Oxidative stress biomarkers versus Magnetic Resonance Imaging (MRI) assessment in patients with tempromandibular disorders

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Abstract: TMJ pain is a significant part of the symptoms in patients with TMJ disorders and a common source of orofacial pain. Interests in the recognition and management of TMD have increased dramatically in the past few years. Mechanical stresses are generated during functional or parafunctional movements of the jaw, adaptive mechanisms of the TMJ may be exceeded by free radical accumulation leading to a dysfunctional state. Ten patients selected with symptoms of TMJ pain, joint noises, limitation of jaw opening, tenderness located in the articular region and six healthy volunteers served as controls. Patients had full or nearly full complement of natural teeth. Clinical and radiographic (MRI) examination was done to determine disc displacement and success of treatment. Arthrocentesis (lavage) TMJ of TMD patients was carried out. Oxidative stress biomarkers of SOD, MDA and GSH of synovial fluid of TMD patients was measured. Our results revealed the success rate of improvement of sign and symptoms of joint tenderness,clicking and the range of mandibular movement before and after arthrocentesis. Oxidative stress of SOD and MDA was elevated while GSH was diminished. Based on these results we concluded that Oxidative stress are linked in the pathogenesis of the temporomandibular joint disorders. The antioxidant agents might be considered in management of TMJ pain and dysfunction to prevent possible increased oxidative stress.
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1. Introduction

Temporomandibular disorders (TMDs) represent a major field in dental and oral medicine. Temporomandibular disorder is a collective term used to describe a group of signs and symptoms involving the temporomandibular joints (TMJs), masticatory muscles, and associated facial structures. Interests in the recognition and management of TMD have increased dramatically in the past few years. Disorders of TMJ have correlation to several factors including dental occlusion,stress and psychosocial elements thereby necessitating a multidisciplinary approach for management and assessement.(1)

TMJ pain is a significant part of the symptoms in patients with TMJ disorders and a common source of orofacial pain. However, we have little knowledge about the pathophysiologic mechanism that underlies the development of the local pain in the TMJ. Temporomandibular joint (TMJ) is a complex, sensitive, and highly mobile joint that regulates the movement of the jaw.(2,3,4). TMJ is anatomically structured to withstand loading during mastication due to its mechanism of stress absorption and energy dissipation. The cancellous bone of the mandibular condyle can resist compressive and tensile deformations during loading of the TMJ with minimum amount of bone mass due to its plate-like trabecular structure.(5,6)

Excessive loading and the subsequent biomechanical imbalance are suggested as major factors in destructive and degenerative changes of joint components(6). The disc displacement induces the change of stress distribution in the disc and the increase of frictional coefficients between articular surfaces, resulting in the secondary tissue damage. The TMJ can adapt to changing biomechanical stresses, However, this adaptability may be adversely affected by several factors including advanced age, tissue perturbations caused by previous traumatic injury, enhanced sympathetic tone and hormonal influences. Mechanical stresses are generated during functional or parafunctional movements of the jaw, adaptive mechanisms of the TMJ may be exceeded by free radical accumulation leading to a dysfunctional state (i.e., disease state). Free radicals are very reactive and unstable molecular fragments that have an unpaired electron and they can produce new free radicals by means of chain reactions. Anterior disc displacement is one of the most common features of internal derangement in individuals with TMJD, the posterior part of the synovium in the TMJ with anterior disc displacement is thought to be subjected to direct mechanical compressive force during chewing, resulting in inflammation (7 -10)

Although oxygen freee radicals participate in many physiological processes,they can be harmful to tissues when their action left uncontrolled.The most

common source of free radicles in biological system is oxygen. The elevation of reactive oxygen species (ROS) lead to oxidative stress that causes molecular damage to vital structures and functions. ROS are generated on a regular basis in biological pathways as by products or as signal transducers However, excessive production or inefficient scavenging of ROS can cause over accumulation, which can injure or kill cells. All basic molecules in living organisms can be attacked by ROS, e.g., lipids, carbohydrates, proteins, and nucleic acids.(11 -13)

The unsaturated fatty acid of cell membrane lipids are susceptible to peroxidative reaction. Lipid peroxidation of cell membrane has been implicated in a wide range of tissue injuries and diseases. Accumulation of lipid hydroperoxides in a membrane disrupts its function and causes it to collapse and have range of cytotoxic radicals. The most series of which are the aldehydes. They may also react with transition metals like iron or copper to form stable aldehydes such as malondialdehyde (MAD) that will damage cell membranes Because hemoglobin constitutes the largest iron store in the body, it is speculated to be a potential source of redox active iron which can catalyze the formation of free radicals that might be damaging to the joint. (14).

Antioxidant defense mechanisms involve both enzymatic and non enzymatic strategies. Common antioxidants include the vitamins A, C, and E, glutathione, and the enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. They work in synergy with each other and against different types of free radicals. Antioxidant enzymes in living organisms have evolved as very sophisticated and effective scavengers of ROS. Superoxide dismutase (SOD) is an essential antioxidant enzyme protecting many cellular components by converting two superoxide anions into a molecule of hydrogen peroxide and one of oxygen. SODs are found in many organisms including all oxygen-consuming organisms. (15)

The aim of this study was designed to measure the levels of oxidative stress marker of Glutathione (GSH), Malondialdehyde (MPA),and Superoxide dismutase (SOD) in the synovial fluid of TMJs of patients with TMD.

2. Materials and methods:

This study included 10 patients selected from the prosthodontic clinics of King Abdulaziz University, Faculty of Dentistry (KAUFD) with symptoms of TMJ pain, joint noises, limitation of jaw opening, tenderness located in the articular region and six healthy volunteers served as controls. Their age ranged from 25-45 years. Patients had full or nearly full complement of natural teeth.

Clinical examination was done by measuring the range of mandibular movement (maximium mouth opening MMO, lateral and protrusive movement) and hearing sound when opening or closing the mandible, tenderness on palpation of TMJ and muscles. (16) Pain score was assessed from 0-3 respectively according to Hirota (17). Diagnostic imaging (panoramic radiograph and magnetic resonance imaging (MRI)was done). MRI was used to determine the disk derangement. All patients were treated by arthrocentesis. The estimation of oxidative stress markers in the the synovial fluid (SF) of the superior compartment of TMJ in TMDS patients was done. Clear hard acrylic mandibular anterior repositioning splint was constructed for TMD patients, the splint was inserted after 7days of arthrocentesis The splint was tried in the patient's mouth while closing in the forward position.. All adjustments were performed with simultaneous contact in the stabilized forward position. The patients were instructed to wear the appliance continually all the times and were allowed to discontinue only during brushing their teeth and during meal. The patients were seen at weekly intervals for necessary adjustment of the occlusal splint.

Before treatment all the patients were briefly informed about the study protocol. Informed Consent was obtained from each patient in order to participation in the study according to Research Ethics Committee at the Faculty of Dentistry, King Abdulaziz University.

Arthrocentesis technique and collection of SF sample (18,19)

Arthrocentesis was done under strict aseptic technique. Two points were marked over to skin of the affected joint indicating the arthicular fossa and eminence (Fig.1). This was followed by 10 ml of lidocaine (10 mg/ ml) local anesthesia, injected to block auriculotemporal nerve posterior to condylar neck and to infiltrate the subcutaneous tissues lateral to the joint. After local anesthesia, 2 ml of normal saline solution was injected to superior joint space.The patient was asked to open and close mouth to mix the saline with synovial fluid. Samples were obtained by washing the joint cavity with saline using push-and-pull technique performed with two syringes; one used for washing the solution that was injected and the other for aspiration. About 14.5 ml diluted SF was collected. The fluid and cellular components were immediately separated by centrifugation (2000) rpm for 10 minutes) and the supernatant was transferred to polypropylene tubes and stored at -20°C until used. Oxidative stress biomarker analysis of the aspirated synovial fluid from the superior joint space was done.

A final evaluation was done before and after eight weeks (two months) of treatment. Evaluation of the

occlusion in the final position without the splint was done by occlusal adjustment.

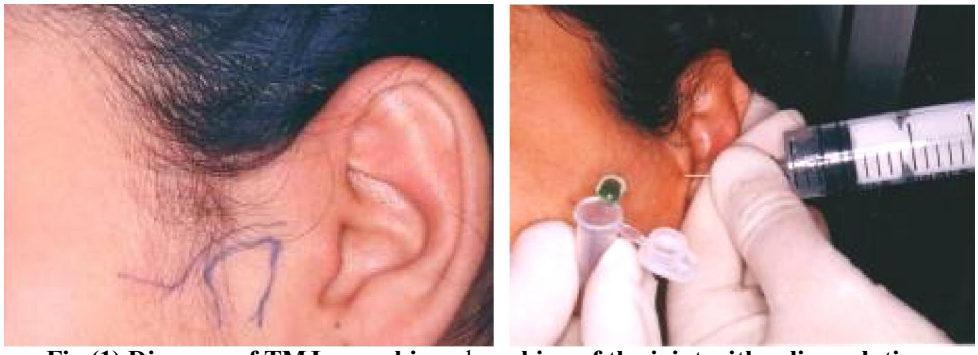


Fig.(1) Diagram of TMJ over skin and **washing of the joint with saline solution**

Magnetic Resonance Imaging (MRI) Assessment

MRI was performed twice for all patients as follow. MRI was performed with 1.5 Tesla systems (MRI Philips) Gyroscan* with TMJ surface coil (6.0 cm in diameter). The scan parameter was adapted (TR 450, E20 FOV 130/1.7). The TMJ was scanned for detection of disc erosion, confirming disc displacement and grading of this displacement. The scanning was done with closed and open mouth for both right and left joints. Rescanning of the patient after two months from starting of the treatment was performed with mandibular repositioning appliance.

We considered the grading of severity of the TMJ as follow: less than half the disc displaced anteriorly to the anterior turbercle of glenoid fossa (grade I)), all the disc displaced (grade III), Less than full disc displacement (grade II).

The criteria of disc capture was used to assess the success of our treatment methods; (1) complete splint capture, (2) partial splint capture, (3) no splint capture (20).. Erosion of the disc was searched for and tabled.

Estimation of Oxidative stress parameters in the synovial fluid:

Protein quantification

Protein-containing solutions were quantified using a bicinchoninic acid (BCA) protein assay kit (Pierce), according to instructions provided by the manufacturer.

-Thiobarbituric Acid Test:

The concentration of MDA, a marker of lipid peroxidation, was assessed in samples by means of the thiobarbituric acid (TBA) reaction using the method of Rowley. (21).

-Assay of SOD Activity

SOD activity was determined by using a commercially available kit (Sigma-Aldrich). The assay was used to measure total SOD (Cu/Zn-, Mn-, and Fe-SOD) in the samples.

- GSH Assay:

To determine intracellular levels of reduced glutathione (GSH), cells were lysed with protein lysis buffer. GSH levels were determined using the Glutathione assay kit (Biochain) following the manufacturer's instructions.

Statistical analysis:

Data was collected and analyzed. The Statistical Package for the Social Science software (version 16.0) was used for all analyses (SPSS, Chicago, IL, USA).Descriptive statistical as mean and standard deviation were used. Mann Whitney test and Wilcoxon matched pair rank test was used for comparison between finding before &after arthrocentesis of TMD subjects,while Student t-test was used for comparing the means of the control group and TMD subjects.

3. Results:

Tables (1,2 and 3) showed assessment of clinical examination of TMD patients as regard tenderness, click, pain and mandibular movement were done before arthrocentesis and then eight weeks posteoperatively.

Table (1): The success rate of improvement of signs and symptoms of joint tenderness and clicking of TMD patients.

Assessment criteria	Preoperative No=10	Postoperative 2 months
Lateral joint tenderness	9	8
Clicking	9	7

Before arthrocentesis nine patients had joint tenderness, eight patients had improvement (8/10), while one patients had no improvement, 9/10 patients showed sound joint (clicking) and marked improvement on seven patients 7/10 was observed (Table 1).

Table (2): Comparison of pain score in selected patient before and after arthrocentesis.

	Pain score							
	Extreme= 3		Moderate= 2		Little= 1		NO= 0	
	Selected patients	%	Selected patients	%	Selected patients	%	Selected patients	%
Before arthrocentesis	10	100%	_	_	_	_	_	_
After 24 hours of arthrocentesis	3	30%	4	40%	_	_	_	_
After 1 week of (day of insertion of splint)	3	30%	4	40%	_	_	_	_

Before arthrocentesis (lavage) extreme pain was predominant in all selected patients, while after 24 hours of lavage 30% of the selected patients were complaining of extreme pain and 40% were complaining of moderate pain. After one week of lavage all patients were not suffering from any pain (Table 2).

Table (3): Range of mandibular movement (mms) before and after arthrocentesis.

Parameter	Before arthrocentesis	At follow up 2 months
Maximum mouth opening(MMO)		
Mean ± SD	30.75 ± 0.79	36.44 ± 0.81
P	<0.001*	
Lateral movement in mms		
Mean ± SD	3.33 ± 0.08	5.53 ± 0.19
P	<0.001*	
Protrustive movement in mms		
Mean ± SD	3.46 ± 0.21	4.15 ± 0.09
P	<0.001*	

p: *p* value for Mann Whitney test *: Statistically significant at $p \leq 0.05$

The mean of MMO, the lateral movement and the protrusive movement measurement (mm.) were increased after two months follow up than before lavage. A statistically significant difference was found after two months follow up periods at 5% level (Table 3).

Table (4): Comparison between the two studied groups according to serum SOD (U/mg), MDA (nmol/mg) and GSH (mmol/mg) protein parameter

	Control (n = 6)	TMD patients (n = 10)	*p*
GSH			
Mean ± SD	0.62 ± 0.01	0.39 ± 0.02	<0.001*
MDA			
Mean ± SD	0.06 ± 0.04	0.11 ± 0.03	<0.030*
SOD			
Mean ± SD	4.98 ± 0.45	9.09 ± 0.39	<0.001*

p: *p* value for Student t-test for comparing between the two studied group *: Statistically significant at $p \leq 0.05$

The mean value of serum SOD, MDA and GSH in the synovial fluid of TMDs patients were showed in (Table 4). A statistical significant difference was observed in the serum SOD and MDA in TMD patients and the control group.(p <0.001) while the serum level of GSH was statistically significant decreased.

Table 5: MRI assessment of TMD patients anesthesia.(16)

Patients number	Before arthrocentesis	After two months of arthrocentesis
1	Grade I	Complete repositioning
2	Grade I	Complete repositioning
3	Grade II	Complete repositioning
4	Grade II	Complete repositioning
5	Grade II	Complete repositioning
6	Grade II	Complete repositioning
7	Grade II	Grade I
8	Grade III	Grade III
9	Grade III	Grade III
10	Grade III	Grade III
P1	2.43*	

P1: Wilcoxon matched pair rank test for comparison between finding before lavage &after two months in each group

Before lavage the finding of MRI showed two patients showed grade I (Fig.2) and five patients showed grade II (Fig. 3) and three patients showed grade III(Fig. 3). After two months follow up two patient showed complete repositioning (complete disc capture). In grade II five patients showed improvement (complete repositioning) with the splint appliance. The last three patients showed grade III before and after lavage and no change was noticed (no disc capture). (Table V). There was a significant difference between MRI before lavage and after two months follow up at 5% level (P1=2.43)

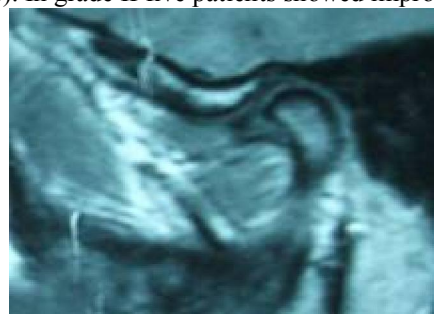


Fig. (2)Complete splint capture (grade I)

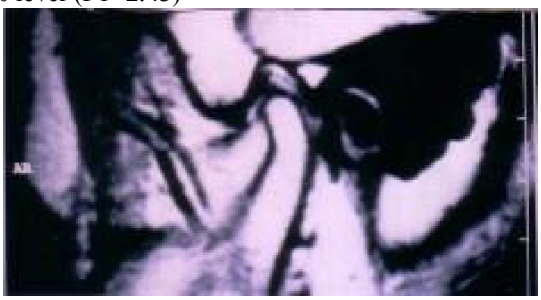
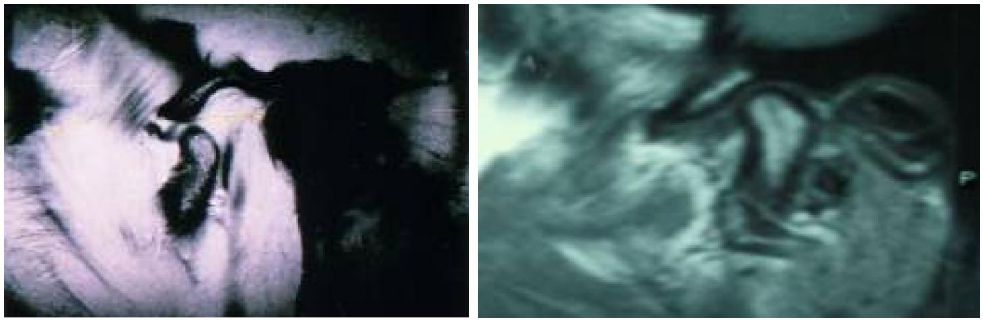


Fig.(3)Partial disc displacement (grade II),partial disc capture



Grade III disc displacement and erosion)no disc capture)

4. Discussion:

The most common manifestation of TMD patients was pain in the TMJ that might be due to an alteration of the disc-condyle relation. Limited and irregular mandibular movements and joint sounds were also observed. Combined therapies have been proposed in the management of symptoms of TMD. This interdisciplinary approach is necessary because of the multifactorial aspect of these problems.

TMJ fluid analysis (SF) was used in this study because it is a method of great future potential to obtain information about local joint pathology. It may help to develop more specific diagnostic and prognostic tools to identify markers of disease.(22) Arthrocentesis is a safe procedure, less invasive

modality and less costly than arthroscopy or open surgery. Arthrocentesis procedure has been of much interest to clinicians because of its relative high success as well as its unique treatment manner, it is a borderline procedure between nonsurgical and surgical intervention and can be performed under local anesthesia.(16)

A significant increase in the measurement of mandibular movement may be attributed to wearing mandibular repositioning splint that reduces the muscle hyperactivity by forward repositioning of the mandible thus keeping a normal disc- condyle-glenoid fossa relationship. The mandibular repositioning splint also prevented clicking resulting from jumping of the condyle below the posterior margin of the anteriorly displaced disc (20).

Extreme pain was decreased in intensity after lavage. This results might be due to the hypothesis of lavage procedure removes inflammatory agents. It is an effective treatment for alleviation of TMJ pain dysfunction in the pathologic joint fluid followed by elimination of the vacuum cap effect in the locking joint. The efficacy of lavage of upper compartment of the TMJ will improve the translation of the disc along the eminence allowing the condyle to complete its natural path and decreased or eliminate pain in the joint (without relocation of the disc) through reduction of the joint loading. ((23,24)

The MRI assessment was used in this study to assess disc capture because high incidence of false negative results was judged by clinical examination. MRI is very fast, curative single screening method to assess the success of the treatment. The clinical and MRI findings before and after arthrocentesis treatment were matched together, patients with grade I was cured clinically and manifested complete disc capture, while patients with grade II were changed to grade I which can be cured by lavage and anterior mandibular repositioning splint. The patients with erosions showed the least improvement by both methods of treatment (arthrocentesis or disc repositioning appliance). These clinical and MRI results were in agreement with the results of Kurita. (1998). (20)

Many researches report that inflammatory reaction may lead to internal derangement. The recent advances in technology enabled us to use biochemical substances recovered from synovial fluid (SF) to diagnose the intra-articular pathology associated with ID.(2) But the recent researches found that mechanical injury, or stresses lead to the accumulation of damaging free radicals in affected articular tissues that damaged the cellular and extracellular molecules Oxygen free radicals and oxidative stress may be participate in the pathologic changes in TMD. (25,26,27)

Oxidative stress in TMDs is evidenced by the elevated levels of MDA, and SOD and the reduced concentration of GSH observed in the synovial fluid of TMJs. It can be postulated from these results that there is a direct relation between inflammation associated with TMD and the increased production of free radicals that characterizes oxidative stress. The increase of (MDA) levels enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms (GSH) to prevent formation of excessive free radicals. Glutathione (GSH) is a non enzymic antioxidant essential for removal of free radical species such as hydrogen peroxide, superoxide radicals, and maintenance of membrane protein. Therefore, an antioxidant therapy retarding the proliferation of reactive molecular species might be useful in decreasing inflammation that accompanies this condition. The overall effect of antioxidants is to slow down the progression of TMD. (28,29,30)

Conclusion:

Oxidative stress is linked to the pathogenesis of temporomandibular joint disorders. The antioxidant agents might be considered in management of TMJ pain and dysfunction to prevent possible increased oxidative stress.

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