Influence of different setting positions of maxillary major connectors on salivary stress markers

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We produced experimental plates covering different areas of the palatal mucosa with different shapes, and attempted to evaluate them objectively by measuring stress reactions in terms of salivary amylase activity and secretory immunoglobulin A (slgA) concentration. All subjects were examined under the four experimental conditions of no plate (C), an anterior plate (A), a middle plate (M) and a posterior plate (P). Saliva samples were collected at 0, 5, 10 and 20 min after the start of each condition. Subjects were then asked to complete a Visual Analogue Scale (VAS) during each condition. Amylase activity of saliva collected at 5 min was significantly higher for P than for C, A or M. The VAS score was significantly higher for P than for A or M. However slgA concentration in saliva collected at 5 min was significantly lower for P than for C or M. Different patterns were seen for both amylase activity and VAS scores. Evaluation of amylase activity and the VAS of discomfort showed that covering the posterior region of the palate caused severe discomfort in the subjects. The use of salivary stress markers is a valuable tool for the objective evaluation of the design of maxillary partial dentures. (J Osaka Dent Univ 2012; 46: 127-135)

Key words: Saliva; α-Amylase; slgA; Stress; Discomfort; Palatal mucosa

INTRODUCTION

Upper partial dentures are often made using major connectors running along the palate. The broader the major connector, the more capable it is of sustaining occlusal pressure, but the more likely it is to inhibit sensation in the mouth. Depending on its positioning, the major connector may also cause phonation disorders or trigger a gag reflex. To date, evaluation of the shape and positioning of major connectors has been based on the degree to which they elicit a foreign-body sensation or pronunciation difficulties. However, this method of evaluation relies on the subjective reporting of the patient or the subject’s perception of discomfort.¹ In fact, subjective modes of evaluation are common for both major connectors and conventional dentures,²-⁴ while objective assessments are rare.

Measurement of biochemical stress markers in saliva is a simple and non-invasive method to quantify and objectively evaluate stress.³-⁷ We found that eliminating discomfort by adjusting the denture led to a decrease in the concentration of the salivary stress marker cortisol. However the extent of the decrease exhibited considerable individual variation and subjective evaluations of the patients were not always consistent.⁶ Meanwhile, Ito et al.⁷ used an experimental palatal plate to recreate the pain stimulus caused by an actual denture, and discovered that salivary cortisol concentration and amylase activity increased.

In the present study, instead of major connectors, we used experimental palatal plates that differed both in terms of shape and position of palatal mucosal coverage, and attempted to objectively evaluate the stress reaction resulting from fitting these
plates by measuring the salivary biomarkers of amylase activity and secretory immunoglobulin A (slgA) concentration.

MATERIALS AND METHODS

Subjects

Subjects were healthy, dentulous undergraduate and postgraduate students enrolled at Osaka Dental University, and residents at the Osaka Dental University Hospital who provided informed consent to participate in the study (5 men and 5 women) mean age of 26 ± 3 years. They were instructed to refrain from strenuous exercise and alcohol consumption, and to get adequate sleep the day before the experiment. Subjects who were smokers were requested to refrain from smoking on the day of experiment, and consumption of food and drink was prohibited from 2 h prior to commencement of the experiment until its completion. The experiment was not conducted during the menstrual period of female subjects as biomarker levels are known to fluctuate during this time menstruation. This study was conducted with the approval of the Osaka Dental University’s Ethics Review Board (ERB No.100918).

Equipment

After obtaining preliminary impressions using alginate impression material, individual trays were fabricated and silicone impressions of the maxillary arch were taken. The maxillary palatal plates were fabricated with a thickness of approximately 0.8 mm using denture base acrylic resin (Palapress Vario; Heraeus Kulzer, Hanau Germany) to enable visual inspection of the plate’s undersurface. Three types of plates were prepared, each with different positions; the anterior of the palate (forward of the bilateral cuspsids; plate type A), the middle of the palate (running laterally between the first premolar and the first molar; plate type M), and the posterior of the palate (running laterally from the first molar to the Ah-line; plate type P) (Fig. 1). Wire clasps were also fitted to the first molars on plate types M and P, and the premolars on plate type A to act as retainers. The fit of the plates was tested prior to the experiment in order to adjust them to where their undersurface conformed evenly to the subject’s palate.

Experimental procedure

The subjects each of the following four experimental conditions: (1) no plate (type C); (2) placement of plate type A; (3) placement of plate type M; and (4) placement of plate type P. First, the subjects were instructed to sit in a dental chair (Spaceline EMCIA Type II FT; Morita Corporation, Osaka, Japan) in a room maintained at a temperature of 25°C. Next, after having the subjects remain still for 10 min, the palatal plate was inserted for 5 min. It was then removed and the subjects were again instructed to remain still, this time for 15 min. After undergoing each experimental condition, the subjects were to remain still for 10 min. Salivary amylase activity was measured immediately after fitting the plate (0 min) and upon removal of the plate (5 min), as well as at 10 and 20 min after removal by immersing the tip of an amylase monitor (Salivary Amylase Monitor; Nipro, Osaka, Japan) in the subject’s saliva for 30s. To measure the slgA concentration at the same time as amylase activity, saliva samples were collected by placing a cotton swab in a tube (Salivette; Sarstedt, Rommelsdorf, Germany) under the subject’s tongue for 2 min at each measurement point. While resting at completion of each experimental condition, the subjects were asked to subjectively evaluate the
Enter the room  
Place plate, collect saliva  
Remove plate, collect saliva  
Collect saliva  
Collect saliva  
Fill out VAS

Fig. 2 Protocol for the experiment. Each 20-minute session was repeated three times, with the order of the three conditions randomized. The three 20-minute sessions were repeated on different days.

palatal plate by drawing a line on a 100-mm Visual Analogue Scale (VAS), with 0 indicating “absolutely no discomfort” and 100 indicating “intolerable discomfort” (Fig. 2). The order of experimental conditions C, A, M, and P was selected randomly.

The above-mentioned process was performed on each subject a total of three times on different days at the same time between 11:00 and 19:00. After measuring the weight of each saliva sample, the saliva was separated from the cotton swab as soon as possible. The resulting fluid was dispensed in quantities of 100 μL, which were kept frozen at –50°C until analysis.

On the day of measurement, samples were allowed to thaw at room temperature, and were then reacted using an immunoassay kit (Salivary Secretory IgA Indirect Enzyme Immunoassay Kit; Salimetrics, State College, PA, USA), after which their respective concentrations were measured using a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA) at 450 nm.

Statistical analysis

Measured saliva weight, amylase activity and slgA concentrations were statistically processed by repeated measures analysis of variance (ANOVA) using the subjects, experimental conditions and time points as main effects, and Bonferroni’s test were then performed for multiple comparison. F-ratio variance due to repeated measures ANOVA was subjected to degrees-of-freedom adjustment using the Greenhouse-Geisser method, based on the results of Mauchly’s sphericity testing, and significance levels were determined.

Correlations between each biomarker and VAS discomfort scores were determined for each sampling point, and the correlation coefficient was found for measured amylase activity values and slgA concentrations. A significance level of 0.05 was applied to all tests, and the results were analyzed using statistical analysis software (SPSS 12.0 J; SPSS Japan, Tokyo, Japan).

RESULTS

Saliva weight

Although the mean saliva weight with P (2.16 mg) was significantly greater than that with C (1.76 mg) at 0 min, no significant differences were observed for any subsequent time points (Fig. 3).

Amylase activity

Mean amylase activity of saliva collected at 0 min was significantly higher for P (52.73 KU/L) than for C (39.97 KU/L) or A (39.5 KU/L) (Fig. 4). In addition, there was a significant difference between the amylase activity at 5 min for P (62.83 KU/L) and that for C (42.23 KU/L), A (46.82 KU/L) and M (48 KU/L), and between the amylase activity at 10 min for P (47.77 KU/L) and that for C (40.43 KU/L). However, no significant differences were seen between any of the experimental conditions at 20 min.

slgA concentration

Mean slgA concentration in saliva collected at 5 min was significantly less for P (60.58 μg/mL) than for C (76.56 μg/mL) or M (79.45 μg/mL) (Fig. 5). However, no significant differences were seen between any of the experimental conditions at 0, 10 or 20 min.

Relationship with VAS discomfort scores

Mean VAS score was significantly greater for P (56.53) than for A (32.59) or M (39.78) (Fig. 6).
Correlation coefficients between the VAS discomfort score, amylase activity and slgA concentration revealed a fairly low positive correlation for amylase activity at 0 min (0.363), 5 min (0.277), 10 min (0.288) and 20 min (0.275), whereas correlations between slgA concentration and VAS score were low at every time point (Table 1). Correlations between amylase activity and slgA concentration were also low at each time point (Table 2).
DISCUSSION

Methods

Previous methods used to evaluate stress in clinical settings have included questionnaires\textsuperscript{12-14} and measurement of serum biomarkers.\textsuperscript{15, 20} However, questionnaires may elicit erroneous results due to embellishments by respondents, while measurement of serum biomarkers involves invasive collection of blood samples, which may act as a stressor to the subject. Compared to these methods, collection of saliva samples is a non-invasive method of quantifying and objectively determining stress.\textsuperscript{21} This method was therefore adopted in the present study because it enables evaluation of stress with minimal influence by stimuli other than that caused
by the experimental palatal plates.

**Experimental palatal plates**
The A, M and P palatal plates were positioned in the anterior, middle and posterior of the palate respectively, with each plate fabricated to a uniform thickness of 0.8 mm. If the plate’s mucosal surface is uneven, it can rub painfully against the palatal mucosa, thereby acting as a stressor that can interfere with the measurement of plate-induced stress. The adaptation was checked and the necessary adjustments were made prior to commencing the experiment.

**VAS**
The subjects were instructed to report their perception of pain after each experiment by drawing a line on a 100-mm VAS scale, wherein the left side of the scale indicated “absolutely no discomfort” and the right side indicated “intolerable discomfort”. VAS is also used in the field of dentistry to assess treatments involving pain and was adopted in the present study as a subjective method of evaluating palatal plate-induced discomfort due to its ease of use and suitability for assessing short-term changes.

**α-amylase activity and slgA concentration**
When sympathetic nerves are stimulated by pain and other stressors, the adrenal medulla releases catecholamines such as noradrenalin into the bloodstream (sympathetic adrenal medullary system), and salivary α-amylase (sAA), which is regulated by noradrenalin, is secreted. Secretion of sAA can also be regulated by direct nervous action and has been shown to have a response time of one to several minutes. In the present study, α-amylase was used as a biomarker of stress because its reaction pathway and time are known to some extent, and it can therefore effectively enhance the reliability of objective stress evaluation by simplifying the determination of whether a stress reaction has occurred in response to experimental conditions.

Immunoglobulin A is an antibody produced by B cells in the blood. It binds with a secretory component (SC) produced by epithelial cells to form slgA, which is subsequently incorporated into these cells and transferred to the luminal mucosa whereupon it is secreted. slgA works to prevent the proliferation of pathogens in mucosa within the mouth, respiratory tract and intestinal tract. Because the concentration of slgA fluctuates in response to biological stress, it is used as a stress biomarker. However its reaction pathway remains unclear. Determining the types of stimuli to which slgA concentrations react, as well as their reaction time, would therefore prove useful for future research.

**Results**
The fact that saliva weight for experimental condition P only differed significantly from that of C at 0 min suggests that a stimulus applied to the posterior part of the palate increases secretion of saliva. This can, however, be described as a temporary reaction, as there were no significant differences and no major changes in saliva weight from 5 min onward.

VAS discomfort scores were significantly higher for P than for M and A, indicating that discomfort was subjectively assessed by subjects to be greatest at the posterior palate region of P. Meanwhile, amylase activity readings were similar for C and A and were highest for P, which implies that the anterior palate area of A is is unlikely to get stressed, whereas the posterior palate area of P is susceptible to stress. This also suggests that discomfort affects amylase activity. Nevertheless, comparison of VAS scores and amylase activity values did not reveal any high correlation coefficients, which indicates that although the amylase activity readings partly reflect the effects of discomfort, they also reflect the effects of other types of stimuli.

Although previous studies have adopted different methods of applying stress, it is clear that pain and other invasive stimuli trigger a demonstrable increase in amylase activity, and the findings of the present study also suggest that the sympathetic nervous system was activated by stimulation of the posterior palatal mucosa.
Amylase activity for P was significantly higher than that of C, A or M at 0, 5 and 10 min, although no significant differences between any of the experimental conditions were observed after 20 min. This suggests that the stress reactions reflected by amylase activity had disappeared 15 min after the plate was removed. We therefore believe that the experimental conditions need to be imposed for a longer period in order to confirm the changes in amylase activity over time in response to stress.

slgA concentration is used as an indicator of acute or chronic stress. Therefore, we measured it in the present study based on the assumption that a response would also be observed for palate plate-induced stress. Although there were significant differences in the slgA concentration under each experimental condition at 5 min, these differences ceased to be significant at 10 min and the respective concentrations decreased. This finding implies that approximately 5 min is required for slgA concentrations to exhibit a response to stress stimuli, and that this response is only manifested for a relatively short time. On the other hand, in contrast to amylase activity, the slgA concentration of P was significantly less than that of M or C at 5 min. This outcome indicates that the slgA concentration tends to decline when the palatal plate is fitted in the posterior region. Although slgA concentration is recognized as a useful biomarker of stress, various results have been described with respect to its response, with some studies reporting that chronic stress triggers a reduction in slgA and that acute stress causes it to increase. However, an experiment by Ring et al. exposing participants to acute stress using the cold pressor test found that slgA concentration decreased, which concurs with the results of the present study.

slgA concentration has also been shown to diminish in response to passive stress. If we define passive stress as a type of acute stress that can only be handled by passive means, the cold pressor test and dental treatment can be classified as forms of passive stress. Moreover, taking the finding that slgA concentration tends to decline during strenuous exercise in conjunction with the finding that the decremental response of slgA to passive stress is reduced by α-receptor blocking drugs, it can be inferred that slgA secretions are linked to sympathetic nervous system activity. However, further research and clarification is needed.

Meanwhile, since the correlation coefficient for slgA concentration and VAS discomfort score indicated that there was virtually no correlation between the two, it is possible that slgA concentration reflected a response to factors other than discomfort. In addition, there was no similarity in the variation patterns of stress-induced amylase activity and slgA concentration, and only a very weak correlation between the two, suggesting that these biomarkers are responding to different biological reactions to stress. Although the present study imposed eating, drinking and smoking restrictions on subjects during the experiment, the impact of subject lifestyles and other environmental factors on the results cannot be ruled out. slgA concentration is also affected by secretions in response to daily events and emotions and objective evaluation based on the use of different types of biomarkers would thus facilitate more accurate detection of biological reactions to stress.

Previous subjective evaluations have indicated that stimulation of the posterior palate causes discomfort. In the present study, objective evaluation using salivary amylase activity as a biomarker has also demonstrated that this form of stimulation causes stress, and that stimulation of the anterior and medial regions of the palate is unlikely to cause stress. On the other hand, our evaluation of stress based on slgA concentration yielded a different outcome to that of subjective evaluations. The findings of the present study that stress biomarkers can be used to evaluate differences in the extent of stress experienced by subjects, and that the combined use of different stress biomarkers enables measurement of biological reactions to stresses other than those perceived in subjective evaluations demonstrate that objective evaluation of stress using salivary markers is useful for the design and placement of palatal plates in clinical prosthetics.
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