Frontiers in Dentistry

Relationship between Parafunctional Habits and Salivary Biomarkers

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Article Info

Article type:
Original Article

Article History:
Received: 19 June 2019
Accepted: 8 October 2019
Published: 20 December 2019

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A B S T R A C T

Objectives: Parafunctional habits, as one of the etiological factors of temporomandibular disorders (TMD), are an individual's response to increased stress. During stress and depression, biomarkers such as cortisol and salivary alpha-amylase (SAA) are secreted in the saliva. The present study aimed to investigate whether there is a correlation between salivary stress biomarkers and parafunctional habits.

Materials and Methods: Thirty-two cases, from May to September 2015, were selected based on two standard stress questionnaires, namely the depression anxiety stress Scale-21 (DASS-21) and the Beck Anxiety Inventory (BAI). Saliva samples were collected to examine the level of unstimulated salivary cortisol and SAA. The significance of the results was assessed via student's t-test and Mann-Whitney test (α=0.05).

Results: The mean concentrations of cortisol and SAA in unstimulated saliva were significantly higher in the case group than in the control group (P=0.01 and 0.44, respectively). The mean scores of anxiety, stress, and depression were significantly higher in the case group than in the control group (P<0.05).

Conclusion: It seems that the levels of salivary cortisol and SAA, as well as stress, anxiety, and depression scores, are higher in patients with parafunctional habits.

Keywords: alpha-Amylases; Anxiety; Cortisol; Depression; Habits

➢ **Cite this article as:** Khayamzadeh M, Mirzaii-Dizgah I, Aghababainejad P, Habibzadeh S, Kharazifard MJ. Relationship between Parafunctional Habits and Salivary Biomarkers. *Front Dent*. 2019;16(6):465-472. doi: 10.18502/fid.v16i6.3446

INTRODUCTION

Parafunctional habits are unusual, unconscious, and sometimes, involuntary activities of the masticatory system that do not have functional purposes [1]. They are believed to cause complications and problems in the teeth and the temporomandibular joint (TMJ), exerting recurrent microtraumas on the masticatory system [2]. Bruxism and clenching are among the most common forms [3]. Although the cause has remained largely unknown, it is believed to be multifactorial [4]. According to various studies, different psychosocial, systemic or genetic factors are

responsible for bruxism. Secondary factors, such as drugs, tobacco, and alcohol, and behavioral and cognitive factors, such as stress and anxiety, are also believed to be among the contributing factors [4,5].

Mental stress is an undeniable part of everyday life [6]. Studies have shown that people under different degrees of stress often show increased muscular activity [7]. Research has shown that metabolic and endocrine changes occur in the body with pain, anxiety, and stress [8]. In general, stress is an individual's response to external pressures or inappropriate conditions, and anxiety is one of its most common side effects. Anxiety is an overwhelming, unpleasant, and vague feeling, often accompanied by symptoms such as headache, sweating, palpitations, restlessness, and shortness of breath [9].

Stress forces the body to escape from the stressful situation, leading to increased hormonal secretions, including catecholamines and glucocorticoids [6]. Saliva is one of the most powerful tools in medical examinations and diagnoses, which can be evaluated at a low cost [1]. Measuring salivary cortisol level, therefore, might be useful in evaluating psychological stress, which is thought to have a role in parafunction [3,5,10]. Salivary alpha-amylase (SAA) is considered a biomarker in response to psychological stress [6]. Recent studies have shown that SAA specifically represents the sympathetic system activity. For example, SAA secretion is suppressed by the administration of propranolol, which is an adrenergic blocker [11].

In dentistry, salivary cortisol and SAA have been used to evaluate the role of stress in the anxiety level of patients undergoing dental treatment, as well as periodontal diseases, dental caries, and temporomandibular disorders (TMD) [10,12]. Secretion of cortisol and SAA in the saliva can be considered a physiological response of the body to oral parafunctional movements, including bruxism, which may cause pain in the TMJ and other damages over time [3]. Perceived stress is associated with TMD; for instance, sleep bruxism, chewing and swallowing malfunctions, and higher concentrations of salivary cortisol have been noticed in children with asthma [13]. However, it is still unclear whether stress biomarkers are elevated in individuals with TMD [14]. Kobayashi et al [15] studied salivary stress biomarkers and anxiety in children with signs of TMD and noticed that, despite the higher score of anxiety symptoms in children with TMD, no difference was noticed between children with and without TMD regarding the salivary stress biomarkers levels. Despite greater perceived stress, individuals affected by TMD had lower cortisol concentrations than the controls in a study by Lambert et al [14].

Apart from the controversies, no previous study has addressed the association between stress biomarkers and parafunctional habits; therefore, the present study aimed to investigate salivary stress biomarkers, namely cortisol and SAA, in patients with a history of oral parafunctional habits. Given that salivary cortisol and SAA secretion is enhanced in stress, their increase in individuals with parafunctional habits compared to the controls can biologically prove the effect of stress in parafunction.

MATERIALS AND METHODS

In this case-control study, which was conducted from May to September 2015, patients were selected from among those attending the Oral and Maxillofacial Medicine Department of the School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran, for routine dental work. Sixty-four subjects were selected randomly: 32 for the case group [age- and sex-matched subjects with at least one parafunctional habit (Appendix 1)] and 32 for the control group (systemically healthy subjects with no parafunctional habit). Individuals with any of the following criteria were excluded from the study:

- History of previous injuries to the head and face (severe blows that cause TMJ dislocation or fractures of the jaw)
- Alcohol or caffeine consumption during the last hour
- Eating or drinking during the last hour

- Brushing or flossing during the last hour
- History of taking corticosteroid medications or antidepressants during the last year
- Current use of pharmaceutical supplements, such as contraceptives or glucocorticoids
- Pregnancy
- Autoimmune diseases
- Neoplasms (malignant or benign tumors)
- Occlusal disharmony (premature contact, deep bite, crossbite or open bite)
- Current orthodontic treatment
- Toothache resulting from pulpal infection
- Tenderness or muscle stiffness associated with systemic diseases, such as neuralgia, myalgia or local infection
- Loss of more than two posterior teeth
- Inflammation of the joint or rheumatoid arrhythmia
- Diabetes
- Any other systemic diseases.

Study groups received general explanations on the project, how it was planned, its duration, and their collaboration in the study, and if the patient agreed to sign the informed consent, subsequent steps were taken. The protocol was approved by the Ethics Committee of the School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.VCR.REC.1396.2158). To verify stress in the participants, all patients were asked to fill out two standard and valid questionnaires, namely the depression anxiety stress Scale-21 (DASS-21) [16] and the Beck Anxiety Inventory (BAI) [17]. The DASS questionnaire contains three self-report scales to measure depression, anxiety, and stress [16]. Each of the scales comprises seven items "divided into subscales with similar content" [16].

The BAI questionnaire also comprises 21 items "with a Likert scale ranging from 0 to 3 and raw scores ranging from 0 to 63". It only addresses anxiety, and "the scores are classified as minimal (0 to 7), mild (8 to 15), moderate (16 to 25), and severe anxiety (30 to 63)" [17].

Patients were asked to drain their saliva into dry 10-ml conical centrifuge Falcon tubes (Corning Inc., Corning, NY, USA) between 9 and 11 a.m. Samples were collected on two alternate weekdays to examine the diurnal secretion profile of cortisol and SAA. Subjects were instructed not to perform physical exercises or ingest caffeinated beverages on the day before saliva collection and to abstain from food, beverages, and tooth brushing before sampling [10]. The unstimulated saliva samples were centrifuged and stored at -20°C. The cortisol level was measured by enzyme-linked immunosorbent assay (ELISA) based on the manufacturer's instruction (DRG Instruments GmbH, Marburg, Germany). The photometric method was used to determine the SAA level (Pars Azmoon Co., Karaj, Iran). Data were analyzed using SPSS version 22 (SPSS Inc., Chicago, IL, USA). The parametric data were expressed as mean ± standard error of the mean (SEM), and nonparametric data were expressed as median ± interquartile range (IQR). Statistical analyses were performed via Student's t-test, Mann-Whitney test, and Spearman's Rho test ($P<0.05$).

RESULTS

The average levels of salivary cortisol and SAA in patients with parafunctional habits (case) and healthy subjects (control) are presented in Table 1. The mean unstimulated saliva cortisol and SAA levels were significantly higher in the case group than in the control group ($P=0.011$ and $P=0.044$, respectively; Table 1).

Table 1. The average levels of salivary cortisol and salivary alpha-amylase in patients with parafunctional habits (case) and healthy subjects (control)

	Control	Case	P-value
Cortisol (ng/ml)	3.68±0.19	4.28±0.14	0.011*
SAA (U/ml)	267.0±21.1	404.2±62.3	0.044*

SAA:Salivary alpha-amylase *Data are expressed as Mean±Standard Error of the Mean and analyzed by unpaired student's t-test. ($P<0.05$)

The median score of anxiety in the case group was significantly higher than that in the control group ($P=0.038$ and 0.039 based on the BAI and

DASS-21 questionnaires, respectively; Table 1). The median scores of stress and depression were also significantly higher in the case group than in the control group (P=0.007 and 0.01, respectively; Table 2).

Table 2. Mean anxiety, stress, and depression scores in patients with parafunctional habits (case) and healthy subjects (control)

	Control	Case	P-value
Anxiety (BAI) score	24±6	27±10	0.038*
Stress (DASS) score	11±4.5	14±7	0.007*
Depression (DASS) score	8±2.75	10±4	0.01*
Anxiety (DASS) score	8±2.75	9±5	0.039*

DASS: Depression Anxiety Stress Scale, BAI: Beck Anxiety Inventory (BAI) *Data are expressed as Median± Interquartile Range and analyzed by Mann-Whitney-U test (P<0.05)

Statistical analysis by Spearman's Rho test revealed that there was no correlation between salivary cortisol and SAA levels (P=0.793), however, their levels significantly correlated with stress, anxiety, and depression scores (P<0.05; Table 3).

DISCUSSION

Cortisol is a hormone secreted by the hypothalamus-pituitary-adrenal axis, which has been used as a stress biomarker for many years. Cortisol secretion increases significantly for several times over a short period after the onset of stress. The same is true for salivary cortisol [6]. SAA is also secreted by the parotid glands in response to adrenergic activity, and although previously considered in digestion, it has been introduced recently as a promising candidate for a reliable and non-invasive marker of psychosocial stress [11]. Based on psychological questionnaires, stress is usually higher in individuals with parafunctional habits as a psychophysiological response; therefore, we expected salivary cortisol and SAA levels to be higher in these individuals. In this study, both salivary cortisol and SAA levels were higher in the case group and showed a significant increase in patients with parafunctional habits compared to healthy subjects. Salivary cortisol is higher in patients with stress and TMD [18-21]. Assessment of cortisol concerning TMD among a sample of dental students revealed that its levels have a significant but weak association with muscular pain and TMJ pain on palpation [18]. Research on the relationship between SAA and stress has focused more on acute stress; few studies have examined its role in chronic stress, such as increasing SAA in women involved in hurricane Katrina or women who were anxious and shy [19].

Moreover, inconsistent results regarding the SAA reactivity to psychological stimuli have been obtained from previous studies [15,19]. In 2010, Karakoulaki et al [9] evaluated the relationship between sleep bruxism and perceived stress through the estimation of salivary biomarkers and showed a moderate positive correlation between the bruxers' BiteStrip score and the salivary cortisol level; however, no such correlation existed regarding SAA levels [9].

Table 3. Correlation of stress, anxiety, and depression scores with salivary cortisol and salivary alpha-amylase (SAA) in the studied groups

	SAA	Anxiety (DASS)	Stress (DASS)	Depression (DASS)	Anxiety (BAI)
Cortisol (ng/ml)	r=0.032	r=0388*	r=0.436*	r=0.356*	r=0.631*
SAA (U/ml)		r=0.492*	r=0.423*	r=0.333*	r=0.315*

SAA: Salivary alpha-amylase; DASS: Depression Anxiety Stress Scale; BAI: Beck Anxiety Inventory (BAI) *Data were analyzed by Spearman's Rho test (P<0.05)

Similarly, Kobayashi and colleagues [15] reported that children with TMD scored higher in anxiety symptoms but no difference was observed in salivary stress biomarkers with or without TMD. On the contrary, SAA level in dental subjects with TMD, 80% of whom had parafunctional habits, was higher than that of the controls in a study by Ali and

Hadi [20] in 2015. Nater and Rohleder [21] also stated that those with chronic stress had a higher SAA level compared to other subjects. It should be noted that the first two studies were on bruxism in children, and due to the lack of scientific evidence on its rather unknown risk factors and pathophysiology, childhood bruxism is something that has not been carefully evaluated [10].

Anxiety is interpreted as a transient emotional reaction to everyday stressful events; this unpleasant feeling is activated by the autonomic nervous system [22]. Increased state anxiety in people with bruxism occurs when nociceptive stimuli reach the central nervous system (CNS), which evaluates and affects painful emotional experiences in the brain's cortex [23,24]. Earlier studies investigating the relationship between bruxism and general anxiety levels have shown contradictory results [5]. Recently, researchers have sought to determine the relationship between bruxism and psychological factors, which revealed that anxiety can play an important role in stimulating and enhancing parafunctional habits [3-5]. Bruxism is defined as "an anxiety response to environmental stress" [4,5]. Emotional factors, such as anxiety, fear, disappointment, and stress, are associated with excessive muscular activity [4,5]. It has been indicated that psychological traits, such as anxiety and depression, are significantly different in people with bruxism compared to healthy individuals [21]. Also, individuals with bruxism and clenching have a higher stress level than healthy subjects [25]. It has also been indicated that the mean anxiety and depression is higher in subjects with daily bruxism than in those without bruxism [26]. In this study, the mean anxiety and depression scores, based on the DASS-21 and BAI questionnaires, were also higher in subjects with parafunctional habits, which is in line with most previous studies [25-27].

It seems that salivary and blood cortisol do not necessarily show a correlation with SAA after stress, and these two biomarkers are complementary [28]. In 2011, Engert et al [29] suggested a reliable association between SAA and cortisol stress responses at various time lags throughout a stressful situation. In our study, on the contrary, there was no significant correlation between cortisol and SAA. One possible explanation for this could be the fact that temporary changes in the level of these hormones are not considered [28]. However, the significant correlation of both salivary cortisol and SAA with stress and anxiety in our study corroborates the findings of other studies [19,28-31].

There are several limitations to this study. Firstly, our criteria in selecting individuals for the case group were based on a self-report questionnaire. There is a standard questionnaire outlined by the American Academy of Sleep Medicine (AASM) for bruxism [9]. However, we could not find a more reliable and valid questionnaire for defining patients with parafunctional habits in the literature, and those found were only valid for a localized population [32]. Although self-reported parafunction has been used by previous studies [5,21], the results of our study would have been more reliable if an objective estimation had been used. Secondly, sample size determination would have been more accurate if the prevalence of parafunctional habits in the general population had been considered, which unfortunately, this prevalence was not available for our population. Thirdly, since there was no information regarding the duration of parafunctional habits, anxiety, and depression in the case group, it was not possible to determine whether chronic cases were more likely than recent-onset ones to have higher cortisol and SAA concentrations. Another issue is the day-to-day variation in salivary cortisol measurements, which is a known phenomenon [33]. We tried to overcome this phenomenon by taking cortisol measurements at a fixed collection time; however, some studies suggest measurements to be taken at various times of the day [15].

CONCLUSION

It seems that the levels of salivary cortisol and SAA and scores of stress, anxiety, and depression are higher in patients with parafunctional habits. Within the limitations of the present study, no apparent significant correlation was found between cortisol and SAA.

ACKNOWLEDGMENTS

This work is based on the thesis submitted to school of dentistry, Tehran University of Medical Sciences, International Campus, in partial fulfillment of the doctorate degree in dentistry.

CONFLICT OF INTEREST STATEMENT

None declared.

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Appendix 1. Parafunctional Habits Questionnaire

Habit	Yes	No
Do you chew gum regularly?		
Do you have the habit of nail-biting?		
Do you have the habit of biting hard objects, e.g., sewing needles, pencil, etc.?		
Do you have a habit of playing the appliance (dental appliance) or prosthesis in your mouth?		
Do you like sucking the cheeks?		
Do you like to suck the tongue?		
Do you like to suck your tongue and cheeks at the same time?		
When you say "copet, cepat, dapat" rapidly and repeatedly, is the tip of your tongue on the lower edges of the front teeth (tongue thrust)?		
Do you like to bite the upper lip?		
Do you like to bite the lower lip?		
Do you clench your teeth at night?		
Do you grind your teeth during the day?		
Do you grind your teeth at night?		
Do you clench your teeth at night?		
Has anyone heard you grinding your teeth frequently during sleep?		
Do you like to play the jaw?		