Study of the Plasmatic Oxidative Stress Markers in Temporomandibular Joint Disorders

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Oxidative stress is considered to have an important role in the pathogenesis of many inflammatory diseases. This investigation sought to determine whether the appliance of etiologic factors for temporomandibular joint disorder is associated with modified values of oxidative stress as measured by biomarkers in plasma. A case-control study design was used to compare oxidative stress biomarkers (malondialdehyde and glutathione) in plasma from Wistar rats with different etiologic factors (biomechanical stress, estrogen hormones and emotional stress), compared to non-temporomandibular joint controls. Etiologic factors were applied in five groups (n=10) of mature female Wistar rats, individually (biomechanical stress, estrogen hormones and emotional stress) in three groups or in combination for two groups (biomechanical stress-emotional stress and biomechanical stress-emotional stress). No factor applied for the control group. Blood samples were taken after 30 and 60 days of experiment. Malondialdehyde and glutathione levels in plasma were evaluated. The most important modifications in malondialdehyde levels were recorded for the fifth group (biomechanical stress-emotional stress) where values increased significantly from 30 to 60 days plasma analysis (p = 0.003). No statistical differences were recorded in glutathione plasmatic levels between control and experimental groups at 30 days analysis. At 60 days, glutathione plasmatic evaluation recorded higher values than control for all experimental groups. Our findings in this rat experimental model, clearly indicate that biomechanical stress, estrogen hormones and emotional stress, have an impact on oxidative stress development and consequently on the functionality of the temporomandibular joint in rats. Moreover combined actions of these factors, increase the oxidative stress phenomena on temporomandibular joint in rats.

Keywords: biomechanical stress, emotional stress, estrogens hormones, oxidative markers, temporomandibular joint, free radicals

Oxidative stress is caused by the release of free radicals in concentrations that overwhelm the natural scavenging mechanism of the antioxidant defense system and initiates processes involved in the pathogenesis of many inflammatory diseases. Accumulation of excess free radicals in tissue, contributes to pathologic conditions by damaging extracellular and intracellular molecules, as well as excessive activation of cellular processes, such as extracellular matrix turnover, DNA damage, protein denaturation, or lipid oxidation [1-3].

Several studies have been published regarding the presence of oxidative stress phenomena during temporomandibular joint disorders development. Oxidative stress can be generated in the temporomandibular joint, by several pathways which include direct mechanical injury, hypoxia-reperfusion, and arachidonic acid catabolism [4-10].

Oxidative stress affect various molecular species of the temporomandibular joint and deteriorate the articular function, including reduction of the synovial fluid viscosity by depolymerization and/or molecular configuration of hyaluronic acid, reduction of lubrication of the articular surface by deterioration of the surface-active phospholipids layer, which acts as an extremely efficient boundary lubricant and protector of articular surfaces, breakdown of collagen proteoglycans, activation of cartilage degrading enzymes such as matrix metalloproteinases [5, 6, 9, 10].

The etiological factors involved in temporomandibular disorders are biomechanical stress, estrogen hormones and emotional stress. The exact implication of each factor has not yet been established.

In the temporomandibular joint trauma, mechanical stress, disc derangements and degenerative changes can cause the release of free radicals resulting in oxidative stress and an imbalance in resultant biomarkers [7]. We know very little about the characteristics of mechanical stresses generated in human temporomandibular joint with the jaw movement. When the intra-articular pressure goes up above 40 mmHg, it surpasses the peripheral arteriolar pressure and can cause temporary hypoxia followed by reoxygenation on cessation of clenching. [11, 12]. It is certainly reasonable to speculate that, physical stresses impacting vascular tissues of the temporomandibular joint under abnormal conditions (i.e., articular disc displacement), could greatly exceed in magnitude the end capillary perfusion pressures of these tissues. Therefore, the hypoxia-reperfusion model could be feasible for free radicals/reactive oxygen species production, on the basis of inadequate intra-articular pressures. With increasing hypoxia, local cell populations undergo a shift in metabolism that can lead to the production of free radicals, when perfusion is reestablished [12].

Psychological stress also plays a major role in temporomandibular disorders development. Research findings have supported a relationship between anxiety, muscular tension and temporomandibular disorder symptoms [13]. Laskin was the first to suggest that the main factor responsible for temporomandibular disorders

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is the emotional, instead of the physical aspect [14]. During the last decade, numerous investigations have been devoted to understand the relationship between psychological stress and temporomandibular disorder. Patients suffering from this condition, report that their symptoms increases during stressful situations [15-17]. De Leeuw et al. [18] consider that muscle dysfunction and accompanying pain are very often, the result of stress induced by the muscular hyperactivity. Stress-induced muscular dysfunction may induce secondary changes in the temporomandibular joint and accumulation of free radicals/oxidative stress.

It has been postulated that, estrogen could be the primary molecule leading to the higher frequency of temporomandibular joint disorders in women. Endogenous estrogen affects the remodeling processes within the temporomandibular joint, possibly by changing the extracellular matrix in the joint, or by changing bone volume [19, 20]. Such changes can result in internal disorders of the temporomandibular joint. Estrogen modulation of matrix metalloproteinases expression, has also been observed in the cartilage of the temporomandibular joint, high levels of matrix metalloproteinases have been reported to play a role in temporomandibular joint osteoarthritis [21, 22]. Moreover, the level of estrogen in the synovial fluid in animal models has been shown to modulate inflammation in the temporomandibular joint [23, 24].

During inflammation condition in the tissue, the overproduction of free radicals/reactive oxygen species occurs and this results in oxidative stress in the cells. The unsaturated fatty acids of the cell membrane are major targets for free radicals/oxygen reactive species, which result in disruption of membrane function. Lipid oxidation aspect of free radicals/oxygen reactive species activity is detected by the measurement of lipid peroxidation products such as malondialdehyde. To overcome the toxic effects of oxidative stress, the tissues have both enzymatic and non-enzymatic mechanisms. The glutathione is one of the most important endogenous antioxidants and provides a sulfhydryl group, for direct scavenging reactions by glutathione S-transferase and glutathione peroxidase [25].

This investigation sought to determine whether the appliance of etiologic factors for temporomandibular disorders was associated with modified values of oxidative stress as measured by biomarkers in plasma. The null hypothesis is that there is no relationship between biomarkers of oxidative stress and the presence of certain etiologic factors. Thus, a case-control study design was used to compare oxidative stress biomarkers (malondialdehide and glutathione) in plasma from Wistar rats with different etiologic factors, compared to non temporomandibular joint controls.

Experimental part

Sixty white adult female Wistar rats (weight 150g) were divided in six study groups. Three groups were exposed separately to an etiological factor for temporomandibular disorders: biomechanical stress, overdose of estrogen hormones or emotional stress. Two groups were exposed to a combination of two factors (biomechanical stress-estrogen hormones and biomecanichal stress- emotional stress). The control group received no etiologic factor. The experiment lasted for 60 days.

Biomechanical stress was induced by metal crowns in occlusal interference cemented on first mandibular right molar.

In order to control the amount of estrogen hormones in the general circulation, ovariectomy was performed in the study groups which received an estrogen hormone overdose (estrogen hormones and biomechanical stress-estrogen hormones groups) calculated on the basis of the bodyweight of each animal (50μg/kg normal dose, 80μg/kg overdose, values based on previous research) [26]. Hormonal manipulation was performed by daily injection of 17β-estradiol. Hormones were dissolved in propylene glycol.

Emotional stress was induced by a ringing bell for 10 min every hour (100 decibels) [27].

During the period of study, animals were maintained in a temperature-controlled room (±23ºC) and were housed in plastic cages with soft bedding on a 12/12 light cycle, with food and water available ad libitum.

Blood samples were taken after 30 and 60 days of experiment. Malondialdehide and glutathione levels in plasma were evaluated. For malondialdehide determination, a fluorometric method based on the reaction between malondialdehide and thiobarbituric acid was used. Briefly, 50μL of plasma was added to 1 mL of 10 mmol/L diethylthiobarbituric acid reagent in a phosphate buffer (0.1 mol/L, *p*H 3). The mixture was mixed for five seconds and incubated for 60 min., at 95°C. Samples were placed on ice for five minutes, and then 5 mL of butanol was added. The mixture was shaken for one minutes to extract the diethylthiobarbituric acid-malondialdehide adduct and then centrifuged at 1500 × *g* for 10 min at 4°C. Fluorescence of the butanol extract was measured at an excitation wavelength of 539 nm and emission wavelength of 553 nm. Malondialdehide concentration is established based on a calibration curve realized with known malondialdehide concentrations evaluated in the same way. Values were expressed in *p*g/mL [28].

Glutathione dosage by flourescence is based on the evaluation of a fluorescent product obtained by the reaction with o-phtalaldehyde. A volume of plasma is combined with thiobarbituric acid 10%, after 10 min of mixing a phosphate buffer (*p*H = 8) and o-phtalaldehyda are added. 15 minutes later the intensity of the emission is evaluated at wave lenght 420 nm and excitation wave lenght of 350 nm. Glutathione concentration is established based on a calibration curve realized with known glutathione concentrations evaluated in the same way. Values were expressed in *p*g/mL [29].

Statistical analysis of data was performed using SPSS statistical software. Validation of data was verified using repeated measures ANOVA test. The level of significance was set at $P < 0.05$.

The experimental procedures were carried out in accordance with the mandatory principles of the ethical committee of the academic research activity [30, 31].

Results and discussions

Plasmatic malondialdehide levels at 30 days for biomechanical stress group (mean 7.216848±0.6616420) were significantly higher than the control group ($p < 0.001$). The values continued to increase until the end of the experiment (60 days-mean: 7.663564±0.9070764, $p < 0.005$).

In the estrogen hormones group, malondialdehide showed statistical significantly higher levels both at 30 and 60 days comparing to the control group. Increased values of malondialdehide were also recorded for the emotional stress group at the end of experimental period (mean 7.931940±1.5827200, $p < 0.001$; table 1).

Table 1

MALONDIALDEHIDE PLASMATIC VALUES AT 30 AND 60 DAYS EXPERIMENTAL PERIOD

Descriptive statistics		Group 0 Control	Group 1 Biomechanical stress	Group 2 Estrogen hormones	Group 3 Emotional stress	Group 4 Biomechanical stress-estrogen hormones	Group 5 Biomechanical stress-emotional stress
Malon-dialdehide 30 days	Mean	3.405636	7.216848	6.748416	7.210032	7.120972	6.721904
	Std.dev.	0.323635	0.661642	1.987225	3.023547	1.343900	1.045136
Malon-dialdehide 60 days	Mean	3.409036	7.663564	8.146952	7.931940	8.213508	8.340716
	Std.dev.	0.324361	0.907076	0.498663	1.582720	0.625094	1.217968

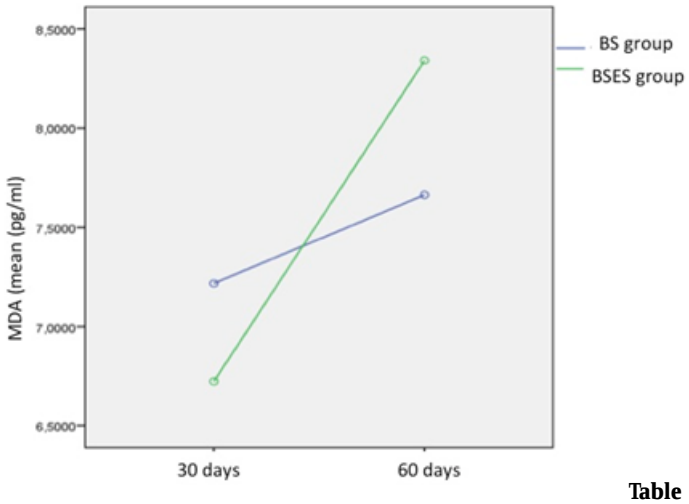

Fig. 1. Evolution of malondialdehide levels (MDA) in biomechanical stress group (blue-BS) and biomechanical stress-emotional stress group (green-BSES). Although there are no statistical differences between the groups at 30 or 60 days, the malondialdehide values increased significantly more for biomechanical stress-emotional stress group comparing to the biomechanical stress group (p = 0.020)

Table 2

GLUTATHIONE PLASMATIC VALUES AT 30 AND 60 DAYS EXPERIMENTAL PERIOD

Descriptive Statistics		Group 0 Control	Group 1 Biomechanical stress	Group 2 Estrogen hormones	Group 3 Emotional stress	Group 4 Biomechanical stress-estrogen hormones	Group 5 Biomechanical stress-emotional stress
Glutathione 30 days	Mean	4.795000	5.239000	4.617000	5.485000	4.705000	5.74000
	Std.dev.	0.819931	0.840650	0.546976	1.157665	0.558391	1.83992
Glutathione 60 days	Mean	5.152000	7.105000	7.582000	5.701000	10.86000	7.06000
	Std.dev.	0.585722	2.738522	1.913928	1.315068	0.79213	1.33848

In the fourth group with associated etiological factors (biomechanical stress-estrogen hormones), malondialdehide was significantly higher than the control group, both at 30 days ($p < 0.001$) and 60 days ($p < 0.001$). The most important modifications in malondialdehide levels, were recorded for the fifth group (biomechanical stress-estrogen hormones) where values increased significantly from 30 to 60 days plasma analysis ($p = 0.003$) (fig. 1).

No statistical differences were recorded in glutathione plasmatic levels between control and experimental groups at 30 days analysis (table 2).

From 30 days to the end of research period glutathione values registered a significant ascendant pathway for biomechanical stress-estrogen hormones group ($p < 0.001$) and biomechanical stress-estrogen hormones group ($p = 0.018$).

At 60 days glutathione plasmatic evaluation recorded higher values than control for all experimental groups. The differences were statistically significant for biomechanical stress-estrogen hormones group ($p< 0.001$) and biomechanical stress-estrogen hormones group ($p = 0.017$).

Plasmatic oxidative stress markers evaluations showed higher values in experimental groups than control, both in 30 days and at the end of experiment.

A study conducted by Fleifel and Alkhiary [32] communicated the evidence of a direct relation between inflammation associated with temporomandibular disorders and the increased production of free radicals that characterizes oxidative stress. The increase of malondialdehide levels enhanced lipid peroxidation, leading to tissue damages. Our results are similar, showing increased values for malondialdehide in all experimental groups, both at 30 and 60 days.

The possible role of biomechanical stress has been stated by numerous studies [11-14]. In our research, for the biomechanical stress group, we determined increased levels of plasmatic oxidative stress markers at 30 days. Values increase even more to the end of experimental period, due to the continuous action of this etiologic factor. Emotional stress and estrogen hormones, have also been considered as etiologic factors for temporomandibular disorders by certain authors [13, 15-24]. We have obtained similar results in oxidative stress markers for estrogen

hormones, emotional stress or biomechanical stress groups, suggesting that we can consider it as possible etiologic factors for temporomandibular disorders.

The absence of statistical differences between the groups at 30 days for glutathione analysis signifies the initial failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. However form 30 to 60 days increasing values of glutathione, were observed for all experimental groups comparing to control, suggesting the effort of organism to remove free radical species such as hydrogen peroxide, superoxide radicals, and maintenance of membrane protein.

The associated action of biomechanical stress and estrogen hormones or biomechanical stress and emotional stress has determinate increased values of oxigen species markers comparing to the individual factor alone. This suggests that temporomandibular disorders development is a consequence of combined actions of these possible etiologic factors.

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

There are several limitations to our experimental model. First because of the involvement of multiple factors and the complexity of underlying mechanisms, it is difficult to extrapolate these results to other models. As with all animal models, there are difficulties in the transition to humans and clinical care. Second because all evaluations were realized from plasma and other co-existing general conditions might influence the results [33-38].

Conclusions

Our findings in this experimental model, clearly indicate that biomechanical stress, estrogen hormones and emotional stress have an impact on oxidative stress development and consequently on the functionality of the temporomandibular joint in rats. Moreover combined actions of these factors increase the oxygen species phenomena on temporomandibular joint. However, further studies with more quantitative methods and other animal models should be done, so that these results may eventually find a clinical application, regarding temporomandibular disorders treatment using antioxidant products.

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