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Evaluation of salivary cortisol levels in relation to dento-maxillary prosthesis adjustment

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Running title: Dento-maxillary prosthesis adjustment and salivary cortisol

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Abstract

Purpose: The purpose of this study was to investigate the influence of dento-maxillary prosthesis adjustment procedure on levels of salivary cortisol.

Methods: Nine participants (six men, three women, mean age 65.9 years) took part in this study. Saliva samples were collected before and after dento-maxillary prosthesis

adjustment during the four different visits. Free cortisol levels were determined using a salivary cortisol immunoassay kit (expanded-range high-sensitivity salivary cortisol enzyme immunoassay kit, Salimetrics, State College, USA). Besides, original self-report sheets, a 35-item food intake questionnaire, the University of Washington Quality of Life (UW-QOL) questionnaire version 4, and the Geriatric Oral Health Assessment Index (GOHAI) questionnaire were also administered. The changes of salivary cortisol levels were analyzed using 2-level multilevel linear regression, with adjustment for age, sex, and time. Wilcoxon signed-rank test was used to compare scores of the food intake questionnaire, UW-QOL questionnaire, and GOHAI questionnaire.

Results: Salivary cortisol levels decreased significantly after carrying out the dento-maxillary prosthesis adjustment procedure. During the third adjustment, the salivary cortisol levels were significantly low. In addition, salivary cortisol levels of participants aged 70 years and over were significantly higher than other aged groups. The total scores for grade III to V of the food intake questionnaire increased significantly. Other questionnaires had a trend toward increasing scores, yet the differences were not significant.

Conclusions: Within the limitations of this study, the results suggest that a reduction in symptoms of discomfort may have an influence on the decrease of salivary cortisol levels in dento-maxillary prosthesis wearers.

1. Introduction

Defects of the head and neck in patients with head and neck cancer (HNC) often lead to functional impairments involving speech, mastication, and swallowing. A dento-maxillary prosthesis can help rehabilitate these impairments, leading to improvements in speech [1], chewing ability [2], and quality of life (QOL) [3] in HNC patients.

However, the structure of a dento-maxillary prosthesis may be complicated based on the patient's defect and the reconstructed site. Furthermore, many patients undergo radiotherapy, which can cause sensitivity of tissues around the area of defect leading to pain and discomfort after delivery of a new prosthesis. Moreover, the occlusion is often complex because of mandibular shift, the presence of flaps, and contracture of scar tissue, even when numerous teeth remain. These issues may extend the adjustment period and delay use the prosthesis, which could be an additional source of stress.

To reduce discomfort of a dento-maxillary prosthesis, a visual inspection or fitness test for traumatic ulcer is useful. Subjective patient reports, the technical skill and experience of the prosthodontist are also important factors in assessment of patient comfort and stability of prosthesis. In addition, an objective evaluation of assessing changes in patient stress levels during the phase of dento-maxillary prosthesis adjustment, provides useful knowledge. This would serve as valuable information to provide to patients when explaining and obtaining consent for treatment.

Questionnaires [4] as well as biological samples such as serum [5], urine [6], and saliva [6, 7] have been used for stress assessment. Saliva sampling in particular is often used because it is noninvasive and less burdensome to obtain. Salivary cortisol is a useful objective marker of stress [8] that is highly correlated with serum cortisol levels [9]. A number of researchers have used salivary cortisol in assessments of stress in various

situations of daily life [10, 11].

In dentistry, deviations in salivary cortisol from the sound patient's levels has been found to be associated with periodontitis [12], temporomandibular disorders [13], and dry mouth [14], and dental anxiety of treatment [15-17]. Morning changes in salivary cortisol levels also have been observed in dento-maxillary prostheses wearers [18]. However, multiple testing and measurements of salivary cortisol during the phase of dento-maxillary prosthesis adjustment has not been investigated.

The aim of this study was to investigate the influence of dento-maxillary prosthesis adjustment procedure on levels of salivary cortisol. The adjustment phase is considered to be a stressful period in dento-maxillary prosthesis wearers. The null hypothesis was that there is no difference in salivary cortisol levels in the following periods: (1) before and after the actual procedure of dento-maxillary prosthesis adjustment in the clinic for each visit; (2) throughout the entire dento-maxillary prosthesis adjustment phase from the first visit.

2. Materials and methods

2.1. Participants

The participants in this study were six men and three women (mean age 65.9 years). The inclusion criteria was: positive history of maxillectomy and/or mandibulectomy due to HNC, receipt of a new dento-maxillary prosthesis, and need for prosthesis adjustment. The exclusion criteria was: habitual smoking; use of oral contraceptives; presence of severe periodontitis or any other general infectious disease; and inability to speak, read, or understand Japanese. All participants received the new dento-maxillary prosthesis and had adjustments performed at the Maxillofacial Prosthetics-Clinic for Oral and

Maxillofacial Rehabilitation, certain University Hospital.

This study was approved by the Ethics Committee of the Faculty of Dentistry in certain two universities (Approval No. 1131 and No. 2015-3-4).

2.2. Measurements

Saliva sampling and cortisol assay

The study design in a flowchart form is shown in Fig. 1. Saliva samples were collected before and after dento-maxillary prosthesis adjustment during clinic visits on each of the four different visits. The sampling time was between 13:00 and 15:00 in consideration of the circadian rhythm of salivary cortisol. Participants were asked to come to the clinic within this time period, and instructed not to eat, drink, brush their teeth, or do hard physical exercise one hour before their appointment to minimize considerable changes in salivary cortisol levels. After arrival, participants were asked to remain quiet and collect saliva for five minutes in accordance with the passive drool method. This was repeated again after the adjustment procedure to get a second sample and total 72 samples were collected. Saliva samples were stored at -20 °C immediately after collection. On the day of analysis, the samples were thawed to room temperature (20–23.3 °C) and free cortisol levels were determined using an expanded-range high-sensitivity salivary cortisol enzyme immunoassay kit (lot 1411508, Salimetrics, LLC, State College, PA, USA). All samples from an individual participant were assayed in the same batch. Inter- and intra-assay variations were below 9% and 5%, respectively.

2.3. Self-report sheets

Original self-report sheets were created for this research, as described in the previous publication [18]. The following aspects were included on the self-report sheets for the

first adjustment visit: (a) oral conditions; (b) medical history; (c) experience with use of dental prosthesis; (d) symptoms of dento-maxillary prosthesis discomfort; and (e) factors that may influence salivary cortisol levels, such as wake-up time, use/nonuse of an alarm clock, menopausal status, medication use, and presence of white coat syndrome.

The following items were incorporated on the self-report sheets for the second, third, and fourth adjustment visits: (a) symptoms of dento-maxillary prosthesis discomfort; (b) other oral symptoms; (c) whether the participant had experienced a stressful event since the last visit; (d) wake-up time, and (e) use/nonuse of an alarm clock. The responses on the self-report sheets filled by the participant on arrival were confirmed by the doctor at the chairside.

2.4. Questionnaires

To achieve a much more versatile evaluation, three kinds of questionnaires were used.

A 35-item food intake questionnaire was used to evaluate the masticatory ability of the participants. Participants were asked to choose one of five categories for each food: 2 (easily eaten, 2 points), 1 (eaten with difficulty, 1 point), 0 (cannot be eaten, 0 points), Δ (do not eat because of dislike, 0 points), and □ (have not eaten since starting to wear dentures, 0 points). The 35 items were also classified into five grades of masticatory difficulty, and a masticatory score was calculated by summing the points for the foods in each grade [19].

Because of its worldwide use in patients with HNC, the University of Washington Quality of Life (UW-QOL) questionnaire (version 4, translated into Japanese) was used to assess the QOL of the participants. This questionnaire contains 12 domains and three global questions [3].

The Geriatric Oral Health Assessment Index (GOHAI) questionnaire was used to assess oral health-related quality of life (OHRQoL) on a five-point scale with 12 questions analyzing physical function, psychosocial function, pain and discomfort. The total GOHAI score was calculated by summing the points ranging from 12 to 60 [20].

Participants were requested to complete the self-report sheet at every adjustment visit. The food intake questionnaire, UW-QOL questionnaire, and GOHAI questionnaire were completed at only the first and fourth adjustments (Fig. 1).

2.5. Statistical analysis

Our data consisted of four sets of measurements from each participant for the four adjustment visits. A set represents a pair of the repeat measurements carried out in one visit. Therefore 2-level multilevel linear regression was used to estimate the effect of carrying out the procedure of dento-maxillary prosthesis adjustment, on salivary cortisol levels. Adjustment for age, sex, and time was done. The Stata software package (Version 13.1, StataCorp LP, College Station, TX, USA) was used for the analysis.

Scores for the food intake questionnaire, UW-QOL questionnaire, and GOHAI questionnaire at the first and fourth adjustment visits were compared using the Wilcoxon signed-rank test. Also, Spearman's rank-correlation coefficient was used to determine the correlation between three questionnaire scores and salivary cortisol levels. The IBM SPSS Statistics Desktop (Version 23.0, IBM Japan, Tokyo, Japan) was used for the analyses. Statistical significance was set at $P < 0.05$.

3. Results

A summary of the participant characteristics is presented in Table 1.

3.1. Self-report sheets

There were no participants who reported: severe previous medical history except HNC, taking medicine regularly, grave oral symptoms, and declared about white coat syndrome. All participants woke up in the morning (six am-nine am). Five participants had prior experience of using dental prosthesis before HNC treatment.

At the first adjustment, all participants complained of dento-maxillary prosthesis discomfort such as pain, difficulty of pronunciation and food residue. Ulcers were seen in the participants who had pain.

At the second adjustment, participants who complained of pain had decreased, but uncomfortable feeling, difficulty in pronunciation and food residue complaints were still reported.

At the third adjustment, one participant complained of pain whereas and few of them complained of slightly uncomfortable occlusion and difficulty in pronunciation. Other participants reported “nothing” for the symptoms/complaints section.

At the fourth adjustment; there were two participants who had difficulty in pronunciation, were conscious of the thickness of the denture base, but none complained of pain.

Regarding the account of any stressful event as required; comments such as “tiredness because of going out for many hours”, ”concern for the results of computed tomography imaging done during a regular medical checkup”, “foot pain because of a foot cramp”, “worry about my son” and “angina treatment” were reported.

3.2. Salivary cortisol levels

Results of the statistical analysis are given in Table 2. Salivary cortisol levels decreased significantly after carrying out the dento-maxillary prosthesis adjustment procedure. During the third adjustment, the salivary cortisol levels were significantly low. Moreover, salivary cortisol levels of participants aged 70 years and over were significantly higher than other aged groups.

3.3. Questionnaires

The mean masticatory score on the food intake questionnaire increased from 47.0 to 54.8. Although, there was no significant difference in the total scores for grade I and II, the total scores for grade III to V increased significantly at the fourth adjustment ($p=0.039$).

Foods for which scores increased are shown in Table 3; Grades I through V indicate masticatory difficulty.

The mean total scores for 12 domains and three global questions on the UW-QOL questionnaire at the first and fourth adjustments stages were 950.0, 984.4, 149.4 and 166.7 respectively. The mean scores on the GOHAI questionnaire at the first and fourth adjustments stages were 42.8 and 45.9, respectively. Although both questionnaires had a trend toward increasing scores, the differences were not significant. Also, there were no correlation between the scores of three questionnaire scores and salivary cortisol levels.

4. Discussion

In the present study, the aim was to measure salivary cortisol levels twice during each of the four dento-maxillary prosthesis adjustment procedures done after delivery of the new dento-maxillary prosthesis. To provide a broad assessment, evaluation of masticatory ability, QOL, and OHRQoL were performed in addition to salivary cortisol measurement.

A significant decrease in salivary cortisol over the dento-maxillary prosthesis adjustment period of study was observed in this study.

Maxillofacial prostheses, of which dento-maxillary prostheses are a part of, are used to replace part or all of any stomatognathic and/or craniofacial structure [21]. Congenital and traumatic factors can cause a wide variety of defects that are often challenging to treat with dento-maxillary prosthesis and requires careful adjustment.

Participants in this study had several types of defects as well as a limited stress-supporting area for their dento-maxillary prosthesis. The first adjustment was therefore scheduled for shortly after prosthesis delivery (8.5 ± 1.4 days). As symptoms of discomfort decreased, the intervals of the second, third and fourth adjustments were set longer than that of the first adjustment. It required 60.1 ± 13.8 days to complete the four adjustment visits. Additional fine adjustments were needed in a few cases, but no participants complained of pain at the fourth adjustment. Decreased salivary cortisol has been reported in certain therapies [10, 17], and consequently relief of symptoms may had a positive effect on the level of salivary cortisol. From this point of view, it was considered that release from the discomfort symptoms may affected the salivary cortisol levels during dento-maxillary prosthesis adjustment.

On the other hand, high levels of salivary cortisol in elderly participants was observed in this study. This result is consistent with previous research reporting a tendency toward high salivary cortisol levels among the elderly [7, 22]. Considering the results of the present study, the complicated structure of dento-maxillary prostheses, decline of adaptability, and reduced wound healing may have caused more stress in elderly participants. Although the number of participants in this study was small, these findings may provide useful information for clinical treatment of patients with HNC.

HNC patients generally face many harsh realities, including the possibility of recurrence or metastasis. Moreover, advanced age, existence of underlying disease, and decrease of saliva due to radiotherapy are also common, and are important to consider when assessing saliva in HNC patients. Factors such as habitual smoking and use of oral contraceptives are also known to affect salivary cortisol levels. The impact of many other medications or treatments is still unknown. Considering the above factors, changes in salivary cortisol levels were investigated during the period of dento-maxillary prosthesis adjustment from many aspects in this study.

Contrary to the expectations, the salivary cortisol level was significantly low only at the third adjustment. As mentioned above, the researchers asked participants whether they had experienced a stressful event since their last visit. Most participants answered “no” to this question at both second and fourth visits. However, at the third visit, five participants reported events as elaborated in the result section. As the salivary cortisol measurement was done only during prosthesis adjustment day, it is difficult to determine the precise reason for the decrease. Nonetheless, there is a possibility that such anxieties may have disturbed their salivary cortisol secretion pattern.

The significant increase in the total scores of grade III to V for food intake questionnaire and Table 3 show that participants were able to eat more tough foods after delivery of the dento-maxillary prosthesis. At the first adjustment visit, five participants replied that chewing was the most important issue for them in the UW-QOL questionnaire. This number decreased to two at the fourth adjustment; thus, improvement of masticatory ability may have improved QOL, though the changes were not statistically significant. Age, sex, neck dissection, and radiotherapy have been reported to affect health-related QOL in HNC patients [3]. In the present study, UW-QOL scores were not significantly

different among the three participants who had undergone neck dissection and the five who had undergone radiotherapy compared with those who did not undergo either procedure. However, these scores were similar to those found in a previous study of patients with laryngectomy [23].

GOHAI questionnaire scores have been reported previously in elderly Japanese individuals [20] and patients who underwent mandibulectomy or glossectomy [2]. Although these scores cannot be simply compared, they are similar to those obtained at the fourth adjustment visit. These findings suggest that specific satisfaction was achieved after dento-maxillary prosthesis adjustment.

Stress factors always exist in daily life, and researchers must consider every possibility. In this study, expanded version of the self-report sheets were used and the researchers tried to communicate well with participants in order to gather as much information as possible. However, it was difficult to pinpoint the factors that influenced changes in salivary cortisol levels and questionnaire scores. Furthermore, the observed changes may be only temporary. A more detailed research plan—for example, including long-term observations and comparison with general denture-wearers—is thus required in the future to further elucidate causes of stress in dento-maxillary prosthesis wearers.

5. Conclusion

Within the limitations of this study, the results suggest that a reduction in symptoms of discomfort may have an influence on the decrease of salivary cortisol levels in dento-maxillary prosthesis wearers.

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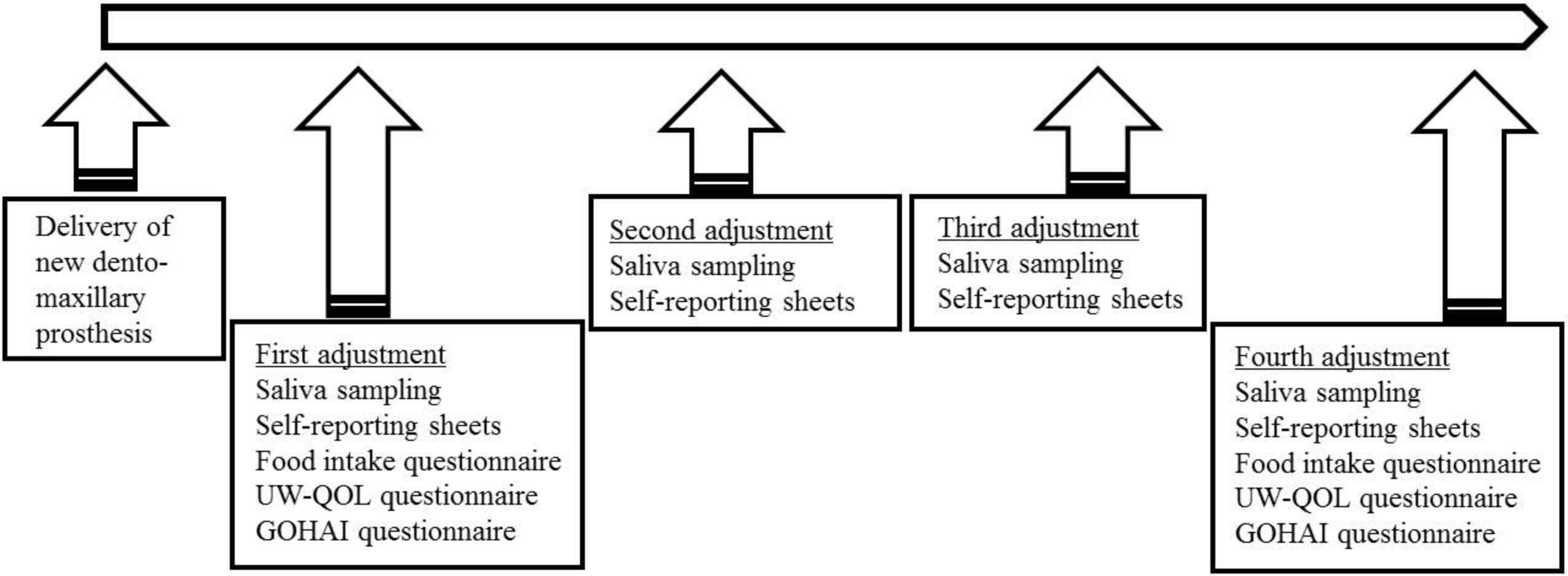

Fig. 1 - Flowchart of the sampling procedure. GOHAI, Geriatric Oral Health Assessment Index; UW-QOL, University of Washington Quality of Life.

Table 1 - Participant characteristics[a]

Participant	1	2	3	4	5	6	7	8	9
Age (years)	76	55	68	65	79	44	66	70	70
Sex (M/F)	M	M	F	M	F	M	M	F	M
Pathological diagnosis	SCC	ECA	SCC	SCC	SCC	GCOC	SCC	AC	SCC
Defect location	MF/MN	MN	MF/MN	MX/MN	MX	MX	MN	MX	MX
Neck dissection (+/-)	(-)	(+)	(-)	(+)	(-)	(-)	(+)	(-)	(-)
Radiotherapy (+/-)	(+)	(+)	(-)	(+)	(-)	(-)	(+)	(+)	(-)
Reconstruction (+/-)	(-)	(+)	(+)	(+)	(+)	(-)	(+)	(-)	(-)
No. of maxillary teeth[b]	0	13	0	7	6	3	9	11	5
No. of mandibular teeth*	0	8	0	14	14	10	5	14	12

[a]SCC, squamous cell carcinoma; ECA, epidermoid carcinoma; GCOC, ghost cell odontogenic carcinoma; AC, adenocarcinoma; MX, maxilla; MN, mandibular; MF, mouth floor.

[b]Number of maxillary/mandibular teeth containing bridge pontic, root cap, and stud attachment.

Table 2 - Salivary cortisol levels for the four dento-maxillary prosthesis adjustment visits

		95% CI[a]		
	B[b]	Lower	Upper	P-value
Day (reference: first adjustment)				
2	-0.139	-1.653	1.374	0.857
3	-1.787	-3.300	-0.273	0.021*
4	0.431	-1.082	1.945	0.577
Time (reference: pre-treatment)	-1.346	-2.417	-0.276	0.014*
Sex (reference: male)	-1.861	-4.323	0.602	0.139
Age (reference: 40–59 years)				
60–69 years	2.915	-0.101	5.931	0.058
≥70 years	4.057	1.041	7.073	0.008*
Constant	3.488	0.998	5.977	0.006

CI[a], confidence interval.

B[b], Coefficient.

P* < 0.05; 2-level multilevel linear regression.

Table 3 - Foods whose intake led to increased scores

	From [0] or [□][b] to [1]	From [1] to [2]	From [0] or [□] to [2]
Grade I[a]	Boiled cabbage	Boiled taro[c], bananas, boiled onions, boiled cabbage	Boiled taro, bananas, boiled carrots, boiled onions
Grade II	Ham, raw cucumber	Boiled fish paste patty, strawberries, ham, raw cucumber	Boiled chicken
Grade III	Boiled beef, apples, pickled eggplant, fried rice crackers	Raw cabbage, fried rice crackers, pickled eggplants	Roast chicken
Grade IV	Pickled radish, rice cakes, pork cutlet	Peanuts, pork cutlet, pickled radish	Roast pork
Grade V	Vinegared octopus	Takuwan[d], raw carrots, jellyfish, dried cuttlefish, vinegared octopus	Raw abalone

[a]Grades I to V indicate increasing masticatory difficulty.

[b]□, have not eaten since starting to wear dento-maxillary prosthesis.

[c]Taro, Japanese taro potato.

[d]Takuwan, harder to masticate than a regular pickled radish.