Original Article

Relationship of Occupational Stress and Temporomandibular Disorders by Measuring Salivary Cortisol and Immunoglobulin Levels in Iranian Dental Students

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Abstract

Background and Aim: The present study aimed to investigate the relationship between stress and temporomandibular disorders (TMDs) by quantification of occupational stress using the Dental Environment Stress (DES) questionnaire as well as measuring the stress markers in the saliva, including cortisol and immunoglobulin A (IgA), in fourth to sixth-year dental students of Qom Dental School.

Materials and Methods: In the present study, 60 students were included. A clinical examination was first performed using the Research Diagnostic Criteria/Temporomandibular Disorder (RDC/TMD) criteria in order to determine the temporomandibular joint (TMJ) status, and then the DES questionnaire was completed in order to determine the occupational stress level of dental students. Finally, saliva samples were obtained from the students, and the levels of salivary cortisol and IgA were measured using enzyme-linked immunosorbent assay (ELISA). Independent t-test, ANOVA, Chi-square test, and Pearson's correlation coefficient were used for data analysis (alpha=0.05).

Results: The mean salivary levels of cortisol and IgA were found to be 4.74 ± 2.80 ng/mL and 220.26 ± 121.22 µg/mL, respectively. The mean DES score was 2.48 ± 0.58. Of all students, 24 (40%) suffered from TMDs. There was no correlation between cortisol and IgA levels and the DES score (P>0.05). No significant relationship was found between TMD and salivary cortisol, salivary IgA, or DES score (P>0.05).

Conclusion: Stress is one of the most important causes of TMDs. Although salivary biomarkers are known as useful markers for detection of stress in patients with TMDs, this issue was not confirmed in the present study.

Key Words: Occupational Stress; Temporomandibular Joint Disorders; Saliva; Cortisol; Immunoglobulin A, Secretory

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Introduction

Temporomandibular disorders (TMDs) are among the most common patient complaints, and about 40-60% of the population have at least one significant symptom related to TMDs (1). TMD is a term that includes a number of clinical complaints related to the masticatory muscles, temporomandibular joint (TMJ), and the associated structures (2). The most common symptom of TMD is pain, which is usually present in the masticatory muscles, areas around the ears, eyes, throat, and TMJ, and in the head and neck areas. Besides pain that increases during mastication, patients with TMDs usually experience limited or asymmetrical jaw movements along with articular sounds such as clicking, popping, and crepitus (2). A variety of factors, including functional, structural, psychological, neuroendocrine, emotional, and behavioral factors, are involved in TMD etiology (3). Stress is also considered as one of the main etiological factors effective on dysfunction of the masticatory system.

Occupational stress is defined as a mismatch between one's occupational needs and his/her abilities, capabilities, and desires. Accordingly, it has various physical, psychological, and behavioral effects (4). The duration of dental educational curricula is relatively long. Dental students are often more stressed during their training period and more anxious compared to the general population (5). Many students are in a constant conflict between the need to study or rest and doing something other than dental work. Stress is a part of every person's life, and it is sometimes considered as a motivation for doing great things. Therefore, it is a double-edged sword that can either motivate students to deliver maximum performance or reduce their efficiency and effectiveness (6). If stressors persist for a long time, they can lead to development of physical and mental problems such as anxiety, depression, fear, insomnia, and excessive sweating. Students with high levels of stress mostly have a lack of self-confidence, are poorly able to continue their education, and are unable to compensate for their clinical weaknesses (7).

Using saliva, as a diagnostic fluid, is a relatively new method. There is a wide range of measurable biomarkers in the saliva, including heavy metals, hormones like cortisol, metabolites, enzymes, immunoglobulins like immunoglobulin A (IgA), proteins, and DNA (8). Collecting saliva is a non-invasive method compared to serum collection; therefore, it is more acceptable to patients. Since saliva is easy to collect, it can be collected by the patient himself and does not require trained staff. In addition, when collecting saliva, there is no risk of needlestick injury. The process of saliva collection is less stressful compared to other invasive methods like serum collection. This is also important when using biomarkers to investigate stress. Salivary samples can reflect the level of biomarkers (9). Stress, known as a major cause of both anxiety and depression, is associated with variations in levels of several salivary biomarkers. These biomarkers include cortisol, alpha-amylase, beta-endorphins, chromogranin A, and IgA. Chronic stress is related to the activity of the hypothalamic-pituitary-adrenal (HPA) axis, which can be measured by measuring the salivary cortisol. Chronic stress also suppresses the immune system activity, which can be measured by measuring the salivary levels of IgA and salivary lysozyme. Moreover, acute stress is related to the activity of the sympatho-adreno-medullary system, which is reflected by alpha-amylase and salivary chromogranin A (9).

Cortisol is considered as the physiological biomarker of stress. When stress is perceived by an individual, the HPA axis is activated resulting in secretion of cortisol from the adrenal glands. Measuring the salivary cortisol level is a reliable method for assessing the activity of the HPA axis. Nowadays, salivary cortisol is used as a stress index (10).

Salivary secretory IgA level is known as the main immunological factor in the mucosal surfaces and has been shown to be sensitive to psychological variables. Moreover, it is believed to indicate the function of the mucosal immune system (11). It is noteworthy that the circadian rhythm of salivary secretory IgA concentration is closely related to the circadian rhythm of

salivary cortisol. However, it is not clear whether the circulatory changes that occur in IgA secretion are in response to cortisol secretion or they are controlled by other mechanisms (9). Salivary secretory IgA has been previously used as a stress marker in several studies (11).

This study aimed to investigate the relationship between stress and TMDs in fourth- to sixth-year dental students of Qom Dental School by examining their occupational stress level using the Dental Environmental Stress (DES) questionnaire subjectively, and measuring their salivary stress markers, including cortisol and IgA levels, objectively.

Materials and Methods

Study design:

The current study was performed on 60 fourth to sixth-year dental students of Qom University of Medical Sciences in April 2019. The institutional ethical committee of Qom University of Medical Sciences approved the study protocol (IR.MUQ.REC.1397.166). Students with systemic diseases affecting the joints, those taking medications such as steroids, oral contraceptives, and antipsychotics, students with a history of trauma to the face or jaw, Cushing's syndrome, Addison's disease, pregnancy, and removable prostheses, and TMD patients with severe pain were excluded from the study. The DES questionnaire was used to subjectively measure the students' stress level. This questionnaire asked for demographic information of the participants and had 37 questions on stressors in the dental environment with 5 options (including not stressful = 1 point, slightly stressful = 2 points, moderately stressful = 3 points, severely stressful = 4 points, and highly stressful = 5 points) based on a Likert scale.

To determine the condition of the TMJ and TMDs, a clinical examination was performed for all the participants by a single examiner. This examination was based on the simplified version of Research Diagnostic Criteria/Temporomandibular Disorder (RDC/TMD), which includes a questionnaire as well as clinical examinations. The information was then recorded in a checklist. The initial assessment included some questions on demographic information such as gender, age, and type of response to stress as well as TMD subjective symptoms such as fatigue and tightness of the facial muscles, neck muscle pain, chronic headaches, and ear symptoms. In the second part, the included students underwent clinical examination either in the group with no maxillary TMD or in one or more diagnostic groups with TMDs using the RDC/TMD criteria (12). Objective assessment of the students' stress was done by measuring the stress-related salivary factors. For this purpose, each student relaxed for 20 to 40 minutes and then non-stimulating saliva samples were collected. To avoid the circadian changes, all the samples were collected at the same time of the day. Furthermore, to obtain a non-stimulated saliva sample, the students were asked to avoid doing exercise, eating, drinking, or using any oral stimulant such as mouthwash or tooth brushing for 90 minutes before saliva collection. Moreover, they were taught to avoid speaking and doing any head movements while collecting the saliva. The collected saliva samples had to be free of food, lipstick, or blood. Saliva was collected using the spitting method. The students were placed in a position with 45-degree angulation of their head and neck. After rinsing the mouth with water and swallowing saliva once, they were asked to collect their saliva in the floor of their mouth and requested to spit their saliva into a Falcon tube 1 to 2 times per minute to complete a 5-minute duration (13).

Statistical analysis:

The obtained data were analyzed using independent t-test, ANOVA, Chi-square test, and Pearson's correlation coefficient by SPSS version 26. The level of statistical significance was set at 0.05.

Results

The study sample included 60 students, including 27 males (45%) and 33 females (55%). Of them, 14 students were in the fourth year (23.3%), 13 in the fifth year (21.7%), and 33 students were in the sixth year (55%) of

their academic education. Moreover, 40 students were single (66.7%) and 20 students were married (33.3%). In terms of their living status, 13 students had their own house (21.7%), 17 students reported living with parents (28.3%), and 30 students were living in a dormitory (50%). In terms of personality traits, 30 (50%) students were sensitive and irritable and 30 (50%) were logical and balanced. Based on the subjective symptoms and objective examinations according to the RDC/TMD protocol, 24 (40%) students were confirmed to have TMDs while 36 students (60%) did not have TMDs.

In terms of articular sounds, 38 (63.3%) had no sound, 19 (31.7%) had clicking, one (1.7%) had popping, and 2 (3.3%) had crepitus.

Assessment of the RDC/TMD protocol revealed that 20 students (33.3%) had clicking during both jaw opening and closing in 2 out of 3 repetitions. Additionally, 12 students (20%) had clicking in lateral and protrusive movements in 2 out of 3 repetitions. The details of the results of these examinations are given in Table 1.

Also, the prevalence of TMD was higher among females (45.5%) than males (33.3%). However, according to the results of the Chi-square test, this difference was not significant (P=0.24). The prevalence of TMD was equal among both single and married students, and in both study groups. Also, 40% of students had TMDs. According to the Chi-square test, there was no significant relationship among TMD prevalence, academic year, and residency status (P=0.44).

The prevalence of TMD was higher among susceptible and irritable individuals (50%) compared to rational and balanced individuals (30%); however, this difference was not significant (P=0.09).

Based on the results of the DES questionnaire, having financial responsibility in the family (compulsory employment with education) caused minimum (1.81), and fear of failure to pass course units (3.10) caused maximum stress in students. Furthermore, in terms of the score of each factor of the questionnaire based on ANOVA, a significant difference existed between the academic years only in terms of stress related to assessment of clinical performance of students by mentors (P=0.03); the stress level in this regard in the fifth-year students was significantly higher than that in fourth- and sixth-year students.

The mean cortisol level of students was 4.74 ± 2.80 ng/mL (range 0.40 to 16 ng/mL). Additionally, the mean IgA level of students was 220.96 ± 121.23 μg/mL (range 48.60 to 537.80 μg/mL). The mean DES score of students was 48.2 ± 58.0 (range 24.1 to 35.4).

According to the Pearson's correlation test, no significant correlation was found between cortisol and IgA levels and the questionnaire score (P=0.44). Moreover, the mean cortisol and IgA levels and the mean score of DES were compared among different academic years. According to t-test, there was no significant difference in terms of the above-mentioned variables among different academic years (P=0.23).

The correlation coefficient between the questionnaire score and cortisol was -0.10; this value was 0.01 for the correlation of questionnaire score and IgA level.

Based on t-test, there was no correlation between having TMD and cortisol and IgA levels, cortisol level and DES score by gender, cortisol and IgA levels and marital status, cortisol and IgA levels and the mean score of the DES, and the personality traits of the individual from his/her own point of view, or cortisol and IgA levels and clicking sound in vertical jaw movements (P=0.29). In addition, DES questionnaire score in cases with TMD was higher than that in those without TMD, and the score was higher in cases with clicking sound in vertical movements compared to those without clicking. IgA levels were higher in males than females. Additionally, the mean score of the questionnaire in married students was higher than that in single students but these differences were not significant (P=0.21). There was no significant relationship among cortisol and IgA levels, the mean score of the DES questionnaire, and clicking in lateral and protrusive movements (P=0.53).

According to ANOVA, there was no significant relationship among cortisol and IgA levels, DES questionnaire score, and living status (P=0.47).

Table 1. RDC/TMD symptoms of students

Symptom	**Present Number** (%)	**Absent** Number (%)
Pain in jaw, temporal area, face, around or inside the ear during rest or function	7(11.7%)	53(88.3%)
Opening the mandible without any assistance or pain less than 4 cm	0(0%)	60(100%)
Opening the mandible with the assistance less than 5 cm	0(0%)	60(100%)
Clicking of the joint when the jaw is vertically opening and closing- twice in 3 repetitions	20(33.3%)	40(66.7%)
Clicking of the joint during lateral and protrusive movements- twice in 3 repetitions	12(20%)	48(80%)
History of jaw locking with eating interference	1(1.7%)	59(98.3%)
Lateral movements less than 7 mm	0(0%)	60(100%)
Uncorrected deviation	0(0%)	60(100%)

Discussion

In the present study, the mean score of the DES questionnaire in the students was 2.48 ± 0.58 and among the questionnaire items, the highest score belonged to "fear of not passing the course" and the lowest score belonged to "having financial responsibility in the family (compulsory employment with education)". In the study performed by Tangade et al, in India, "fear of not passing courses" was found to put the most stress on students (14). In studies by Harikiran et al, and Sangiorgio et al, exams were the most stressful factor (15, 16). Altogether, such similar results showed that exams and obtaining a passing score and the fear of failing and not passing the courses are the most stressful factors among the students in different dental schools.

In the present study, there were no significant relationships between the level of stress based on the DES questionnaire score and the academic year, which was different from the results reported in the study by Sangiorgio et al, who reported second and fifth years of academic education as the most stressful years for students (16). Second and third years are prone to be more stressful because of the intense laboratorial/pre-clinical loads, and fifth-year students face anxieties about the future and may be uncertain about some features of their dental education, which are common in undeveloped and developed countries (16, 17). Lower stress level of senior dental students can be due to gaining more experience and skills in the clinical work, decreased volume of theoretical courses, and their increased confidence and self-esteem (16). Such differences may possibly be due to differences in dental curricula in different areas and variations in sample size.

According to the present findings, there was no significant relationship between the stress levels with the DES questionnaire score or gender, which was different from the results of Sangiorgio et al, and Polychronopoulou et al (16, 18). In their studies, it was reported that female students had a higher overall stress level compared to male students. Higher stress among women can be attributed to some issues such as feeling more pressured to succeed, receiving less support from friends, and being more vulnerable. Moreover, failure to express concerns or emotions by men can also play a role in this regard (18). Lack of significant differences between males and females in the present study may possibly be due to the statistical population or different sample sizes.

In the present study, no significant relationship was observed between stress based on the DES questionnaire score and living status. Al Saleh et al, and Muirhead and Locker reported that students who were living with their parents had higher stress scores (19, 20). Tangade et al. found that students who lived in their own house had less stress compared to those living in a dormitory (14). Harikiran et al, also

reported that students living in a dormitory were more stressful than those living in their own house (15). Living with family has a protective effect. On the other hand, this leads to separation of students from the university conditions by spending more time with their family and social activities. Since living conditions mostly depend on regional, religious, and cultural issues, it is not possible to accurately compare the present results in this regard with those of the available studies.

In the present study, there was no correlation between stress level and the DES questionnaire score or cortisol and IgA levels. In a study by Pani et al, it was shown that the correlation between the sources of academic stress measured by the DES questionnaire and cortisol levels was negative (21). A study by Vivian et al, also found that students had more stress and higher salivary cortisol levels before taking their exam (22). In another study, Yang et al. reported that emergency department nurses who reported more work stress had significantly lower IgA salivation compared to public department nurses (11). The differences between the results of the abovementioned studies and the present investigation may possibly be due to the use of different methods of stress assessment, different statistical populations, and different sample sizes.

The present study showed the prevalence of TMDs among students to be 40%. This rate was 54% in a study by Wieckiewicz et al (12). In the present study, 36.7% of the students had articular sounds, 31.7% had clicking, and 3.3% had crepitus, which were somewhat similar to the rate reported by Poveda Roda et al (23). Additionally, based on the DES questionnaire score, the stress level was higher among the students with TMDs than those without TMDs, but this difference was not statistically significant. This may be due to the small sample size. A study by Cynthia et al. found that people with TMD mostly experienced more stress (24). Ebtisam et al, also showed that people with TMDs had significantly higher levels of stress perception (25). These findings suggested that stress may be one of the most important causes of TMDs. According to the present study, the prevalence of TMDs was higher in females (45.5%) than males (33.3%); however, this difference was not significant. A study by Pedroni et al, also found a higher prevalence of TMDs in females (26). It can be stated that higher prevalence of TMDs in females can be attributed to psychological differences between different genders and more stress in women, hormonal differences, or higher referral of women for both counseling and treatment of TMD-related problems (26). In the present study, the prevalence rate of TMDs was reported to be 50% among sensitive and irritable individuals and 30% among rational and balanced individuals. No significant association was found between salivary cortisol levels and TMDs. However, Ebtisam et al, in their study reported a significant difference between salivary cortisol level among patients with and without TMD, and it was higher among TMD patients (25). It seems that lack of significance in the present study may be due to the use of different study methods, different statistical populations, and smaller sample size. Moreover, there was no significant relationship among cortisol and salivary IgA levels, gender, and marital status in the present study. In the study by Pani et al, salivary cortisol levels were equal among both men and women in the last week of the clinic, but they were higher in men than women on the day of the test. Moreover, in this study, the mean salivary cortisol levels were higher in married people compared to singles (21). According to the above-mentioned statements, it seems that no significant relationship exists among gender and IgA levels, or marital status and IgA levels but the difference in cortisol may probably be due to different methodologies and statistical community. In the present study, there was no significant relationship between salivary cortisol levels and living status. In the study by Pani et al, cortisol levels were significantly higher in students who were living alone compared to those who were living with their parents or in a dormitory (21). Differences in these studies can be due to statistical populations or different sample sizes.

Conclusion

In the present study, 24 patients (40%) had TMDs. There was no correlation between cortisol and IgA levels and DES questionnaire score. There was no significant relationship between TMDs and cortisol level, IgA level, and DES questionnaire score. For more detailed evaluation, more studies with larger sample sizes are required.

Ethical Issues

The research followed the Declaration of Helsinki. The Ethics Committee of Qom University of Medical Sciences approved this study. The institutional ethical committee at Qom University of Medical Sciences approved all study protocols (IR.MUQ.REC.1397.166). Accordingly, written informed consent was taken from all participants before any intervention. Besides, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Conflicts of interest

The authors declare that they have no competing interests.

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