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ORIGINAL ARTICLES

Circadian variation in the concentration of salivary α-amylase in healthy humans

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In the present study, we investigated circadian variation in the concentration of salivary α-amylase (CsA), which is known to be affected by stress, using a salivary amylase monitor with a chip. The CsA was measured three times a day (diurnal; morning, noon, and evening), three times a week (daily; Monday, Wednesday, and Friday) and three times a month (weekly; 1st, 2nd, and 3rd weeks). Ten healthy male participants aged 25-30 years were recruited.

A three-way analysis of variance (ANOVA; [diurnal, daily, and weekly]) was used. Multiple comparisons between groups were performed using the Tukey honest significant difference post-hoc test. The ANOVA found a significant main effect in diurnal and daily measurements. When the CsA was compared within the diurnal groups, post-hoc test found that the values in the noon and evening were significantly higher than those in the morning ($p<0.01$). Similarly, the CsA values on Fridays were significantly lower than those on Mondays. These results suggest that CsA shows a diurnal and daily fluctuation.

Key words : α-salivary amylase, stress, circadian variation

Introduction

Masticatory muscle pain in temporomandibular arthrosis is commonly caused and exacerbated by psychological (e.g., stress), social, and environmental factors[1)]. Therefore, it is necessary to establish a reliable method for assessing stress in the clinical setting. A typical method of assessing stress includes biochemical analysis of blood[2, 3)]. However, blood withdrawal may in turn result in additional stress[2, 3)].

Stress also affects the autonomic nerve system and increases the sympathetic activity[4, 5)]. It also affects the concentration of salivary α-amylase (CsA) and the flow rate of saliva[4, 5)]. Therefore, the measurement of CsA is a useful and reasonable method to assess stress in humans. For this purpose, a salivary amylase monitor with a chip has been developed[2)], and used in certain situations[2, 5)].

Stress is also affected by differences among individuals[4)]. Thus, CsA may also show varying trends in individuals. To measure stress in this scenario, we believe that the salivary amylase monitoring device may be feasible and easy to use.

In the present study, we measured the circadian variation in CsA diurnally, daily, and weekly using this device.

Methods

Ten human male participants (age: range, 25-30 years, mean age, 26.7 ± 1.6 years) were recruited. They had no loss of teeth other than the third molar, no abnormalities in the stomatognathic system, no chronic disease, and no history of endocrine disease.

The subjects were prohibited from eating and drinking anything other than water 2 hours prior to their test. Just prior to the test, they were instructed

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to gargle sufficiently using distilled water.

To measure CsA, an enzyme analyzer, including the salivary amylase monitor (Nipro, Osaka) and a salivary amylase monitor chip (13 × 6 × 120 mm, approximately 3 g; Nipro) was used.

The chip monitor, which contained 2-chloro-4-nitrophenyl-4-O-β-d-galactopyranosyl maltoside (Gal-G2-CNP) as the substrate for detecting α-amylase[6], was inserted under the tongue, and the sublingual saliva was collected for 30 s (Fig. 1(a)). The CsA (UNIT/L; kU/L) was displayed on the screen of this device (Fig. 1(b)).

The CsA was measured three times a day (diurnal: morning [8:30-9:00], noon [12:00-13:00], and evening [16:00-17:00]), three times a week (daily: Monday, Wednesday, and Friday), and three times a month (weekly: 1st, 2nd, and 3rd weeks).

Three-way analysis of variance (ANOVA; diurnal × daily × weekly) was used to analyze the mean CsA in each group. When a significant effect was detected, the Tukey's honest significant difference post-hoc test was used for multiple comparisons. All analyses were performed using the IBM SPSS Statistics Ver 27 (IBM, Tokyo, Japan), with a significance level set at $p < 0.01$.

This study was conducted with the approval of the Asahi University Ethics Committee (Approval No. 25158). The study adhered to the tenets of the Declaration of Helsinki, and informed consent was obtained from all study participants.

Results

Three-way ANOVA

Table 1 shows the results of three-way ANOVA (diurnal × daily × weekly). We found significant differences in the diurnal ($F[2, 243] = 21.20$, $p = 2.98 \times 10^{-9}$) and daily ($F[2, 243] = 4.74$, $p = 0.01$) CsA but not in weekly CsA ($F[2, 243] = 0.19$, $p = 0.83$). There were no between-group differences (Table 1).

Diurnal variations in the CsA

Fig. 2 shows diurnal CsA values. CsA in the noon (37.1 ± 19.4 kU/L) and evening (40.5 ± 23.9 kU/L)

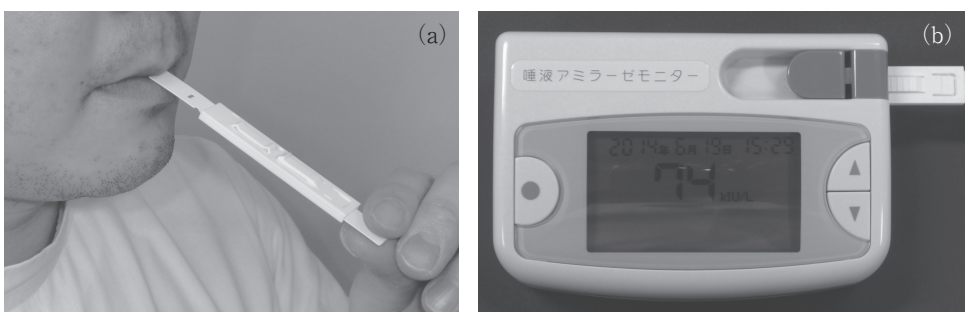

Fig. 1 (a) Collection of saliva with a chip (b) Salivary amylase monitor showing for determining the concentration of salivary α-amylase (CsA)

Table 1 Three-way analysis of variance (ANOVA) results

Source	SS	df	MS	F	p
Diurnal	15024.96	2	7512.48	21.20	2.98×10^{-9} *
Daily	3363.00	2	1681.50	4.74	0.01 *
Weekly	134.10	2	67.03	0.19	0.83
Diurnal x Daily	1855.56	4	463.89	1.31	0.27
Diurnal x Weekly	169.58	4	42.39	0.12	0.98
Daily x Weekly	51.24	4	12.81	0.04	1.00
Diurnal x Daily × Weekly	242.60	8	30.30	0.09	1.00
Error	86125.70	243	354.40		

SS=sum of squares, df=degrees of freedom, MS=mean square

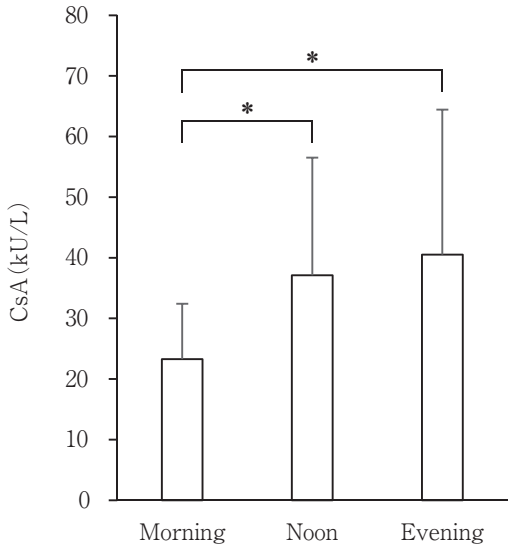

Fig. 2 Concentration of salivary α-amylase (CsA) recorded diurnally (morning, noon and evening) (*: $p<0.01$)

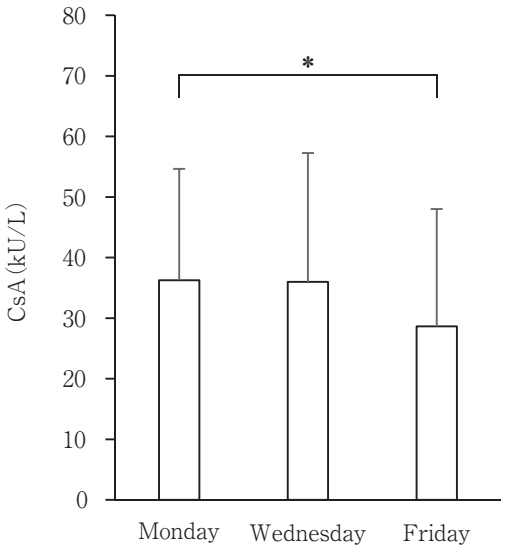

Fig. 3 Concentration of salivary α-amylase (CsA) recorded daily (Monday, Wednesday, and Friday) (*: $p<0.01$)

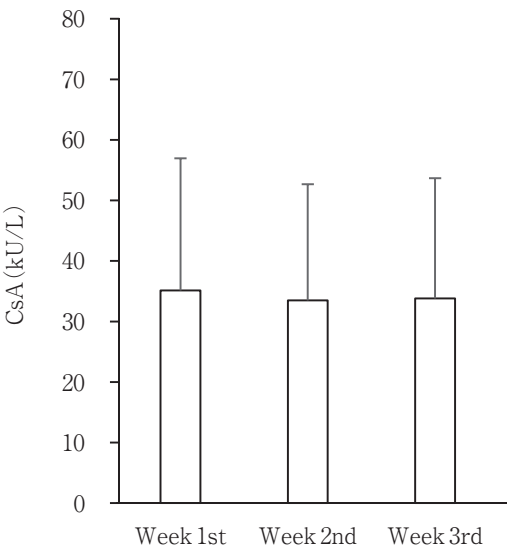

Fig. 4 Concentration of salivary α-amylase (CsA) recorded weekly (week 1, week 2 and week 3) (No significant difference)

were significantly higher than that in the morning (23.3 ± 9.1 kU/L; Tukey test; $p < 0.01$). However, there was no significant difference between CsA values in the noon and evening.

Daily variations in the CsA

Fig. 3 shows daily CsA values. The CsA on Monday (36.3 ± 18.4 kU/L) was significantly higher than that on Friday (28.6 ± 19.4 kU/L; Tukey test; $p < 0.01$). However, there was no significant difference between CsA values recorded on Monday and Wednesday (36.0 ± 21.3 kU/L) and on Wednesday and Friday.

Weekly variations in the CsA

Fig. 4 shows weekly CsA values. The CsA in week 1, 2, and 3 was 34.6 ± 21.5 kU/L, 33.0 ± 18.9 kU/L, and 33.3 ± 19.5kU/L, respectively.

Discussion

This study was conducted to evaluate CsA for the detection of stress in humans using a salivary amylase monitor at chair side clinically. The CsA was measured and compared diurnally, daily, and weekly.

In this study, when CsA values were compared for 3 weeks on the same day of the week, there was no significant differences. This shows that CsA did not depend on subjects' habituation to the experiment in this study. Therefore, the significant difference observed within days and weeks in this study was reliable.

When diurnal CsA values were compared, those in the noon and evening were higher than that in the morning. Nicolas et al[7] used a standard ELISA reader developed by Bosch et al[8] and reported that CsA decreased after waking up and increased thereafter, reaching the highest level in the afternoon and evening. Using a photochemical assay, Dennis et al[9] also reported that CsA is lower in the morning and higher in the evening. Rohleder et al[2] and Nater et al[10] used a photometric assay and determined that

CsA showed a pronounced decline within the first 30 minutes after waking, followed by a steady rise in the afternoon, and peaking in the late afternoon or evening. Our results are in line with these previous findings[2, 7, 9–12] CsA decreases sharply after waking up and increases thereafter, peaking at in the afternoon and evening[2, 7, 9–12].

When daily CsA values were compared in our study, the values on Friday were significantly lower than those on Monday and Wednesday. CsA is sensitive to psychological stressors[2, 10]. Kobayashi et al[13], using a subjective symptom survey, reported that subjective fatigue was higher on Monday than on Friday. They also reported that high subjective fatigue on Monday was greatly affected by resting on weekends and lack of motivation at the beginning of the week[13]. We hypothesized that CsA is higher on Monday than on Friday due to subjective fatigue.

In dental clinical settings, it is important to manage patients' stress. Responses to stress are broadly divided into the endocrine response of the hypothalamic–pituitary–adrenal axis (HPA) system and the autonomic nervous system (ANS) response of the sympathetic–adrenal–medullary axis system[14]. Moreover, CsA has emerged as a valid and reliable maker of ANS activity in the field of stress research[10].

As CsA is a more reliable indicator of individual responses to stress than cortisol[15], and can be measured quickly and noninvasively through saliva collection. CsA analysis may be a valuable tool for managing stress in dental patients.

In this study, we compared diurnal, daily, and weekly CsA values as an indicator of stress. The CsA monitoring device used in this study may be useful to detect stress in humans in the clinical setting.

Conclusion

We compared diurnal, daily, and weekly CsA values to evaluate whether stress could be measured objectively and easily. The results of this study showed that there were significant fluctuations in diurnal and daily CsA values.

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