Influence of Oral Cavity Characteristics and Life Style Factors on Salivary Alpha-Amylase

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Abstract

Background: Salivary α-amylase (SAA) has become a popular fluid for research and clinical diagnostics in recent years. While many reports have examined SAA, these studies have few participants and did not conduct gender analysis. This study aimed to compare gender differences in SAA based on intraoral symptoms, intraoral status, life style, and the saliva sampling time in common medical examination participants.

Methods: A self-administered questionnaire and dental examination were carried out on 1074 (Male: 474, Female: 600) participants of the Japan Multi-Institutional Collaborative cohort study in Kyoto field. SAA was measured using a portable salivary amylase monitor.

Results: In males, there was a significant difference in SAA when compared by age and denture use. In females, SAA was significantly different when denture use, teeth condition and gingiva were used for comparison.

Conclusion: Our results suggest that denture use significantly raises SAA in males. In females, denture use and perceived bad teeth condition or gingiva significantly raised SAA. For males, when SAA is used to measure stress in a routine medical examination, age is a necessary consideration.

Key Words: Saliva, Alpha-amylase, Stress, Oral hygiene, Biomarker

Introduction

Gorza et al. [1] and Speirs et al. [2] reported that salivary α-amylase (SAA) activity and salivary flow rates increase with increased activity of the sympathetic nervous system. In addition, SAA secretion is controlled by the sympathetic nervous adrenal medullary system, primarily norepinephrine, via hypothalamic stimulation. Furthermore, Chatterton et al. [3] suggested that there was a significant positive correlation between SAA and plasma norepinephrine in response to exercise and suggested the use of SAA as a marker for sympathetic nervous system activity.

Since then, several independent studies have confirmed this response of SAA to psychosocial stress and physical exercise, and additional evidence for this association of SAA responses with sympathetic activation has been reported [4-6]. Takai et al. [7] reported that unpleasant stimulation increases while comfortable stimulation decreases SAA activity. It has therefore been suggested that SAA activity may be used as an index to determine stress load.

Nater et al. [8] reported that SAA has a pronounced and distinct diurnal rhythm with a strong drop in activity in the first hour after awakening, and a steady increase towards the evening. Previous data have also shown that SAA levels are higher in males , with gender differences in the SAA response to stress [9,10].

An association between oral cavity health and SAA has been reported in periodontal disease and burning mouth syndrome [11-14]. However, there are no reports comparing SAA with oral conditions other than periodontal disease and intraoral subjective symptoms. While there have been many articles examining SAA as a stress marker, these studies had few participants and did not conduct gender analysis. Therefore, this study aimed to compare gender differences in SAA based on intraoral symptom, intraoral status, life style, and saliva sampling time (morning or afternoon) in routine medical examination participants.

Material and Methods

Participants

Participants included subjects from the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study [15]. The J-MICC Study evaluated the general Japanese population in ten research areas, using genetic and clinical data to detect and confirm gene-environment interactions related to life style-associated diseases. We have conducted a baseline survey on approximately 6500 inhabitants aged above 35 years old in the Kyoto prefecture between 2007 and 2013. We enrolled 1418 subjects who participated in the follow-up survey five years after the baseline survey. The survey was performed between November 2013 and March 2017. Of the 1418 participants, 110 did not complete the questionnaire, 234 did not measure saliva. Thus, 1074 participants were included in the present study for analysis.

All participants provided written informed consent for our study, and this study was approved by the Committee for Ethical Matters in Medical Research of Kyoto Prefectural University of Medicine (Authorization Number ERB-E-68 and ERB-E-31-1).

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Questionnaire

The questionnaire was administered prior to dental examination, and included questions on gender, age, smoking and drinking status, daily medication (Antihypertensive, Diabetes and Antihyperlipidemic drug), subjective intraoral symptoms and dental health behaviors, including frequency of tooth brushing and frequency of dental scaling visits. Those who were smoking every day were designated as the smoking group. Those who were currently drinking alcohol every day were designated as the alcohol drinking group.

Measurement of Salivary alpha-amylase

This study was conducted from 10:00 a.m. to 4:00 p.m.. SAA was measured to reduce stress effects after obtaining informed consent and height and weight measurements. SAA was measured using a portable salivary amylase monitor (NIPRO, Osaka, Japan), which provided completely automated measurements [16-18]. Saliva was collected by a test strip placed under the tongue for 30s and immediately measured, with the result being displayed within 60s. The SAA monitor used had been thoroughly examined and assessed to be valid and reliable.

Dental examination

Clinical examination of dental status was performed by general dentists. Participants sat facing a dentist, and were examined using a dental mirror and explorer. The number of remaining teeth was quantified during examination.

Statistical analysis

Participant characteristics were compared between males and females using unpaired t-test, chi-squared test and Mann–Whitney U test. SAA associated with a difference based on life style and saliva sampling time (morning or afternoon) were investigated using Mann-Whitney U test and Kruskal-Wallis test in males and females. Salivary markers associated with a difference based on oral condition, hygiene, and subjective intraoral symptoms were investigated using analysis of covariance with age as covariates in males. In females, salivary markers associated with a difference based on oral condition, hygiene and subjective intraoral symptoms were investigated using Mann–Whitney U test. The level of significance was set at less than 0.05 and all analyses were performed using SPSS Statistics 21 for Windows (SPSS Japan Inc.).

Results

The characteristics of the study subjects are shown in *Table 1*, in which Significant differences were observed upon comparison of age and SAA between male and female subjects. Significant differences were also observed when prevalence of smoking and drinking status, saliva sampling time and daily medication were compared.

The comparison of SAA by life style and saliva sampling time is shown in *Table 2*, a significant difference based on age in males was observed by the team.

The comparison of SAA by oral condition and hygiene in males is compared in *Table 3*. SAA was significantly different based on denture use.

The comparison of SAA by oral condition and hygiene in females has been compared in *Table 4*. SAA was significantly different based on denture use, teeth condition and gingiva.

Table 1. Participant characteristics

Participant characteristics	Male (n = 474)	Female (n = 600)	p value [1)]
Age (mean ± SD)	55.7 ± 9.3	54.6 ± 9.4	0.041
Smokers	83 (17.5%)	30 (5.0%)	< 0.001
Drinkers	360 (75.9%)	298 (49.7%)	< 0.001
Salivary alpha-amylase (kU/L)	52.0 ± 53.2	43.3 ± 48.9	0.003
Saliva sampling time			
Morning	302 (63.7%)	302 (53.8%)	0.001
Afternoon	172 (36.3%)	298 (46.2%)	
Number of remaining teeth (mean ± SD)	26.5 ± 4.8	26.9 ± 4.0	0.182
Daily medication			
Antihypertensive drug	102 (21.5%)	56 (9.3%)	< 0.001
Diabetes drug	25 (5.3%)	7 (1.2%)	< 0.001
Antihyperlipidemic drug	76 (16.0%)	73 (12.2%)	0.069

[1)] t-test: Age, Number of remaining and treated teeth Mann-Whitney U test: Salivary alpha-amylase Chi-squared test: smokers, drinkers, Frequency of dental scaling visit

Table 2. Comparison of salivary alpha-amylase by life style and sampling time of saliva

Age, life style and sampling time	Male (n = 474)		Female (n = 600)	
	SAA (kU/L)	p value 1)	SAA (kU/L)	p value 1)
Age (Male number / Female number)				
37 ~ 49 years old (130 / 215)	37.9 ± 33.8	0.014	38.0 ± 38.4	0.138
50 ~ 59 years old (161 / 198)	46.8 ± 45.9		42.9 ± 40.2	
60 ~ 77 years old (183 / 187	58.3 ± 51.3		40.7 ± 46.0	
Smoking status				
Current smoker	53.4 ± 49.4	0.463	31.4 ± 32.7	0.456
Non-smoker	47.9 ± 45.1		40.9 ± 41.9	
Drinking status				
Drinker	47.8 ± 45.5	0.298	40.1 ± 42.4	0.553
Non-drinker	51.9 ± 47.1		40.8 ± 40.6	
Everyday medicine				
Take medicine	50.5 ± 46.7	0.694	36.2 ± 40.5	0.078
Nothing	48.0 ± 45.5		41.4 ± 41.7	
Sampling time of saliva				
Morning	48.6 ± 44.9	0.927	40.4 ± 41.0	0.83
Afternoon	49.2 ± 47.6		40.5 ± 42.1	

1)Mann-Whitney U test: Smoking and Drinking status, Everyday medicine, Sampling time of saliva; Kruskal-Wallis test: Age; SAA: Salivary alpha-amylase

Table 3. Comparison of salivary alpha-amylase by oral condition and oral hygiene in males

Oral condition and Oral hygiene	SAA (kU/L)	
	mean±SD	p value 1)
Number of remaining teeth		
0 ~ 19 teeth (29)	63.2 ± 53.9	0.488
20 ~ 32 teeth (445)	47.9 ± 45.2	
Use of dentures		
Yes (68)	65.7 ± 56.3	0.026
No (406)	46.0 ± 43.3	
Frequency of tooth brushing		
Once or Twice a day (368)	49.9 ± 45.9	0.344
3 or more times a day (106)	45.2 ± 46.0	
Frequency of dental scaling visit		
None (259)	49.0 ± 45.1	0.491
Once or more times a year (215)	48.6 ± 46.9	
Self-reported Condition of teeth and gingiva		
Good (264)	48.9 ± 46.5	0.75
Bad (210)	48.8 ± 45.3	
Did you feel any tooth or gum pain within three months?		
Yes (215)	47.6 ± 46.1	0.463
No (259)	49.8 ± 45.7	
Self-reported Difficult to chew hard foods.		
Yes (86)	57.3 ± 49.1	0.263
No (398)	46.9 ± 45.0	
Having noises from the joint during mandibular movement or TMD has been diagnosed.		
Yes (68)	47.1 ± 44.0	0.907
No (406)	49.1 ± 46.2	
Feel dry mouth.		
Yes (72)	46.8 ± 47.3	0.62
No (402)	49.2 ± 45.7	
Pointed out as having a bad breath by another person.		
Yes (113)	55.7 ± 50.0	0.14
No (361)	46.7 ± 44.4	

1)Analysis of covariance; covariate: age; SAA: Salivary alpha-amylase, TMD: Temporomandibular joint dysfunction

Table 4. Comparison of salivary alpha-amylase by oral condition and oral hygiene in females

Oral condition and Oral hygiene	SAA (kU/L)	
	mean±SD	p value[1)]
Number of remaining teeth		
0 ~ 19 teeth (23)	54.8 ± 60.2	0.55
20 ~ 32 teeth (577)	39.9 ± 40.5	
Use of dentures		
Yes (70)	52.8 ± 51.0	0.038
No (530)	38.8 ± 39.8	
Frequency of tooth brushing		
Once or Twice a day (363)	41.1 ± 42.3	0.738
3 or more times a day (237)	39.4 ± 40.3	
Frequency of dental scaling visit		
None (247)	41.3 ± 43.9	0.559
Once or more times a year (353)	39.9 ± 39.7	
Self-reported Condition of teeth and gingiva		
Good (353)	38.3 ± 41.8	0.03
Bad (247)	43.5 ± 40.8	
Did you feel any tooth or gum pain within three months?		
Yes (274)	41.6 ± 40.8	0.414
No (326)	39.5 ± 42.1	
Self-reported Difficult to chew hard foods.		
Yes (113)	45.9 ± 44.5	0.133
No (487)	39.2 ± 40.7	
Having noises from the joint during mandibular movement or TMD has been diagnosed.		
Yes (120)	38.7 ± 41.8	0.482
No (480)	40.9 ± 41.4	
Feel dry mouth.		
Yes (129)	43.9 ± 45.4	0.384
No (471)	39.5 ± 40.3	
Pointed out as having a bad breath by another person.		
Yes (100)	43.0 ± 43.4	0.457
No (500)	39.9 ± 41.1	

[1)] Mann-Whitney U test; SAA: Salivary alpha-amylase, TMD: Temporomandibular joint dysfunction

Discussion

In this study, we report that denture use significantly raises SAA in males. In females, denture use, and a perceived bad teeth condition or gingiva significantly raised SAA. In addition, older males were associated with higher SAA.

Pain at the denture-bearing mucosa is one of the most frequent problems in patients with dentures [19, 20]. 21% of denture wearers reported soreness on the denture-bearing mucosa while chewing [21], while 30% of complete or partial denture wearers experienced pain when using dentures [22]. Moreover, other issues regarding dentures included concerns about functional aspects such as decreased chewing efficiency and dissatisfaction with the aesthetics of the dental prostheses [23]. A previous study has also reported that SAA increases significantly with the thickness of the interference [24]. Therefore pain, a sense of incongruity and the occlusal deficiency associated with denture use may raise SAA. While

a questionnaire on sensations associated with denture use will be needed in future, denture use is associated with increased SAA in both males and females.

Our results showed that a perceived bad teeth condition or gingiva significantly raised SAA in females. A previous study reported that SAA level was significantly correlated with tooth pain severity assessed by a visual analogue scale [25]. Furthermore, higher SAA was associated with periodontal disease and burning mouth syndrome [26, 27]. Other studies also concluded that SAA levels could be a reasonable biomarker for objective evaluation of pain [28, 29]. In addition, Rashkova et al. [30] reported that SAA may be used as a biomarker for objective evaluation of the psychosomatic state of individuals in a stressful environment. Therefore a rise in SAA may reflect a perception that one's teeth condition and gingiva is bad.

Some studies [31, 32] have reported increased SAA concentrations in older participants compared with younger participants. The hypothalamus-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) interact with each other and exert numerous systemic effects. A previous study suggested that psychobiological mechanisms that are thought to underlie age-related alterations results in increased activity of the HPA axis and the ANS [33, 34]. This was similar to a study by Nater UM et al. [31], who reported that higher SAA levels with increasing age were in accordance with age-related increases in activity of the HPA axis and ANS. Our data suggested that SAA correlated with age in males. Previous data suggest that SAA levels are higher in males, and the SAA response to stress is different based on gender [9, 10]. This may be reflected in the differential rise in SAA in older males.

There are some limitations associated with this study. First, it is possible that our results reflect, to some extent, the condition of the subjects in a clinical setting, who may have been affected by a variety of stress factors such as travel on the day of testing. Second, the measurement of saliva flow rate and the comparison of individual differences in SAA may be necessary. However, our study included subjects over a wide age range and larger numbers of subjects as compared to previous studies.

Conclusion

Our results suggest that denture use significantly raises SAA in males. In females, denture use, a perceived bad teeth condition and gingiva is significantly associated with raised SAA. When stress is measured using SAA in males, it is necessary to consider age.

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