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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Salivary cortisol responses to acute stress in students with myofascial pain

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SUMMARY

Introduction/Objective Temporomandibular disorders (TMD) are characterized by the appearance of musculoskeletal pain and dysfunction of the masticatory system.
The aims of this study were to evaluate the salivary cortisol levels in students with chronic myofascial pain (MFP) related to TMD during oral exam, as well as to analyze the correlation between salivary cortisol levels, TMD-related MFP, the level of anxiety, depression symptoms, somatization, and perceived stress.
Methods The study included 60 university students, who were allocated either into the group of students with MFP (n = 30) or into the control group of healthy students (n = 30). The level of salivary cortisol was measured on the exam day and during the control day when the students had no exams. Depression symptoms, somatization, perceived stress and anxiety were evaluated according to Axis II RDC/TMD, Perceived Stress Scale and State–Trait Anxiety Inventory.
Results Levels of salivary cortisol were significantly higher in the group of students with MFP in all phases of measurements compared to the control group ($p < 0.01$). Students with MFP also showed significantly higher depression symptoms, somatization, and trait anxiety scores than the control group. No significant group differences were found on the scales measuring state anxiety and perceived stress. The level of salivary cortisol was found to be in correlation with depression symptoms, state anxiety, and perceived stress, but not with chronic pain, somatization, and trait anxiety in students with TMD.
Conclusion Salivary cortisol could be an important indicator of psychological distress in TMD.
Keywords: temporomandibular disorders; saliva, chemistry; hydrocortisone, metabolism; stress, metabolism

INTRODUCTION

Temporomandibular disorders (TMD) represent a set of musculoskeletal disorders embracing a number of clinical problems that involve masticatory muscles and/or temporomandibular joints. The most common symptom is myofascial pain (MFP) exacerbated by mandibular movement and stomatognathic functions [1]. An integrated approach that covers the whole biopsychosocial spectrum is needed to enhance TMD-related pain treatment and prevention [2].

Chronic TMD shares many common features and often co-exists with other syndromes such as fibromyalgia, chronic fatigue syndrome, irritable bowel syndrome, migraine, and dysmenorrhea, leading to the suggestion that it is part of a spectrum of disorders, mainly psychologically determined. It is interesting to notice that these functional disorders tend not only to cumulatively affect an individual, but also to present central sensitization and amplified pain perception. Central sensitization may be influenced by the autonomic nervous system and might lead to pain despite the absence of pathologies or peripheral pain stimuli [1, 3]. Several studies revealed that TMD patients experience depression and anxiety more often compared to healthy individuals and highlighted that suffering from depression and anxiety increases the risk of feeling joint and muscle pain [4–9].

The etiology of TMD has been extensively studied and is considered multifactorial. In addition to genetic association, deleterious body posture, bruxism, occlusal features, hormonal changes, various external stimuli such as trauma and stress (acute or persistent) have been temporary associated with the development of TMD [3, 10]. Psychosocial stressors are considered to play a significant role in the development of masticatory muscle pain [11, 12], and patients with TMD commonly report that their pain increases during stressful situations [1, 7, 11, 13]. The relationship established between stress and MFP could be explained by the greater contraction of masticatory muscles, since muscle hyperactivity is one of the most frequent mechanisms influencing MFP [1, 14].

Dysfunction of hypothalamic-pituitary-adrenal (HPA) axis plays an important role in pathophysiology of TMD. The repeated exposure

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to stressful stimulation leads to rapid habituation of HPA axis responses [15, 16]. Severe pain may induce excess stimulation of HPA axis, causing elevated serum cortisol concentration. On the other hand, serum cortisol levels may be low if some components of the HPA axis diminish over time due to exorbitant stimulation [17]. Hereupon, cortisol has been used as an indicator of stress in research [18, 19]. The salivary cortisol evaluation provides measurement of unbound cortisol compared to serum, while collecting saliva is quite stress-free and does not require any special training, environmental conditions or sterility. Although several studies have investigated the levels of cortisol following exposure to acute psychosocial stressor in patients with chronic MFP [5, 20], the knowledge on how alterations in HPA axis lead to the response to acute stressors in patients with chronic MFP related to TMD is still limited.

As noted earlier, many studies have reported changes in daytime cortisol levels, but only a few have investigated the levels of cortisol following the exposure to acute psychosocial stress in patients with chronic pain related to TMD [5, 20]. The advantage of laboratory stressors is the very standardized stress induction; however, it may lack external validity and is questionable whether the stress reactions induced by certain experimental standardized stressor represent real-life stress reactions. Hereupon, academic exams have often been used in stress research because they are standardized and discrete examples of a real-life stressor [21]. Undergoing academic exams has been associated with changes in mental and physical health studies, which suggests that academic examination stress can have a significant impact on a student's overall well-being [22].

The primary objective of this study was to evaluate salivary cortisol levels in students with chronic MFP related to TMD during oral exams. The secondary objective was to analyze the correlation between salivary cortisol levels and TMD-related MFP, the level of anxiety, depression symptoms, somatization, and perceived stress.

METHODS

Respondents and setting

This study was conducted after approval by the Ethics Committee of the Faculty of Medicine in Foča, University of East Sarajevo (Code No.116/15). At the beginning of the study, 620 students of medicine and dentistry were invited to the Department of Oral Rehabilitation for TMD screening. The screening was performed by two researchers using a brief questionnaire on difficulties during chewing, swallowing, opening and closing the mouth, and experiencing MFP. The students with positive screening results were invited back to the Department for further clinical evaluation. The TMD was diagnosed using the Research Diagnostic Criteria for TMD (RDC-TMD) Axis I, group I. The students with the presence of MFP according to a proposed diagnostic classification and criteria were allocated into the study group. Those who were wearing any intraoral appliance, taking any muscle-relaxing medication, having painful joint sounds, joint arthralgia or osteoarthritis, disc displacement or pain upon digital palpation of the lateral pole of the right or left condyle, and students using other treatment modalities, were excluded from the study group, as well as female students who had their menstrual cycle at the time of research. After the exclusion criteria were applied, the study group consisted of 30 students with MFP. The control group included 30 healthy students matched in age and gender, who did not have previous history of MFP symptoms or other TMD. All the students were informed about the objective of the study, and their written informed consent was sought and obtained.

The study took place in 2015 and was conducted in accordance with the World Medical Association Declaration of Helsinki, as revised in 2008.

Experimental protocol

An oral exam was used as the trigger for acute stress. The students underwent the oral exam in Physiology at the end of the summer semester (June or July). The examiner was opposite the students. The students were instructed not to take any pharmacological agents such as oral contraceptives, beta blockers, benzodiazepines or analgesics prior to the exam. The exams started between 2 PM and 5 PM (when salivary cortisol levels are considered to be stable on the basis of circadian rhythm) and lasted for at least 30 minutes.

The students were asked not to chew gum, eat or drink any liquids except water two hours prior to the sampling of saliva. Unstimulated salivary samples from all the students were collected just before (T1) and directly after (T2) the oral exam. At the time of sampling T2, the students were not informed about their exam results. Thirty days after the exam, the students were asked to collect two additional saliva samples. The first control sample was taken at the time which coincided with the estimated time of the oral exam in Physiology (T3) and the second control sample was collected 30 minutes afterwards (coinciding with the end of the exam) (T4). The students were instructed to rest and avoid stressful events prior to T3 and T4 sampling.

Salivary cortisol measurement

The salivary cortisol was collected using a Salivette (Sarstedt Inc., Rommelsdorf, Germany). The students were instructed about saliva sampling using salivate tubes containing a polyester wool swab. Students chewed the swab for up to three minutes, and put the soaked swab into the tube. Swabs soaked with saliva were centrifuged at 1,800 rpm for 20 minutes. (within 15 to 30 minutes after sampling) and immediately frozen at -20°C. Salivary cortisol level was measured (μg/dl) using a commercially available enzyme-linked immunosorbent assay – ELISA (IBL, Hamburg, Germany). Analyses were carried out at the Biochemistry Department, Foča University Hospital. Correct sampling was controlled by one of the researchers (NI or ĐB).

Assessment of psychological factors

Before the exam, the students were asked to complete several questionnaires. Testing for chronic MFP was performed in accordance with the RDC/TMD, the dual-axis diagnostic procedure developed by Dworkin and LeResche [23]. The Axis II involved depression symptoms and somatization measurements as well as the Graded Chronic Pain Scale.

To evaluate the anxiety, we used State–Trait Anxiety Inventory (STAI) [24]**.** This questionnaire has two subscales. The State Anxiety Scale evaluated the current state of anxiety, asking how respondents feel "right now," using items that measure subjective feelings of apprehension and activation of the autonomic nervous system. The second subscale, the Trait Anxiety Scale included relatively stable aspects of proneness to anxiety, including calmness, confidence, and security. The STAI has 40 items, 20 items allocated to each of the subscales. Responses for the State Anxiety scale assessed intensity of current feelings at the current moment with the responses ranging from "not at all" to "almost always."

The Perceived Stress Scale (PSS) was used to assess the degree to which situations in a respondent's life are appraised as stressful [25]. The questions were designed to measure how unpredictable, uncontrollable, and overloaded respondents find their lives and also to measure current levels of experienced stress.

Data analysis

Statistical analyses were carried out using the SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software. Tests of differences between groups were carried out using the Mann–Whitney U-test. The comparison between the levels of salivary cortisol at each time point in the same group was performed using the Wilcoxon test. Nonparametric tests were used due to ordinal-scaled values. Relationships between the psychological variables and the levels of salivary cortisol were examined by Spearman's correlation coefficients. P-value < 0.05 was considered statistically significant.

RESULTS

Salivary cortisol concentrations

Distribution of students did not significantly differ between the groups according to age (mean = 19.2, range = 18–20 in the study group; vs. mean = 19.5, range = 18–21 in the control group) and gender (six men and 24 women per group).

As shown in Figure 1, the levels of salivary cortisol in the study group were significantly elevated after the oral exam, T2 (2.8 μg/dl), compared to the levels measured before the exam, T1 (1.3 μg/dl), during the first control, T3 (0.2 μg/dl), and the second control measurement, T4 (0.2 μg/dl). A statistical difference was observed between T1 and T3 measurements ($p < 0.001$). However, no significant difference was observed in the salivary cortisol level between the first and the second control measurements ($p = 0.457$).

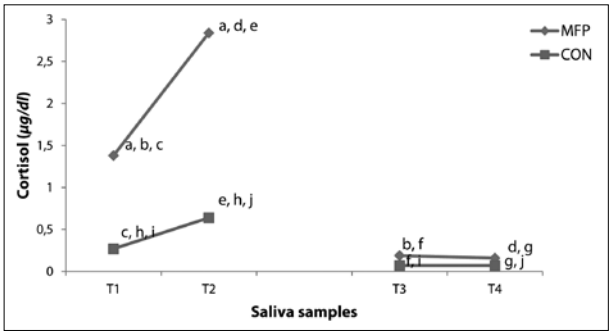

Figure 1. Salivary cortisol levels in students with myofascial pain and controls during oral examination and control day; data are presented as the mean value; $^{a,b,d,h,i,j}p < 0.01$ – significant main effect within groups for levels of salivary cortisol; $^{c,e,f,g}p < 0.01$ – significant main effect between groups for levels of salivary cortisol; T1 – saliva sample before the oral examination; T2 – saliva sample after the oral examination; T3 – the first control saliva samples (thirty days after the exam – coincides with T1); T4 – the second control saliva samples (thirty days after the exam – coincide with T2)

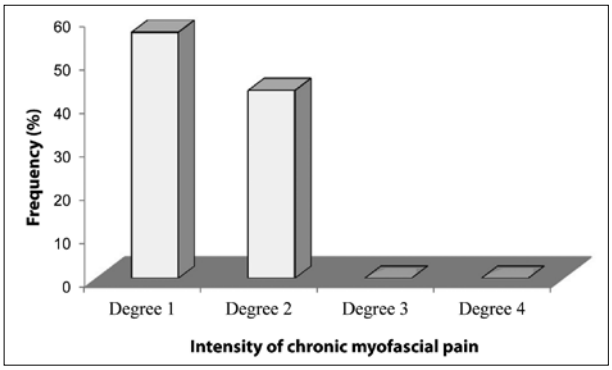

Figure 2. Frequency of the university students with TMD according to intensity of chronic myofascial pain (n = 30)

Similar results were observed in the control group. The levels of salivary cortisol were found to be significantly statistically higher after the exam, T2 (0.6 μg/dl) compared to the level of salivary cortisol before the exam, T1 (0.3 μg/dl), during the first control, T3 (0.1 μg/dl) and the second control measurement, T4 (0.1 μg/dl). The levels of salivary cortisol were also higher before the exam compared to the T3 measurement ($p < 0.001$). No significant differences were observed between the first and the second control measurements ($p = 0.538$).

The levels of salivary cortisol were found to be significantly higher in the study group compared to the control group in T1 ($p = 0.001$), T2 ($p = 0.004$), T3 ($p < 0.001$), and T4 ($p = 0.001$) (Figure 1).

Pain and psychological variables

According to the RDC/TMD, the intensity of chronic pain in the study group was classified as degree 1 in 56.7% (n = 17) and as degree 2 in 43.3% (n = 13) of the students. None of the students had either III or IV grade of MFP (Figure 2).

Table 1. Psychological characteristics of students with myofascial pain (MFP group) and controls

Questionnaire scale		MFP group (n = 30)	Control group (n = 30)
		Mean SD	Mean SD
RDC/TMD	Depression Somatization	1.3* ± 0.7 1.1** ± 0.9	0.9 ± 0.7 0.5 ± 0.5
STAI	A-trait A-state	3.3** ± 0.6 2 ± 0.7	2.7 ± 0.8 1.8 ± 0.4
PSS	Stress	1.8 ± 0.6	1.9 ± 0.3

*p < 0.05, **p < 0.01
RDC/TMD – Research Diagnostic Criteria for Temporomandibular Disorders; STAI – State Trait Anxiety Inventory; PSS – Perceived Stress Scale

The mean scores of depression symptoms, somatization, anxiety, and perceived stress level assessment are presented in Table 1. Students with MFP reported higher depression symptoms (p = 0.044) and somatization (p = 0.008) scores compared to healthy students. Based on the results obtained from Spielberger's trait anxiety inventory, 60% of students with MFP reported anxiety, mostly of a high level, while 40% of students in the control group reported moderate levels of anxiety. Hence, a statistically significant difference was observed in the presentation of trait anxiety between the two groups (p = 0.008). No significant differences between the two groups were observed on the scales measuring state anxiety (p = 0.158) and perceived stress (p = 0.688).

Correlations between salivary cortisol and psychological variables

Statistical analysis failed to show any significant correlation between the degree of chronic pain and salivary cortisol response in the students with MFP (r = -0.004, p > 0.05).

In the same group, a positive correlation was found between salivary cortisol and the following variables: perceived stress (r = 0.396, p = 0.030), depression symptoms (r = 0.366, p = 0.047), and state anxiety (r = 0.666, p = 0.001), but there was no statistically significant correlation between salivary cortisol and somatization (r = 0.248, p = 0.186) and trait anxiety (r = 0.162, p = 0.392) in this group.

Statistically significant, positive correlation was found between cortisol levels and perceived stress (r = 0.381, p = 0.038) in the control group. However, no significant correlation was found between salivary cortisol and depression symptoms (r = 0.120, p = 0.527), somatization (r = 0.278, p = 0.124) and trait anxiety (r = -0.134, p = 0.480).

DISCUSSION

The current study showed that the levels of salivary cortisol after exams were higher compared to other measurements in both groups. The measurements before and after oral exam as well as two control measurements of salivary cortisol were significantly higher in the group of students with MFP compared to the control group. The level of salivary cortisol was found to be in correlation with psychological factors in students with TMD, but not with the control group.

These results are in accordance with another study, which found an increased cortisol level in response to an experimental stress protocol in a subset of patients with disc-related symptoms [20]. The larger increase in the level of cortisol in students with MFP compared to healthy students could be explained by the fact that MFP represents an important stimulus to HPA axis activation [12]. However, it is noteworthy that students with MFP had grade I and II of pain and that the pain did not correlate with cortisol levels as measured in this study, so it is unclear whether the observed HPA axis abnormalities in students with MFP reflect a preexisting vulnerability to functional pain disorders as a response to other psychological factors [26].

Psychological factors have been associated with TMD and may be a component of its clinical presentation [27]. The MFP students reported higher levels of depression symptoms, trait anxiety, and somatization compared with students without MFP, which is in accordance with other studies [28]. The psychological factors might explain why only a small percentage of people are troubled by MFP related to TMD and why just a small number of symptomatic individuals seek treatment [13].

The study has shown that cortisol responses to acute stressors did parallel subjectively perceived stress, without any statistical difference in the perceived stress level being found between students with and without MFP. This is not in accordance with studies showing statistically significant difference between these groups at several measures of psychosocial stress, suggesting that psychosocial stress plays an important role in etiopathogenesis of TMD [29]. Stress can profoundly affect the pain transmission processes and perception, so inappropriate adaptation responses could act as stressors themselves [30]. It has been proposed that reduced hippocampal volumes may be a predisposition to the maladaptive stress response and allostatic load, in individuals showing more stress vulnerability, when facing prolonged pain [31].

Jasim et al. [32] reported that patients with chronic MFP show significantly higher scores of depression symptoms, somatization and perceived stress compared to patients with acute pain. In the current study, the differences in anxiety and depression levels were considered clinically significant rather than just statistically significant. The positive correlation between cortisol level and self-reported depression symptoms and trait anxiety in the MFP group, as well as the lack of any significant correlation between these variables in the control group, could indicate that there are links between physiological and psychological factors. Although it is difficult to determine if low mood represents a causal component or is a consequence of a chronic pain condition, the data do support recommendations toward treating the ongoing symptoms of depression itself [33].

It was proved that anxiety is positively associated with the process of temporal somatization, which suggests that anxiety might contribute to central pain processing [6]. Yoshihara et al. [5] found that state-anxiety levels, increased plasmatic cortisol levels, adrenalin, and noradrenaline significantly correlated after psychologically-induced stress in patients with myofascial pain; however, such

correlations were not found in healthy individuals [5]. Results suggest that trait anxiety levels might be associated with greater sensitivity in the HPA axis and sympathetic adrenal medullary system in patients with MFP [5].

The current study has several limitations. Sample size was small and the respondents were students who did not consult the doctor about the MFP. More experimental research, with larger groups, and in particular prospective longitudinal studies, is needed to further elucidate the role of HPA axis activity in the process of chronic MFP development in young adults.

CONCLUSION

The current study shows that salivary cortisol could be an important indicator of psychological distress. Although significant correlation was not found between MFP and salivary cortisol, higher salivary cortisol levels were found among the students with TMD who reported higher scores of depression symptoms, state anxiety, and perceived stress. The results indicate that TMD occur due to interaction of physiological and psychological factors and that salivary cortisol plays an important role in TMD development. Integrated biopsychosocial, patient-oriented approach to diagnosis and treatment of patients with TMD-related pain and associated symptoms is required.

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Ниво кортизола у пљувачки у акутном стресу код студената са миофасцијалним болом

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САЖЕТАК

Увод/Циљ Темпоромандибуларне дисфункције (ТМД) одликују се појавом мускулоскелетног бола и дисфункцијом мастикаторног система.

Циљеви ове студије су били да се утврди ниво саливарног кортизола код студената са хроничним миофасцијалним болом (МФБ) повезаним са ТМД за време усменог испита, као и да се анализира корелација између нивоа саливарног кортизола, МФБ повезаног са ТМД, анксиозности, депресивних симптома, соматизације и доживљеног стреса.

Методе Студија је обухватила 60 студената, који су распоређени или у групу студената са МФБ (n = 30) или у контролну групу здравих студената (n = 30). Ниво саливарног кортизола је мерен на дан испита, као и за време контролног дана када студенти нису имали испите. Симптоми депресије, соматизације, доживљеног стреса и анксиозности испитивани су према *Axis II RDC/TMD*, Скали доживљеног стреса и Инвентару тренутне и опште анксиозности.

Резултати Нивои саливарног кортизола били су знатно већи код групе студената са МФБ у свим фазама мерења у поређењу са контролном групом ($p < 0,01$). Студенти са МФБ су такође показали више симптома депресије, соматизације и опште анксиозности него контролна група. Мерењем тренутне анксиозности и доживљеног стреса није пронађена знатна разлика у резултатима међу групама. Ниво саливарног кортизола био је у корелацији са симптомима депресије, тренутном анксиозношћу и доживљеним стресом, али не и са хроничним болом, соматизацијом и општом анксиозношћу код студената са ТМД.

Закључак Саливарни кортизол може бити важан показатељ психолошког дистреса у ТМД.

Кључне речи: темпоромандибуларна обољења; пљувачка; кортизол; стрес; метаболизам