysfunction and therapeutic cascade varies between patient education, anti-inflammatory medications, splint therapy, arthrocentesis, and surgical

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interventions depending on the severity of illness and patient response to treatment [1, 7].

Current concepts reveal that TMJ pain and dysfunction is strongly dependent on adaptive capacity of patients rather than the position of the disk [8, 9]. Mainly, pressure changes, contents of synovial fluid (SF), and biochemical changes are thought to play role on the pathogenesis of TMJ pain [9–11]. However the molecular events that underlie TMJ diseases are poorly understood. Recently, biochemical analyses of the contents of SF offer new concepts with regard to the pathophysiology of TMJ diseases. SF of the TMJ has been analyzed for the attendance of various mediators and free radicals that may be used as markers of joint disease [10–14]. It has been reported that the source of inflammation within TMJ which may subsequently cause pain and dysfunction may be related with accumulation of free radicals [2].

The expression oxidative stress is adopted to describe any condition that results in an accumulation of free radicals which are deactivated by molecules known as antioxidants [11]. Oxidative stress is also claimed to have a major effect in processes such as osteoarthritis, disk displacement, internal derangement, and others [15].

Various antioxidants can be measured separately within various body fluids; however, their antioxidant effects would be additive and measurements would be time consuming, labor intensive, and not practical. For this reason, total antioxidant capacity (TAC) which is also referred to as total antioxidant activity, and total oxidant status (TOS) which is also named as total peroxide, serum oxidation activity, or oxygen metabolites can be measured. A standard hydrogen peroxide solution is oxidized with free radicals and an increased yellow-brown color is achieved. Subsequently, antioxidants in the sample suppress the oxidation and color formation. This reaction is monitored by spectrophotometry and TAC is measured. Similar to TAC measurement, oxidization of ferrous iron with oxidants in the sample causes a calculable color change allowing TOS measurement [16–22]. The aim of this study was to investigate whether a relationship exists between TAC and TOS values of synovial fluids of patients with localized TMJ pain.

Patients and methods

Patients with complaints of TMJ pain and/or dysfunction were included in this study. This study was approved by the local ethic committee of Erciyes University Faculty of Dentistry and informed consent was obtained from subjects before the procedure. TMJ pain of the patients was evaluated with visual analogue scale (VAS) of 0 to 100 mm, which

was designed as 0 being no pain and 100 being the worst pain ever experienced [23]. Maximum voluntary interincisal mouth openings (MMO) of the patients and the duration of the symptoms were recorded as well. Patients receiving therapy for neurological and/or psychiatric disorder, and previous history of interventional TMJ management and/or patients with major bony deformities or any other diseases like osteoarthritis, fibromyalgia, rheumatoid arthritis, and painful masticatory muscles were excluded. Based on clinical and radiographic examinations (panoramic radiographs and lateral TMJ scans), 42 patients with localized TMJ pain in whom conservative treatment modalities (patient education, anti-inflammatory medication, and physical treatment modalities) failed (34 females and 8 males, mean age=31.0±13.9) were included in this study. All patients received the same medications (Table 1). Patients with TMJ pain and limited mouth opening (ranged between 19–32 mm), jaw deviation to the affected side during mouth opening, and history of previous clicking were classified as TMJ pain and dysfunction (TMJPD) group and patients with TMJ pain and clicking without limited mouth opening (>35 mm) were classified as TMJ pain (TMJP) group. TMJ pain may exist without any sign of dysfunction as well as it may usually cause limited mouth opening which has been defined as less than 36 mm MMO [24]. Management of the patients was performed by the first author (OAE). All patients underwent TMJ arthrocentesis which is known as the initial intervention for TMJ pain for patients who does not respond conservative treatment [25]. TMJ arthrocentesis procedure was performed according to Nitzan [26] under local anesthesia. Before arthrocentesis, SFs of the patients were collected by ten times injection–ejection process of 2 ml of sterile saline solution into the upper joint compartment of TMJ after subcutaneous local anesthesia with Articain HCL with 0.005 mg/ml epinephrine (Maxicaine, VEM, Ankara, Turkey). SFs of the patients were centrifuged under 4,000 rpm for 5 min and stored at –80 until biochemical analysis.

Table 1 Data of conservative treatment protocol before arthrocentesis

	Number	Anti-inflammatory drugs (3 weeks)	Physical therapy and instructions (3 weeks)
TMJPD	21	Naproxen sodium (275 mg/day)	Soft diet + self-traction mouth opening exercises + moist warm application
TMJP	21	Naproxen sodium (275 mg/day)	Soft diet + moist warm application

TMJPD temporomandibular joint pain and dysfunction, *TMJP* temporomandibular joint pain

Measurement of TAC and TOS of the SFs were measured using a new colorimetric method, which was introduced by Erel [27, 28]. Results were expressed as millimolar Trolox equivalent per liter for TAC and micromolar hydrogen peroxide equivalent per liter for TOS.

The results were expressed in mean ± standard deviation. Independent *t* test and Pearson's correlation were used for statistical analysis (SPSS 11.0, SPSS Inc., Chicago, IL, USA). A *P* value of less than 0.05 is considered to be significant.

Results

Twenty-one of the patients had TMJPD while the remaining 21 had TMJP. The mean MMO value of the patients with TMJPD was 26.9±4.2 mm while the mean MMO value of the patients with TMJP was 41±4.8 mm. TAC and TOS values of the subjects were given in the Table 2. There was no significant difference between patients with TMJPD and TMJP in terms of TOS levels of SF. However TAC of SF was significantly reduced in patients with TMJPD (Table 2). There was no correlation between VAS scores of the patients and TAC and TOS of SFs. In addition, VAS scores of the females were significantly higher than males and TAC of SFs in female patients was significantly lower (Table 3). There was also no correlation between the levels of TAC and TOS. However there was a significant negative correlation between TOS and duration of the symptoms of the patients (*r*=−0.33, *P*=0.03, Fig. 1)

Discussion

The oxidant/antioxidant imbalance in pathogenesis of TMJ and related diseases has been implicated in many studies [2, 29–32]. Free radicals in normal TMJs may not lead to a pathologic state if endogenous free radical scavenging mechanisms prevent their accumulation. However, if scavenging competence of affected articular tissues is exceeded

Table 2 TAC and TOS values of the subjects

	Number	TAC	TOS	MMO (mm)	VAS (mm)
TMJPD	21	0.34±0.1	1.16±0.2	26.9±4.2	65.1±24.7
TMJP	21	0.48±0.2	1.16±0.3	41.0±4.8	66.6±30.0
<i>P</i>		0.03	0.63	0.001	0.86

TAC total antioxidant capacity (was measured as micromolar Trolox equivalent per liter), *TOS* total oxidant status (was measured as micromolar H₂O₂ equivalent per liter), *OSI* oxidative stress index, *MMO* maximum mouth opening, *VAS* visual analogue scale, *TMJPD* temporomandibular joint pain and dysfunction, *TMJP* temporomandibular joint pain

Table 3 VAS scores of the patients and TAC and TOS of SFs

	Number	TAC	TOS	VAS (mm)
Female	34	0.38±0.2	1.16±0.3	70.1±24
Male	8	0.53±0.1	1.15±0.1	47.8±30
<i>P</i>		0.05	0.14	0.03

TAC total antioxidant capacity (was measured as micromolar Trolox equivalent per liter), *TOS* total oxidant status (was measured as micromolar H₂O₂ equivalent per liter), *VAS* visual analogue scale

by an overpowering production of free radicals, distinct tissue damage could occur. Free radicals can promote chronic inflammation, leading to more tissue damage and pain [2].

Milam et al. [2] proposed that oxidative stress which is induced by free radicals might initiate degenerative changes in TMJ. They reported that free radicals in healthy TMJs would not lead to TMJ disease as long as increased oxidative stress is avoided by antioxidant enzymes. Hence possible tissue damage within TMJ is dependent on the local scavenging capacity of articular tissues against free radicals [2]. It has also been reported that increased oxidative stress caused by free radicals in the affected TMJ impairs local antioxidant defense deteriorates normal lubrication of TMJ (15). Sumii et al. [30] found increased activity of superoxide dismutase (SOD) which is an important antioxidant enzyme in the SF from patients with rheumatoid arthritis than healthy control subjects. Similarly Cai et al. [31] reported that SOD levels were significantly higher in patients with TMJ diseases than in healthy control subjects. The rise of SOD in affected TMJs might be thought as excessive response to increased oxidative stress. Conversely, Güven et al. [9] reported that the activity of SOD seemed progressively reduced as the stage of the disease increased. The authors mentioned that reduction of SOD activity might be related with insufficient scavenging capacity of free radicals. Several antioxidant enzymes may exist within SF and other body fluids and measuring certain antioxidants separately might be the reason of conflicting findings of aforementioned studies. Additionally, controversial findings of the studies with regard to antioxidant enzymes in SF might be related with the variable range of oxidative changes and antioxidant response as well.

Previously increased TOS and reduced TAC of serum were reported in patients with myofascial pain [32]. It has also been reported that plasma TOS value was significantly lower in patients with osteoporosis [33]. Same authors have also found a significant correlation between TOS and bone mineral density. Similarly Altindag et al. [34] found significant relationship between reduced TAC and knee osteoarthritis and mentioned possible relationship between hard tissue destruction and TOS. However the abovementioned studies are concerned with serum or plasma analyses and

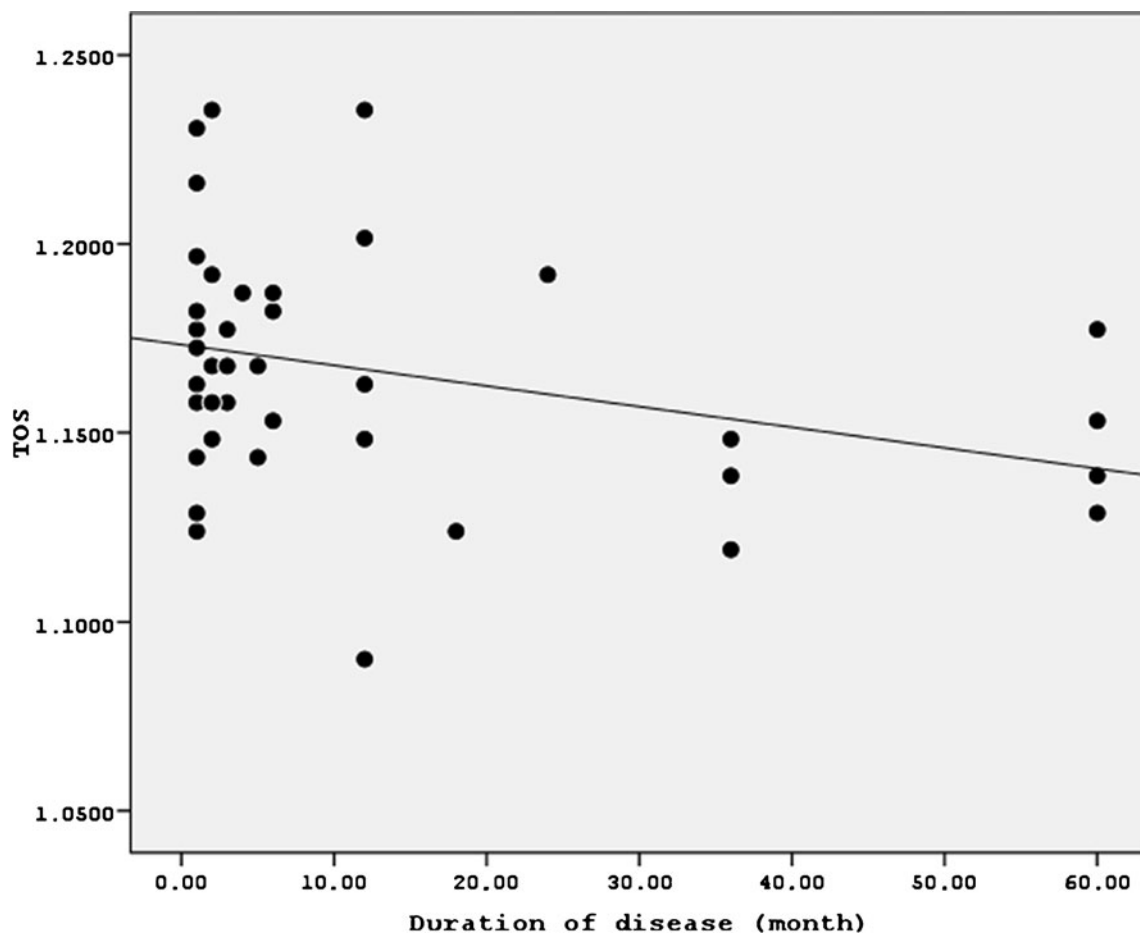


Fig. 1 There was a significant negative correlation between total oxidant status (TOS, in micromolar H₂O₂ equivalent per liter) and duration of disease ($r=-0.33$, $p=0.03$)

results of the studies might be affected by general condition of subjects. The present study is the first in which it was aimed to analyze SF of patients with TMJ pain and/or dysfunction in terms of TOS and TAC. For this aim, we collected SFs of the patients prior to arthrocentesis which has been known as a favorable therapeutic option for many patients with TMJ pain and/or dysfunction. We found that TOS of SFs significantly reduced in the course of disease. This finding supports the knowledge that oxidative stress is decreased by scavenging mechanisms of free radicals by antioxidant response and occurrence of disease might strongly be dependent on antioxidant capacity of TMJ. In addition TAC of SF in patients with TMJPD was significantly lower than TMJP. However TOS levels of patients with TMJPD and TMJP were similar. Local antioxidant defense seemed to be decreased as the stage of clinical condition worsened and antioxidant capacity of SF might be considered as the determining factor rather than increased oxidative stress. The present study might be considered as a preliminary report in which it can be concluded that local antioxidant capacity is strongly related with dysfunction

rather than pain. Although demographic features of the patients in this study might also be associated with internal derangements of TMJ (TMJPD group—irreducible disk displacement, TMJP group—reducible disk displacement), according to the results of the present study, we may not be able to conclude on the exact relationship between the type of TMJ disorder (reducible/irreducible disk displacement or anchored disk phenomenon, fibrous adhesion, and synovial chondromatosis which has overlapping symptoms with other TMJ diseases [35]) and severity of total antioxidant/oxidant imbalance in SF as lack of magnetic resonance imaging scans of the study group. It is also not clear whether lower antioxidant response is a result or cause as the present study did not comprise longitudinal assessments and SF analyses of healthy control subjects was not provided due to ethical concerns

In addition, TAC of SF in females was lower than males. Although this finding was not statistically significant, this might be a descriptive data to understand why TMJ disorders are more common (and also VAS scores are significantly higher) among females.

Within the limitations of the present study, local oxidative changes are thought to play a role in the pathogenesis of TMJ pain and dysfunction. Further studies should be performed to analyze the exact effect of antioxidants in certain TMJ disorders and local treatment modalities to improve antioxidant capacity of SF might be considered in management of TMJ diseases.

Conflicts of interest The authors declare that they have no conflict of interest.

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