Relationship Between Sleep Bruxism and Stress Determined by Saliva Biomarkers

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Purpose: The aim of this study was to evaluate the relationship between sleep bruxism (SB) and perceived stress through the estimation of stress-related biomarkers (cortisol, α-amylase) in saliva. ***Materials and Methods:*** Forty-five volunteers (20 men, 25 women) participated in this study. Participants were divided into two groups (bruxers and nonbruxers) according to their answers in a standard bruxism assessment questionnaire outlined by the American Academy of Sleep Medicine. To confirm the preliminary diagnosis and to determine the severity of SB in the group of patients who had a positive self report for SB, a miniature, single-use electromyographic (EMG) device for SB detection (BiteStrip) was used. The perceived stress of the 45 participants was measured using the Perceived Stress Scale questionnaire. Unstimulated whole saliva was collected and levels of salivary cortisol and α-amylase were determined by enzyme-linked immunosorbent assay test and enzyme kinetic reaction, respectively. Nonparametric statistical methods were applied for data analyses. ***Results:*** Bruxers showed higher levels of perceived stress than nonbruxers ($P < .001$). There was a moderate positive correlation between the 25 bruxers' BiteStrip scores and the salivary cortisol levels (Spearman rank correlation = 0.401, $P = .047$). Additionally, bruxers showed higher levels of cortisol than nonbruxers ($P < .001$). On the contrary, salivary α-amylase levels were not significantly different in bruxers and nonbruxers ($P = .414$). ***Conclusions:*** These findings suggest that SB activity was related to higher levels of perceived psychological stress and salivary cortisol. Despite the limitations of the EMG recording device, a moderate positive correlation between BiteStrip score and cortisol levels was observed in bruxers. *Int J Prosthodont 2015;28:467–474. doi: 10.11607/ijp.4296*

Bruxism has been defined as "a repetitive jaw-muscle activity characterized by unconscious clenching or grinding of the teeth and/or by bracing or thrusting of the mandible."[1] It is considered a sleep or waking parafunctional activity that may have detrimental consequences for dental, periodontal, and musculoskeletal tissues and cause complications with prosthetic restorations.[2–4]

Nowadays, the multifactorial etiology of bruxism is widely accepted.[5,6] Several authors have proposed a conceptual shift from peripheral (eg, occlusal interferences) to central etiologic factors, such as psychosocial (eg, perceived stress, anxiety, personality characteristics) and pathophysiological factors (eg, sleep-related microarousals).[5–8] Furthermore, bruxism activity seems to be modulated by various neurotransmitters (norepinephrine, serotonin, dopamine) in the central nervous system.[5]

Psychological stress is increasingly considered an initiating or predisposing factor for bruxism activity during wakefulness and sleep, although the relationship has not been completely determined to date.[7,9,10] It has also been reported that the majority of data on the associations between bruxism and psychological symptoms derived from studies that adopted self-reporting questionnaires or clinical diagnosis of

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bruxism.[10] The shortcomings of these subjective diagnostic methods are that they do not provide detailed insight into bruxism in relation to sleep, so only possible sleep bruxism (SB) activity may be assessed.[1] For this reason, a disposable miniature self-contained electromyographic (EMG) detector-device (BiteStrip, Scientific Laboratory Products) that can evaluate masseter muscle activity in a patient's home environment was introduced.[11] The BiteStrip has a simple design, low cost, and acceptable accuracy, and it may help the clinician in the diagnosis of SB. It has been shown that BiteStrip is accurate in detecting the presence or absence of SB but less accurate in assessing SB intensity, suggesting that the use of laboratory polysomnography with audio-video recording (Type 1 PSG) remains the gold standard tool for SB diagnosis.[11]

Stress has been invoked as a cause of major psychopathology, a precipitator or trigger of psychiatric illness, and a contributor to considerable mental anguish.[12] It is considered as a cognitive perception of uncontrollability and/or unpredictability that is expressed in a physical, mental, or emotional adjustment or response.[12] It has been reported that psychological stress increases the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathoadrenal medullary (SAM) system.[12,13] The levels of salivary cortisol, as a biomarker of the HPA axis, and of salivary α-amylase, as a biomarker of the SAM system, have been examined for the estimation of psychological stress.[13–16]

The noninvasive, safe, and readily repeatable procedure of saliva sample collection, which also can be performed outside of laboratories several times per day, makes saliva a useful tool for investigating several markers related to stress. Saliva may be used for diagnostic purposes without much patient discomfort, offering an advantage over other stressful autonomic measures obtained by venipuncture, such as plasma epinephrine/norepinephrine, accompanied with anxiety/pain or laboratory complications.[17]

The aim of this study was to investigate the relationship between SB activity and perceived psychological stress and to detect possible alterations of stress-related biomarkers (cortisol, α-amylase) in saliva. More specifically, the research hypotheses were that *(1)* SB would be related to higher levels of perceived stress, *(2)* SB activity would be positively correlated with salivary cortisol levels, and *(3)* SB activity would be positively correlated with salivary α-amylase levels.

Materials and Methods

Study Sample

Forty-five volunteers (20 men and 25 women) that referred to the Clinic of Fixed Prosthesis and Implant Prosthodontics, School of Dentistry, Aristotle University of Thessaloniki, with missing teeth or bruxism habit as their chief complaint, were selected to participate in this study. The inclusion criteria were *(1)* a history of good health with no psychiatric disorders, epilepsy, or alcoholism; *(2)* good oral health with no extensive prosthetic restorations (no more than a 4-unit fixed partial denture); and *(3)* aged between 25 and 55 years. In contrast, the exclusion criteria were *(1)* medication that may affect the sympathetic or parasympathetic nervous system or affect salivary gland function (including benzodiazepamines, antidepressants, and anticonvulsants); *(2)* disturbances in the structure/function of the pancreas, such as diabetes; *(3)* use of corticosteroids or Cushing syndrome or Addison's disease; *(4)* pregnancy; *(5)* smoking; *(6)* orofacial pain or recent occlusal splint therapy; and *(7)* local or systemic diseases affecting salivary function, such as oral mucosal inflammation or trauma, autoimmune sialadenitis including Sjögren syndrome, sialolithiasis, and neoplasms of salivary glands. All participants provided written informed consent for the study, which was approved by the Ethical Committee of Aristotle University of Thessaloniki School of Dentistry and followed the guidelines of the Declaration of Helsinki.

Assessment of Sleep Bruxism

Participants were initially divided into two groups (30 bruxers, 15 nonbruxers) according to their answers to a standard questionnaire of bruxism awareness based on a previous study by Winocur et al[18] and on the diagnostic criteria of the American Academy of Sleep Medicine (AASM).[19] The questionnaire referred to the participants' self-report of bruxism events during the last 6 months. To confirm the initial diagnosis and to determine the severity of SB in the group of patients who had a positive self-report of SB, a miniature, disposable single-use EMG device for SB detection was used (BiteStrip, Scientific Laboratory Products).[20] It consists of EMG electrodes, an amplifier, and a central processing unit (CPU) with software that detects and analyzes the EMG pattern in real time. The recording device with written instructions was given to each participant for one night during the work week and applied over the masseter muscle to estimate the total number of SB events within a sleeping period of 5 hours. Patients had to make four to five maximum voluntary clenches to assess the individual bruxing threshold. SB episodes were recorded as those that exceeded 30% of maximum voluntary clenching and lasted over 0.25 seconds. According to the manufacturer, the BiteStrip score throughout the night was displayed on a 4-grade scale of 0 to 3 (0 = no or low SB: fewer than 30 episodes; 1 = mild SB: between

30 and 60 episodes; 2 = moderate SB: between 61 and 100 episodes; and 3 = severe SB: more than 100 episodes).

The final study sample consisted of 25 bruxers (those whose initial SB diagnosis was confirmed by a BiteStrip score between 1 and 3) and 20 nonbruxers. In a limited number of patients, loss of connectivity between the BiteStrip and the skin during sleep resulted in a failure to register the EMG masseter events. In 3 patients it was necessary to repeat the procedure the following night before the saliva collection.

Perceived Stress

For the subjective assessment of perceived psychological stress, all 45 participants (bruxers and nonbruxers) were instructed to fill out the Perceived Stress Scale (PSS) questionnaire developed by Cohen et al.[21] The PSS is a valid, brief, and easy-to-administer measure of the degree to which situations in the subject's life are appraised as stressful. It consisted of 14 items and addressed the feelings and thoughts that had caused the patient stress over the previous month. The possible answers for each item were rated on a five-point scale (from 0 = never to 4 = very often). The total score could range from 0 to 56, where higher scores indicated higher level of psychological stress.

Saliva Analysis

Saliva samples from the 45 participants (bruxers and nonbruxers) were used to assess the levels of the specific stress biomarkers α-amylase and cortisol. Saliva was collected immediately after waking, between 7 and 9 am, to avoid changes due to the circadian rhythm.[22] A special vial (Swab Storage Tube, Salimetrics) was used for saliva collection, containing an inert polymer swab (Oral Swab, Salimetrics). In short, the procedure included rinsing the oral cavity with copious amounts of water after awakening and before eating anything and placing the swab under the tongue for 5 minutes. After removal of the swab with clean hands, the swab was placed in the vial and kept refrigerated (−18°C) by the patient until delivery at the laboratory, using freezer packs for transport. The samples were frozen at −30°C at the laboratory no more than 4 hours after the collection. On the day of assay, the saliva samples were prepared by centrifugation at 1500 g (3000 rpm) for 15 minutes and then used for the measurements.

The 96-well salivary α-Amylase Assay Kit (Salimetrics) was used for the kinetic enzymatic measurement of salivary α-amylase levels, as described previously.[23] In summary, this method included the enzymatic action of α-amylase on the chromagenic substrate 2-chloro-P-nitrophenol linked with maltotriose, and the spectrophotometric measurement of this interaction at 405 nm with a cut-off value of detection at 0.01 U/mL. The amount of α-amylase activity given in U/mL presented in the sample was directly proportional to the increase in absorbance at 405 nm. The optical density (OD) of each well was read by an LP400 plate reader (Anthos Labtec Instruments) and measurements were taken per strip of 8 wells. The OD values in each strip were read twice (in the first and third minutes) and were recorded using an EPSON LX 800 printer (Telford). The α-amylase activity of each sample (in U/mL) was determined using the following formula:

$$\frac{\text{DAbs/min} \times \text{TV} \times \text{DF}}{\text{MMA} \times \text{SV} \times \text{LP}} = \text{value (U/mL)}$$

where DAbs/min = absorbance difference per minute; TV = total assay volume (0.328 mL); DF = dilution factor (1/200); MMA = millimolar absorptivity of 2-chloro-P-nitrophenol (12.9); SV = sample volume (0.008 mL); and LP = light path = 0.97 (specific to plate received with kit).

The measurement of saliva cortisol was performed using a Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics) for the ELISA method according to the manufacturer's instructions. The immunoenzymatic reaction indicating the cortisol level was measured by optical density that was then read at 450 nm in an automated plate reader (LP400, Anthos Labtech Instruments). Based on the standards, a calibration curve was drawn to aid in the calculation of the cortisol concentration. The amount of cortisol peroxidase detected, as measured by the intensity of color, was inversely proportional to the amount of cortisol present, with a cut-off value of detection at 0.003 μg/dL.

Statistical Analyses

Data were summarized by calculating descriptive statistical indices of central tendency (mean values), variability (standard deviation), and association (Spearman rank correlation coefficient). The nonparametric Mann-Whitney test was used for the comparison of the two groups (bruxers vs nonbruxers) relative to age, PSS, and α-amylase and cortisol levels. Box plots were created for visual examination of the differences between the two groups. In addition, scatter plots were produced for visual inspection of the reported correlation between BiteStrip and cortisol level. The line of best fit was plotted on the corresponding scatter plots by applying the Loess smoothing method to validate the reported associations.

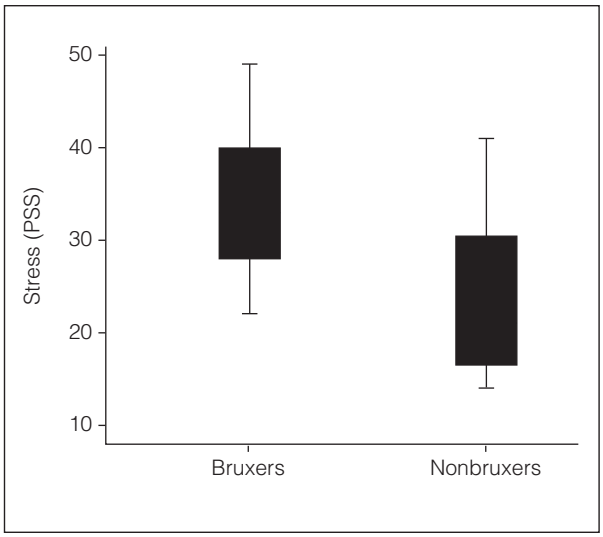

Fig 1 Box-plot of PSS levels per group (bruxers and nonbruxers).

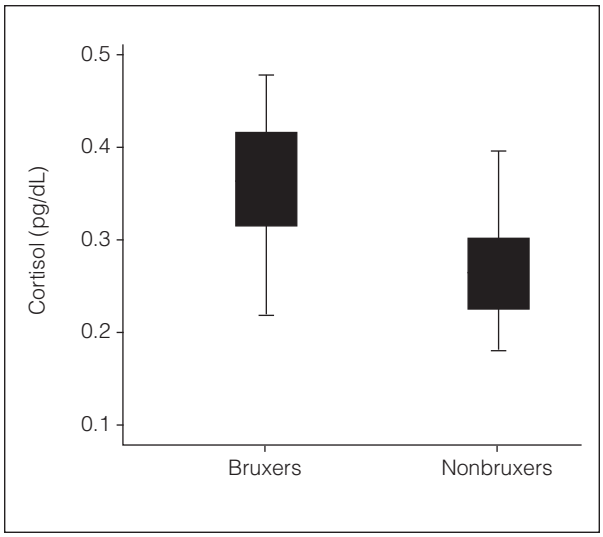

Fig 2 Box-plot of cortisol levels in saliva per group (bruxers and nonbruxers).

The chi-square test was applied to test the differences between the two groups relative to gender distribution. Summary data are reported in the form of mean ± standard deviation. In all hypotheses testing procedures the observed significance level (*P* value) was estimated with the Monte Carlo simulation method using 10,000 resampling circles. This approach leads to valid inferential conclusions even in cases where the methodological presuppositions of the corresponding nonparametric statistical tests are not satisfied. Data were analyzed using SPSS v15.0 statistical software (SPSS) enhanced with the module Exact Tests (for Monte Carlo simulation).

Results

The final study sample consisted of 45 volunteers (age range: 25 to 52 years; mean age: 34.5 ± 6.4 years) of whom 25 were bruxers (those who had the initial diagnosis confirmed by BiteStrip scores of 1, 2, or 3) and 20 were nonbruxers. The two groups showed similar features in terms of sex ($P = .764$) and age distribution ($P = .293$).

In the bruxers group PSS scores ranged from 22 to 49 (34.52 ± 7.98), while among the nonbruxers these scores ranged from 14 to 41 (23.80 ± 8.21). The bruxers showed higher levels of perceived stress than the nonbruxers ($P < .001$) (Fig 1).

The salivary cortisol levels for bruxers ranged from 0.22 to 0.48 μg/dL (0.37 ± 0.08 μg/dL) while for nonbruxers these levels ranged from 0.18 to 0.44 μg/dL (0.27 ± 0.06 μg/dL) (Fig 2). Additionally, the value 0.31 μg/dL could be used as a threshold for cortisol levels since it coincides with the Q25 quartile of bruxers' cortisol level distribution and, at the same time, leads to an optimal division of participants into two groups (bruxers and nonbruxers). According to this cut-off value, the cortisol levels in 19 of 25 bruxers (76%) were greater than 0.31 (sensitivity = 80%). In 16 out of 20 nonbruxers (80%) the cortisol levels were less than or equal to 0.31 (specificity = 83.33%). Bruxers showed higher levels of cortisol in saliva than nonbruxers ($P < .001$). Furthermore, there was a moderate positive correlation between BiteStrip scores in bruxers and saliva cortisol levels (Spearman rank correlation = 0.401, $P = .047$) (Fig 3).

On the other hand, the bruxers group showed salivary α-amylase levels between 5.08 and 127.10 U/mL (39.74 ± 31.86 U/mL), while these values in the nonbruxers group ranged from 10.50 to 82.49 U/mL (38.76 ± 22.70 U/mL) (Fig 4). In contrast to cortisol, differences in α-amylase levels in saliva between bruxers and nonbruxers were not statistically significant ($P = .414$).

Discussion

In this study the link connecting SB activity and perceived stress, and a more subjective estimation of this relationship for diagnostic reasons, were investigated using levels of cortisol and α-amylase in saliva, biomarkers considered indicative of stress. The main findings supported the first and second research hypotheses, that SB activity was related to higher levels

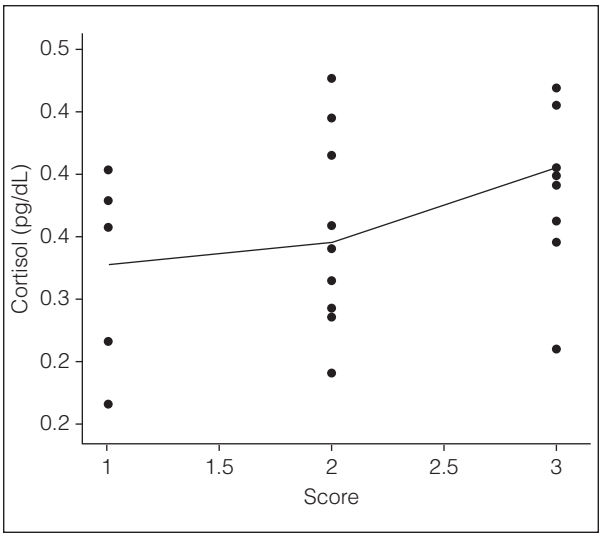

Fig 3 Correlation between cortisol concentration and BiteStrip scores (group of bruxers).

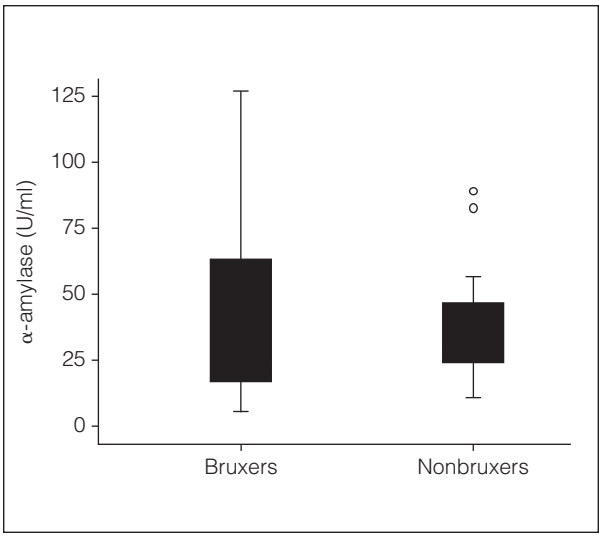

Fig 4 Box-plot of α-amylase levels in saliva per group (bruxers and nonbruxers).

of perceived psychological stress and salivary cortisol. The results also suggested that there were no differences between bruxers and nonbruxers in salivary α-amylase levels; therefore, the third research hypothesis was not confirmed.

Earlier investigations studying the relationship between SB behavior and stress yielded contradictory results.[5,7,24] While many studies reported that subjects under stress are more likely to exhibit bruxism,[5,7–9] others failed to confirm this correlation.[24,25] An earlier study by Rugh and Solberg[26] reported that SB seemed to appear after exhausting and stressful days. Similarly, Hicks and Chancellor[27] showed an association between SB and an overtly ambitious character or behavior (Type A), which in turn is related to a stressful lifestyle. The findings of the present study are in agreement with previous investigations that showed that SB patients experienced higher levels of perceived psychological stress than nonbruxers.[18,28] Furthermore, in another study on 1339 subjects, frequent bruxism was significantly associated with severely stressful situations at work and it was concluded that bruxism may reveal ongoing stress in normal work life.[7] In contrast, Nakata et al[29] examined the relationship between psychosocial job stress and SB in a Japanese population of 1944 male and 736 female factory workers and found that SB was weakly associated with some aspects of job stress in men.[29]

Contradictory results in the literature may be attributable to the fact that different methods have been described for the diagnosis of SB and different psychological factors have been investigated; therefore the findings of these studies are not conclusive. In the present study, SB was evaluated subjectively by a standard questionnaire based on a previous study by Winocur et al[18] and on the diagnostic criteria of the AASM.[19] SB activity was further confirmed by EMG recordings of SB episodes during bedtime with BiteStrip screener.[20,30] BiteStrip is a cost-effective, miniature EMG device that may detect masseter muscle activity during sleep, although no studies apart from the manufacturer's validation findings have been reported.[20] At present, polysomnography with audio-video recording represents the gold standard tool for the diagnosis of SB in small samples.[1,3,6] However, this method is expensive and time consuming and requires the patient to sleep in a laboratory environment, which may influence SB behavior. Saliva is now considered an optional fluid for diagnostic and disease monitoring purposes based on its minimal cost and invasiveness and ease of collection and processing, but also because the current proteomic technologies in biomarker discovery can bring salivary diagnostics into a clinical reality.[17] Compared to blood, saliva contains a smaller quantity of proteins, decreasing any potential risk of nonspecific interference or hydrostatic interactions. Within blood, the protein concentration can vary over several orders of magnitude, with protein half-lives ranging from a few seconds to several months or longer. The composition of saliva, however, is not as complex or varying and should more accurately reflect the current condition of the body at any given time. Ultimately,

saliva may contain locally expressed proteins and other substances that can be used as indicators of diseases.[17] The estimation of stress markers in saliva has some advantages over other methods, including blood tests.[31] The advantages include easy collection several times per day without a need for medical staff and avoidance of the pain and anxiety that can potentially serve as a stressor itself, leading to an acute release of catecholamines and therefore biased data.[31] Subsequently, saliva sampling offers the opportunity to collect data from subgroups of patients that include anxious or sensitive subjects as well as older subjects, children, and even infants.[32]

The assessment of salivary cortisol offers the opportunity to collect the samples stress-free, without medical personnel, and in many different environments. Despite dissociation between saliva and blood measures during the circadian circle, under challenge conditions saliva remains the optimal fluid for free cortisol evaluation. These advantages are of particular relevance for ambulatory assessments, studies in large cohorts, and studies in children.[15,16,32] Additionally, it is suggested that chronic stress is associated with the activation of the HPA axis (measured by salivary cortisol) and acute stress is associated with activation of the SAM system, which is reflected by salivary α-amylase and chromogranin A.[33] However, there is ongoing debate. Some authors doubt that at this stage of research α-amylase could be employed as a valid and reliable marker of sympathetic nervous system (SNS) activity and argue that α-amylase levels might reflect not only sympathetic but also parasympathetic activity.[33,34]

Only a limited number of studies have focused on the relationship between SB and perceived stress and consequently on the relationship between SB and levels of specific markers considered as representative for stress. Animal experiments concerning the relationship between emotional stress and bruxism-like activity of the masseter muscles in rats have suggested a positive correlation.[35] The results of the present study are similar to the findings of a previous study that has shown an association between SB and psychological stress sensitivity.[36] That same study found that the mean salivary chromogranin A (CgA) levels of the bruxism group were significantly increased after a stress task.[36] Furthermore, a correlation between bruxism and stress markers (neurotransmitters) has been described in the urine of adults (epinephrine/norepinephrine) and in children with high levels of sleep masseter muscle activity (epinephrine/dopamine).[37,38] On the other hand, saliva levels of CgA, which is secreted at the initial response to stress, were not significantly increased in bruxers compared to a control group.[39]

Additionally, the detection of saliva levels of cortisol was examined by Tahara et al[40] in chewing/clenching patients under a stress-loading stimulus (arithmetic exercises). A characteristic decrease of cortisol was observed by radioimmunoassay (RIA) in both cases. Chewing force seems to influence the decrease of cortisol but not α-amylase, and these findings could be attributed to the different nature of chewing/clenching and SB.[41] A recent study showed that children with SB were more likely to present low concentrations of awakening salivary cortisol.[42]

The conflicting results in the literature concerning the relationship between SB activity and stress-related biomarkers can be attributed to the multifactorial nature of SB, the different methods for assessing perceived stress, the different stress-related biomarkers studied in saliva, and the confusion between stress as a cause versus a trigger of illness. Moreover, due to HPA-axis influence by multiple extrinsic systems, the coordinated nature of the stress response may explain the heterogeneity of the relevant results.[33] In the present study, perceived stress was assessed subjectively by using the PSS questionnaire developed by Cohen et al[21] and objectively by measuring the levels of suggested stress-related biomarkers (cortisol and α-amylase) in saliva.[21,23,33] However, only cortisol levels showed a significant increase in saliva, indicating an activation of the HPA-axis rather than the SAM system in SB.

Important limitations of this study were the one-night EMG recording using BiteStrip and the one-time saliva collection. It has been reported that the diagnostic validity of the BiteStrip recording device is still lacking, especially as it compares with PSG findings.[43] Moreover, the positive predictive value of the BiteStrip was found to be 59% to 100%, with a sensitivity of 71% to 84.2%.[11,20,43] The drawback of the one-night EMG masseter muscle activity registration is that SB severity usually shows fluctuation from one night to another. Therefore, since the BiteStrip was used on one random night during the work week, its result might not be representative of SB severity. It must also be taken into consideration that with the absence of PSG and audio/video recordings, other orofacial activities such as swallowing, abnormal head movements, sighing, eye opening, and blinking might be registered erroneously as SB activity and might have influenced the results.

Concerning the saliva collection, more saliva samples from each participant would produce more reliable results, eliminating the circadian variability. Findings of the present study need to be supported by further research that should take into account audio-video recording of bruxers' grinding sounds during sleep, night-to-night SB variability, and repeated measurement of post-awakening salivary cortisol.

Conclusions

Within the limitations of this study, it has been found that SB activity was related to higher levels of perceived psychological stress and salivary cortisol. Shortcomings in the diagnostic accuracy of the BiteStrip recording device are readily acknowledged, although a moderate positive correlation between BiteStrip score and cortisol levels in bruxers was observed.

Acknowledgments

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Literature Abstract

Mesenchymal stem/progenitor cell isolation from tooth extraction sockets

Mesenchymal stem/progenitor cells used in regeneration therapy are commonly derived from the bone marrow. However, the procedures for harvesting bone marrow–derived mesenchymal stem/progenitor cells (BMSCs), commonly from the iliac crest, are associated with the risks of infection and pain. Hence, the ability to collect BMSCs from alternative sources may prove to be a better method. This study investigated the possibility of isolating stem/progenitor cells from granulation tissues in the dental extraction socket. Granulation tissue was collected from the dental sockets of six dogs 3 days after tooth extraction. These tissues were subjected to enzyme digestion in a mixture of collagenase type I and dispase in order to isolate the dental socket–derived mesenchymal stem/progenitor cells (DSCs). BMSCs were also harvested from the femur of the same animal for comparison. Using a flow cytometric analysis, DSCs tested positive for CD44, CD90, and CD271 but were negative for CD34 and CD45, similar to BMSCs. DSCs also showed osteogenic, adipogenic, and chondrogenic differentiation similar to BMSCs, with greater potential for colony formation, proliferation, and motility. In addition, DSCs were found to be effective in regenerating cementum-like and periodontal ligament-like tissues as well as alveolar bone when transplanted into a one-walled defect model. The authors concluded that the dental socket could be a novel source for isolating mesenchymal stem/progenitor cells from bone. However, this study was limited by the inability to characterize DSCs in detail. Future studies with human cells may allow for better characterization of DSC and increased understanding of their biological properties.

Nakajima R, Ono M, Hara ES, Oida Y, Shinkawa S, Pham HT, Akiyama K, Sonoyama W, Maekawa K, Kuboki T. *J Dent Res* 2014;93:1133–1140. **References:** 25. **Reprints:** T. Kuboki, Department of Oral Rehabilitation and Regenerative Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan. Email: kuboki@md.okayama-u.ac.jp—*Teo Juin Wei, Singapore*