a new wetness tester for diagnosis of dry mouth and reported its usefulness in the elderly requiring care as measurement of the amount of saliva in these individuals is difficult. In such patients, saliva is found on the sublingual mucosa but the top surface of the tongue is dry. Factors for this dryness have not yet been clarified.

Materials and methods

Subjects were 224 elderly who lived in a nursing home and required individual care. Moisture of the dorsum of the tongue and sublingual mucosa was measured using a wetness tester (L-SALIVO) with a measurement time of 10 s. Measurement was performed in 2 h following a meal from 10:00 to 12:00 in the morning with no eating or drinking in the interim. Dryness was assessed only if the moisture level of the dorsum of the tongue was 0 mm and the level for the mucosa under the tongue was over 3 mm. Personal information (age, disease, regular medication, level of ADL (Activities of Daily Living) and degree of disability) was obtained from the patient's primary physician. The patient's general condition, regular medication, and type of disease were selected as explanatory variables in a chi-square test or Yates's continuity correction. Multiple logistic regression analysis was used to determine significant factors associated with drying only from the dorsum of the tongue. The objective and methods of the study were explained to the patient, when competent, or to a patient representative, in the event of severe dementia, and consent to participate in the study was obtained. Ethical considerations for this study were based on ethical guidelines for clinical studies from the Ministry of Health, Labour and Welfare. The human rights of the subjects were respected, and the study was performed appropriately after the patients or their close kith (the charge person who consents) had been informed on the nature of the study and had given their consent.

Results

The mean age of subjects was 83.6 years (SD: 7.4, age range: 65–101 years). Of the subjects, 22.3% were able to act independently at home, 62.9% were able to sit up and 14.7% were bedridden. In terms of systemic disease, 72.8% of the subjects had dementia, 59.8% had cerebrovascular disease, 33.9% had hypertension, 28.6% had heart disease, 10.7% had diabetes mellitus, 10.3% had urinary disease, 6.3% had Parkinson's disease, 5.8% had cancer and 4% had respiratory illness. Only dryness of the dorsum of the tongue was assessed in 20 persons (8.9%).

Of 224 patients, 202 took regular medication; there was an average of 4.2 ± 2.8 medications per person. Fifty-four types of regular medication were not significantly associated with dryness of the mouth, as were diseases (Table 1). A significant difference in dryness of the dorsum of the tongue was noted with regard to the patient's general condition in terms of the degree of incapacity, level of consciousness, eating (oral ingestion or tube-feeding) and conversation (Table 2).

Multiple logistic regression identified the degree of incapacity ($p = 0.041$, odds ratio (OR) = 3.2, 95% CL = 1.049–9.766) as a significant factor for dryness. The odds ratio for eating was 11.226 ($p = 0.063$, 95% CL = 0.880–143.275) while that for conversation was 3.534 ($p = 0.107$, 95% CL = 0.761–16.420) (Table 3).

Discussion

In the elderly who have dementia and require care, measurement of the amount of saliva with the Saxon test, Gum test, or paraffin[13] and measurement of the amount of resting saliva have traditionally proved difficult. Lopez-Jornet *et al.*[14] and

Table 1 Number of patients with disease and prevalence of dryness only of the dorsum of the tongue.

Disease	*No. of Patients*	*Prevalence of dryness only of the dorsum of the tongue (%)*
Hypertension	76	10.5
Cerebrovascular disorder	134	9.7
Heart disorder	64	10.9
Diabetes mellitus	24	8.3
Renal insufficiency	4	0
Hepatic disorder	3	0
Respiratory illness	9	22.2
Rheumatism	5	0
Parkinson's disease	14	7.1
Urinary disease	23	8.7
Dementia	163	8.6
Depression	8	0
Schizophrenia	5	0
Fracture	38	5.3
Seeing and hearing disorder	11	9.1
Hyperlipaemia	13	7.7
Carcinoma	13	7.7
Osteoporosis	18	5.6
Anaemia	11	18.2

Table 2 Relationship between the patient's general condition and drying only of the dorsum of the tongue.

		Drying only at the tongue dorsum		
Item	*Category*	*Yes*	*No*	*p-value*
Age (years)	65–74	2	24	0.188
	75–84	5	90	
	85+	13	90	
Sex	Male	8	56	0.236
	Female	12	148	
Degree of incapacity	act independently at home	3	47	0.00006
	Able to sit up	8	133	
	Bedridden	9	24	
Upper jaw	Dentulous	6	66	0.83
	Edentate	14	138	
Lower jaw	Dentulous	9	91	0.973
	Edentate	11	113	
Upper and lower jaw	Dentulous	9	105	0.581
	Edentate	11	99	
Level of consciousness	Alert	13	186	0.0001
	Awaking without stimulation	3	13	
	no consciousness	4	5	
Eating	Ingestion	17	203	3E-06
	Tube feeding	3	1	
Conversation	Talking often	2	79	0.007
	Talking when necessary	8	82	
	Talking little	6	30	
	not talking	4	13	

Table 3 Multiple logistic regression analysis of factors affecting dryness only of the dorsum of the tongue.

Item	*Odds ratio*	*95% CL*		*p-value*
Degree of incapacity	3.200	1.049	9.766	0.041
Eating	11.226	0.880	143.275	0.063
Conversation	3.534	0.761	16.420	0.107
Level of consciousness	2.767	0.499	15.329	0.244

Chen *et al.*[15] reported that the Schirmer test paper was useful in assessing dry mouth. Nevertheless, this procedure requires a measurement time of 1–3 min, and elderly who have dementia and require care present with particular difficulties. Kakinoki[12,16] developed a new tester using the Schirmer test and reported its usefulness with a measurement time of 10 s[12,16]. This absorbs water when moisture is present in the area being measured and was developed to become wet at 1 mm in 1 s when immersed in water[1]. The tester remains completely dry when moisture is not present in the area being measured (the surface). The new tester is therefore more appropriate for assessment of dryness of the oral mucosa in the elderly requiring care.

There are reports that dry mouth was diagnosed by assessing dryness of the dorsum of the tongue[11,12,16] and that areas below and at the dorsum of the tongue were the wettest portions of oral mucosa[17]. Drying of the tongue causes difficulty and pain when chewing, so determining the dryness should generally prove useful in assessing dry mouth. About 70% of saliva is secreted by submandibular and sublingual glands and flows from sublingual caruncles[18]. Measurement of dryness of the sublingual region is appropriate for assessment of the presence or absence and extent of salivation in individuals in whom the amount of saliva secretion cannot be measured. Among the elderly requiring care, some individuals have a dry surface to the tongue despite having saliva under the tongue[19], so assessment of the moisture level is crucial. Moreover, case-control studies examining healthy individuals and elderly without motor impairment

are needed. As a consequence, characteristics of dryness only at dorsum of the tongue should become more definitive.

Multiple logistic regression analysis in the current study indicated that the degree of incapacity, tube-feeding, and not talking were important explanatory variables. The ratio of bedridden elderly who had dryness only of the dorsum of the tongue was 3.2 times higher than patients who were not bedridden; the ratio of patients on tube-feeding was 11.2 times higher than patients orally ingesting food. In Japan, the bedridden elderly have less daily activity, meet few other people and tend not to talk. In addition, elderly who suffer dementia and require care, lose interest in doing things and lack spontaneity; they stop talking and laughing. A bedridden person is in a resting state, causing what is known as disuse atrophy, or a drop in physical strength, appearance of various physical symptoms and worsening of psychiatric manifestations.

Saliva is secreted mainly from the sublingual caruncles, sublingual folds and openings of the parotid ducts. Drying of only the dorsum of the tongue in the bedridden elderly is thought to be caused by saliva failing to moisten the tongue when it reaches the mouth as the tongue, jaw and lips rarely move. In a bedridden individual, dryness of the tongue appears to be because decreased functional activity in the mouth results in saliva not moving to the tongue region; instead, it merely accumulates at the bottom of the oral cavity. Elderly requiring care who are tube-fed do not chew, so lack of movement by the jaw and lips is thought to be a factor. In addition, a bedridden individual who is also tube-fed cannot talk, so the dorsum of the tongue tends to be even drier. Drying of the tongue causes difficulty with bolus formation, swallowing and talking as well as halitosis, thus diminishing QOL (Quality of Life)[20].

Based on the current results, having the elderly not remain in bed, actively communicate talk and laugh are critical to preventing drying of the tongue. In addition, forms of rehabilitation such as oral exercises, swallowing training, and tongue exercises are useful forms of eating/swallowing activities in the elderly and may moisten areas in the oral cavity. Tongue exercises such as sticking the tongue out and moving it side to side carry no risk and are simple to perform.

Such efforts are not possible for the unconscious elderly requiring care; instead, an oral spray or artificial saliva and a moisturiser must be used frequently to prevent drying of the tongue. This could inhibit the increase in bacteria and can help prevent respiratory infection.

If a patient is in an institution and able to communicate, he or she should be transported by wheelchair or other means to places where people congregate, e.g. day rooms; talking with others and communicating involves use of the tongue and could reduce dryness. These benefits greatly outweigh the time and effort needed for a caregiver to convey the patient to a common area.

In their report[14], Lopez-Jornet *et al.* assessed dry mouth using the level of saliva moisture at the bottom of the oral cavity. Kakinoki[20] recommended assessing the moisture of both the dorsum of the tongue and the sublingual region to diagnose dry mouth in elderly requiring care. Unlike dry mouth because of decreased saliva secretion, drying only of the dorsum of the tongue involves different clinical findings[2]; so measuring the level of moisture in this area and oral cavity is critical to correctly diagnosing dry mouth. This allows appropriate care for the patient's clinical condition.

Conclusion

This study showed that in the elderly requiring care, dry mouth only of the dorsum of the tongue was associated with the degree of incapacity, tube feeding and level of conversation.

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References

1. **Murray Thomson W, Chalmers JM, John Spencer A, Slade GD, Carter KD.** A longitudinal study of medication exposure and xerostomia among older people. *Gerodontology* 2006; **23:** 205–213.
2. **Gupta A, Epstein JB, Sroussi H.** Hyposalivation in elderly patients. *J Can Dent Assoc* 2006; **72:** 841–846.
3. **Bai JP, Lin M.** Correlation between clinical manifestation and salivary secretion of xerostomia patients. *Hua Xi Kou Qiang Yi Xue Za Zhi* 2006; **24:** 240–242.
4. **Pow EH, Kwong DL, McMillan AS *et al.*** Xerostomia and quality of life after intensity-modulated radiotherapy vs. conventional radiotherapy for early-stage nasopharyngeal carcinoma: Initial report on a randomized controlled clinical trial. *Int J Radiat Oncol Biol Phys* 2006; **66:** 981–991.
5. **Frost PM, Shirlaw PJ, Challacombe SJ, Fernandes-Naglik L, Walter JD, Ide M.** Impact of wearing an intra-oral lubricating device on oral health in dry mouth patients. *Oral Dis* 2006; **12:** 57–62.

6. **Dormenval V, Budtz-Jorgensen E, Mojon P, Bruyere A, Rapin CH.** Associations between malnutrition, poor general health and oral dryness in hospitalized elderly patients. *Age Ageing* 1998; **27:** 123–128.
7. **Fontana M, Zunt S, Eckert GJ, Zero D.** A screening test for unstimulated salivary flow measurement. *Oper Dent* 2005; **30:** 3–8.
8. **Oksenberg A, Froom P, Melamed S.** Dry mouth upon awakening in obstructive sleep apnea. *J Sleep Res* 2006; **15:** 317–320.
9. **Hay KD, Morton RP.** Optimal nocturnal humidification for xerostomia. *Head Neck* 2006; **28:** 792–796.
10. **Matsuzaka T, Inoue H, Nagata N, Kakinoki Y.** Research on psychological factors of mouth dryness, - Preliminary research results among healthy adults. *JJSDH* 2005; **26:** 180–188.
11. **Yamada H, Nakagawa Y, Nomura Y *et al.*** Preliminary results of moisture checker for Mucus in diagnosing dry mouth. *Oral Dis* 2005; **11:** 405–407.
12. **Kakinoki Y, Nishihara T, Arita M, Shibuya K, Ishikawa M.** Usefulness of new wetness tester for diagnosis of dry mouth in disabled patients. *Gerodontology* 2004; **21:** 229–231.
13. **Ikebe K, Matsuda K, Morii K *et al.*** Impact of dry mouth and hyposalivation on oral health-related quality of life of elderly Japanese. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; **103:** 216–222.
14. **Lopez-Jornet P, Camacho-Alonso F, Bermejo-Fenoll A.** A simple test for salivary gland hypofunction using Oral Schirmer's test. *J Oral Pathol Med* 2006; **35:** 244–248.
15. **Chen A, Wai Y, Lee L, Lake S, Woo SB.** Using the modified Schirmer test to measure mouth dryness: a preliminary study. *Am Dent Assoc* 2005; **136:** 164–170.
16. **Kakinoki Y.** Evaluation of dry mouth in older and disabled persons with saliva wet tester. *JJSDH* 2004; **25:** 11–17.
17. **DiSabato-Mordarski T, Kleinberg I.** Measurement and comparison of the residual saliva on various oral mucosal and dentition surfaces in humans. *Arch Oral Biol* 1996; **41:** 655–665.
18. **Edgar WM, O'Mullane DM** eds. *Saliva and Oral Health*, 2nd edn, Translated by Kohno Syouji. Tokyo: Ishiyaku Publishers, 1997: 39–40.
19. **Koloktronis A, Chatzigiannis I, Paloukidou N.** Oral involvement in a case of AA amyloidosis. *Oral Dis* 2003; **9:** 269–72.
20. **Kakinoki Y.** The diagnosis, treatment and care of dry mouth. *Dent Outlook* 2002; **100:** 336–376.
21. **Kakinoki Y.** The clinic picture of dry mouth,- Diagnosis and treatment guidelines. *Dent Outlook* 2004; **103:** 39–46.

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ORAL MEDICINE

Salivary levels of cortisol and chromogranin A in patients with dry mouth compared with age-matched controls

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Objective. To evaluate the salivary levels of cortisol and chromogranin A (CgA) in patients with dry mouth (perceived xerostomia and hyposalivation) compared with age-matched controls.
Study design. We studied 174 subjects, including those with dry mouth, classified into 2 subgroups based on perceived xerostomia and salivation, and those without (control subjects). The control subjects were patients at the same hospital and healthy volunteers. Cortisol and CgA levels in stimulated whole saliva were measured using ELISA kits.
Results. All subjects with dry mouth had significantly higher cortisol and CgA levels than the control subjects. The statistical associations remained significant when they were divided into the 2 subgroups, although somewhat weaker associations were observed. The influences of xerogenic drugs were found to be minimal on salivary flow rate and levels of cortisol and CgA.
Conclusions. We found significant associations between salivary cortisol and CgA levels and symptoms of oral dryness and reduced salivary flow. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:833-839)**

The sense of oral dryness or xerostomia is a major complaint of a number of elderly individuals. Sreebny and Valdini[1] reported that 29% of their subjects stated that they were regularly troubled by the feeling of oral dryness in questionnaires, and Österberg et al.[2] reported that 16% of men and 25% of women complained of oral dryness in their investigation. In addition, Nagler and Hershkovich[3] found that elderly people have significantly reduced salivary secretion with altered composition compared with younger people, and 50% of an elderly population had oral sensorial complaints regarding xerostomia, taste, or burning mouth sensation. Changes in the salivary glands are assumed to occur with aging in many individuals. However, stimulated salivary flow rates in healthy elderly subjects revealed no significant age-related decrease.[4] In a great majority of cases, the causes of salivary gland changes are assumed to be related to various systemic diseases, medication, and psychologic and idiopathic factors, and only a small percentage of xerostomia cases have a known etiology such as radiotherapy or Sjögren syndrome.[5]

Hyposalivation is the most common etiologic factor in xerostomia,[6] though some investigators have

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reported that xerostomia is not necessarily related to decreased salivary flow rate[7] and that the association between xerostomia and hyposalivation is rather weak.[8] Therefore, the sensation of dry mouth, i.e., perceived xerostomia, is not necessarily associated with decreased whole salivary flow, and other assessments in addition to conventional salivary flow rate should be used to better diagnose the presence of dry mouth, i.e., perceived xerostomia and hyposalivation. On the other hand, xerostomia occurs with a particularly high frequency in menopausal women[9,10] and is often associated with mental depression and anxiety.[11,12]

In the present study, we investigated whether the pathophysiologic status of dry mouth is associated with changes in endocrinologic hormones related to depression and anxiety, which are included in disorders associated with dysregulation of the stress system. In general, the stress system consists of brain elements, of which the main components are corticotropin-releasing hormone (CRH) and the locus ceruleus-norepinephrine/autonomic systems, as well as their peripheral effectors, the hypothalamic-pituitary-adrenal (HPA) axis and sympathoadrenomedullary (SAM) system.[13] Salivary cortisol level reliably reflects HPA activity and has long been used in human psychobiologic studies as a biologic marker of stress, depression, and anxiety.[14] Hypercortisolism has also been reported to be associated with major depression and was suggested that as many as 60% of cases of major depression are associated with hypercortisolism,[15] although some reports have found that hypocortisolism is present in some forms of stress-related disorders, such as atypical depression and seasonal depression.[13] On the other hand, chromogranin A (CgA), an acidic glycoprotein that is stored and co-released by exocytosis with catecholamines from the adrenal medulla and sympathetic nerve endings,[16,17] is reported to be released into saliva from salivary glands, including the submandibular gland.[18] In addition, Dimsdale et al.[19] found that plasma CgA levels were correlated with noradrenaline release rate, and CgA levels have also been used as a quantitative index for the SAM system in other studies.[17]

We recently presented a preliminary report showing that the level of salivary CgA was significantly higher in community-dwelling elderly subjects with xerostomia.[20] The purpose of the present study was to investigate the salivary levels of cortisol and CgA in subjects with dry mouth, including xerostomia and hyposalivation, and compare them with control subjects without dry mouth. We also investigated whether administration of xerogenic drugs was linked to changes in the levels of these hormones.

SUBJECTS AND METHODS

Ethics committee approval and informed consent

The study was approved by the Ethics Committee of Kyushu Dental College (no. 04071007). Written informed consent was obtained from each subject after the aims and methodology of the study were explained.

Subject recruitment

We enlisted outpatients being treated for dry mouth at Kyushu Dental College Hospital in Fukuoka Prefecture, Japan. A total of 174 subjects, including dry mouth patients and control subjects, participated in the study. The dry mouth group was composed of 116 patients (mean age 64.7 yrs) whose chief complaint was dry mouth and who were further classified into 2 subgroups: 1) subjects with perceived xerostomia and normosalivation (dry mouth 1; n = 54); and 2) subjects with perceived xerostomia and hyposalivation (dry mouth 2; n = 62). Those with an unstimulated salivary flow rate of <0.1 mL/min were considered to have hyposalivation, according to previously reported criteria.[1,21] Answers regarding perceived xerostomia were elicited by the question, "Does your mouth usually feel dry?," which is often used in surveys of subjective oral dryness.[22] The symptoms were then queried and the following responses noted: "always," "sometimes," and "never." Subjects with perceived xerostomia were defined as having subjective oral dryness ("always" and "sometimes"). In the control group, 2 types of subjects (mean age 63.7 yrs) participated in this study: 1) healthy volunteers receiving regular health checks in the Kitakyushu city area (control 1; n = 37); and 2) patients with oral complaints other than perceived xerostomia, such as burning sensation in the mouth and tasting disturbance (control 2; n = 21). The dry mouth and control groups were matched for age. Subjects were excluded from the study if they had Sjögren syndrome, any other connective tissue disease, or a history of radiotherapy or chemotherapy. Each subject was asked to respond to a survey consisting of questions related to general medical condition, medication usage, and current smoking status. In addition, self-rated overall health status was determined by their answer to the question, "How do you feel about your recent general health condition?," with the following responses noted: "good," "fair," and "poor." Xerogenic drugs were considered to include antihypertensive agents, antihistamines, analgesics, diuretics, hypnotics, antidepressants, and antianxiety drugs.

Saliva sampling

Saliva samples were collected from all subjects between 9 a. m. and 11 a. m. to minimize any circadian rhythm effects, after they had refrained from oral in-

Table I. Descriptive characteristics of all subjects

	Controls	*Dry mouth*	*P value*
Perceived xerostomia	No	Yes	
Number of subjects	58	116	
Female	45 (78)	96 (83)	.413†
Age	63.7 (1.3)	64.7 (1.2)	.271‡
Number of teeth	23.6 (1.0)	22.1 (0.7)	.168‡
Xerogenic drug*	0 (0)	56 (48)	<.001†
Current smoking status	7 (12)	10 (9)	.471†
Hypertension (drug-treated)	0 (0)	26 (22)	<.001†
Diabetes (drug-treated)	0 (0)	5 (4)	.111†
Self-rated health status			
Good	24 (41)	27 (23)	.010†
Fair	29 (60)	61 (3)	
Poor	5 (9)	28 (24)	

Data indicate the number of subjects (%) or mean (SE) (for age and number of teeth).
*Antihypertensive agents, antihisamines, analgesics, duretics, hypnotics, antidepressants, and antianxiety drugs were included.
†Determined using chi-squared test.
‡Determined using Mann-Whitney *U* test.

take, tooth brushing, and smoking for at least 2 h before saliva collection. Subjects with complete or removable partial dentures kept them in their mouth during saliva collection. Each subject was first asked to swallow all saliva in the mouth, then unstimulated saliva was collected except for those in the control 1 group. Next, the subjects were asked to chew a tasteless piece of paraffin (1 g) for 5 min at a constant pace of 60 times per minute, which was monitored with an electric metronome, after which they were asked to expectorate whole saliva into a sterilized plastic tube. Collected samples were placed on ice immediately and the salivary flow rate (mL/min) was estimated by measuring the volume of saliva collected in the tube. Thereafter, the saliva samples were frozen at −30°C until further analysis.

Biomarker analyses

The concentration of cortisol in saliva (nmol/L) was determined using a salivary cortisol enzyme immunoassay kit (Salimetrics, State College, PA), with a lower sensitivity limit of 0.19 nmol/L, and that of CgA (pmol/mL) was determined using a YK070 Chromogranin A (Human) electroimmunoassay kit (Yanaihara Institute, Fujinomiya, Japan), with a lower sensitivity limit of 0.01 pmol/mL. Both biomarkers were also measured as absolute amounts, i.e., the amount secreted into the oral cavity per minute, to determine output. To obtain the output value, the mean flow rate and concentration values were multiplied.

Statistical analysis

Power analysis and sample size estimation were performed using the software G-power, ver. 2.0. The statistical power of this study was found to be 87% (with sample sizes: n1: 58; n2: 116), an effect size of 0.5, and α value of .05 (2-tailed t test with accuracy mode), which showed reasonable power. To assess differences between groups, a χ^2 test was used for categorized variables and a Kruskal-Wallis test for continuous variables, because a normal distribution was not present according to the results of a Kolomogorov-Smirnov test. A Scheffe test and Steel-Dwass test of multiple groups were applied after the Kruskal-Wallis test. All statistical analyses were performed using the statistical software package SPSS (version 11.0 for Windows; SPSS Japan, Tokyo, Japan). The level of statistical significance was set at .05 for all of the analyses.

RESULTS

The demographic characteristics for the 116 dry mouth subjects and 58 control subjects are presented in Table I. There were significant differences between the groups regarding xerogenic drug use, hypertension (drug-treated), and self-rated health status, and the differences were not significant regarding gender, age, number of teeth, current smoking status, and diabetes.

We compared salivary flow rate (stimulated), and salivary levels of cortisol and CgA between the dry mouth and control groups, as shown in Table II. The salivary flow rate was significantly lower in the dry mouth group compared with the controls, and the levels of cortisol and CgA also were significantly higher in the dry mouth group regarding both concentration and output.

Table III shows comparisons between the 2 control subgroups and 2 dry mouth subgroups for the salivary levels of cortisol and CgA. As expected, the subjects with a stimulated salivary flow rate of <1.0 mL/min in dry mouth 2 comprised approximately 80%, indicating that both the unstimulated and the stimulated salivary flow rates were reduced in most of the subjects in that subgroup. Also, the stimulated salivary flow rate in dry mouth 2 was the lowest among all of the subgroups, with significant differences observed between that subgroup and both control subgroups. Furthermore, the levels of and output values for cortisol were significantly higher in the 2 dry mouth subgroups compared with both control subgroups (P = .064 in Scheffe test), although there was a marginally significant difference between control 1 and dry mouth 2 regarding output (P = .086 in Steel-Dwass test). On the other hand, the level of CgA was significantly higher in dry mouth 2 compared with both control subgroups, and there was also a significant difference between control 2 and dry mouth 1, but none between control 1 and dry mouth 1. There were also significant differences between both control subgroups and dry mouth 1 for the output of

Table II. Salivary flow rate and levels of cortisol and CgA

		Controls	*Dry mouth*	*P value**
Number of subjects		58	116	
Stimulated salivary flow rate (mL/min)	Median	1.13	0.70	.001
	25th, 75th percentile	0.83, 1.41	0.40, 1.30	
Concentration				
Cortisol (nmol/L)	Median	4.56	20.00	<.001
	25th, 75th percentile	2.75, 6.65	6.90, 45.45	
CgA (pmol/mL)	Median	2.22	4.19	<.001
	25th, 75th percentile	1.15, 4.56	2.26, 7.79	
Output				
Cortisol (pmol/min)	Median	4.22	15.51	<.001
	25th, 75th percentile	2.57, 7.43	3.75, 43.66	
CgA (pmol/min)	Median	2.01	3.8	.020
	25th, 75th percentile	1.09, 3.87	1.64, 5.24	

CgA, Chromogranin A.
*Determined using Mann-Whitney *U* test.

Table III. Salivary flow rate and levels of cortisol and CgA divided by subgroup

		Control 1 (n = 37)	*Control 2 (n = 21)*	*Dry mouth 1 (n = 54)*	*Dry mouth 2 (n = 62)*	*P value*
Percentage with stimulated salivary flow rate <1.0 mL/min		51	24	41	79	
Stimulated salivary flow rate (mL/min)	Median	0.97	1.30	1.20	0.50*,†,**,††	<.001
	25th, 75th percentile	0.67,1.23	1.00, 1.60	0.60, 1.63	0.30, 0.83	
Concentration						
Cortisol (nmol/L)	Median	4.84	3.03	1.94*,†,**,††	24.69*,†,**,††	<.001
	25th, 75th percentile	3.27,6.22	1.79, 8.83	8.28,44.21	6.00,51.04	
CgA (pmol/mL)	Median	2.55	1.46	3.68†,††	5.30*,†,**,††	<.001
	25th, 75th percentile	1.42,4.56	0.66, 4.37	2.09, 5.94	2.76, 9.85	
Output						
Cortisol (pmol/min)	Median	4.36	4.22	19.70*,†,**,††	9.17	<.001
	25th, 75th percentile	2.60,7.11	2.41,12.3	7.20,46.72	2.23,36.02	
CgA (pmol/min)	Median	2.23	1.55	3.36**,††	2.62	.012
	25th, 75th percentile	1.04,3.94	1.09, 3.67	2.11, 5.96	1.20, 4.39	

Control 1: healthy volunteers; control 2: control patients perceived xerostomia (−)%; dry mouth 1: perceived xerostomia (+), unstimulated salivary flow rate ≥0.1 mL/min; dry mouth 2: perceived xerostomia (+), unstimulated salivary flow rate <0.1 mL/min.
CgA, Chromogranin A. Kruscal-Wallis test.
*Versus control 1, as determined using Scheffe test for multiple comparisons ($P < .05$).
†Versus control 2, as determined using Schaffe test for multiple comparisons ($P < .05$).
**Versus control 1, as determined using Steel-Dwass test for multiple comparisons ($P < .05$).
††Versus control 2, as determined using Stee-Dwass test for multiple comparisons ($P < .05$).

CgA, whereas no significant differences were found between the 2 control subgroups and dry mouth 2.

Next, we analyzed the effects of xerogenic drugs on the dry mouth 1 and 2 subgroups, as shown in Table IV. In dry mouth 1, subjects with a stimulated salivary flow rate of <1.0 mL/min ranged from 37% to 42%, whereas the range was from 75% to 80% in dry mouth 2. When the drug-administered subjects were compared with the nonadministered subjects, no significant differences were found between them regarding stimulated and unstimulated salivary flow rats or concentrations and output values of cortisol and CgA, although the levels of cortisol tended to be higher in the drug-administered subjects.

DISCUSSION

In the present study, we investigated HPA and SAM responses as well as dry mouth status in outpatients and found that elevated levels of cortisol and CgA were

Table IV. Comparison of salivary flow rate and cortisol and CgA levels between dry mouth subjects administered xarogenic drugs and those not administered

		Dry mouth 1			Dry mouth 2		
		Drug (−)	Drug (+)	P value*	Drug (−)	Drug (+)	P value*
Number of subjects		30	24		30	32	
Percent with stimulated salivary flow rate <1.0 mL/min		37	42		80	75	
Stimulated salivary flow rate (mL/min)	Median	1.35	1.05	.055	0.55	0.42	.400
	25th, 75th percentile	0.70, 1.85	0.50, 1.38		0.36, 0.83	0.30, 0.88	
Unstimulated salivary flow rate (mL/min)	Median	0.20	0.18	.539	0.04	0.03	.514
	25th, 75th percentile	0.16, 0.33	0.14, 0.28		0.00, 0.06	0.00, 0.04	
Concentration							
Cortisol (nmol/L)	Median	17.52	22.62	.465	22.76	27.73	.719
	25th, 75th percentile	7.93,42.07	10.83,48.35		7.17,51.04	5.18,58.63	
CgA (pmol/mL)	Median	3.19	4.06	.254	5.95	3.82	.307
	25th, 75th percentile	1.48, 5.37	2.40, 6.71		3.41, 9.85	1.50,10.65	
Output							
Cortisol (pmol/min)	Median	27.49	19.28	.702	10.70	8.90	.568
	25th, 75th percentile	5.97,61.16	11.84,40.28		2.69,35.87	1.92,38.27	
CgA (pmol/min)	Median	3.09	3.73	1.000	3.20	1.83	.111
	25th, 75th percentile	2.11, 7.57	1.98, 5.65		1.69, 5.66	1.03, 3.74	

Dry mouth 1: perceived xerostomia (+), unstimulated salivary flow rate ≥0.1 mL/min; dry mouth 2: perceived xerostomia (+), unstimulated salivary flow rate <0.1 mL/min. *CgA*, chromogranin A.
*Mann-Whitney *U* test.

associated with symptoms of oral dryness and reduced salivary flow.

Cortisol and CgA are well known biologic markers of the stress system, and a number of reports have shown that salivary cortisol is associated with depression and anxiety,[15,23,24] although activation or inactivation of the stress system is dependent upon the depression subtype.[13] On the other hand, salivary CgA has been reported to rapidly and sensitively respond to psychosomatic stressors.[17,25] Several studies of the associations between CgA and psychiatric disorders such as depression have been conducted. For example, Noto et al.[26] investigated the association between levels of cortisol and CgA in saliva and answers to a questionnaire that used a state-trait anxiety inventory. Miyakawa et al.[27] also studied the relationship between noise sensitivity based on salivary CgA levels and responses to a 28-item general health questionnaire. However, no clear associations between salivary CgA and depression or anxiety were demonstrated in those reports. Only 2 known studies have documented associations between the pathophysiology of dry mouth (xerostomia) and stress-related hormones. In one, Rivera Gómez et al.[28] compared the levels of salivary cortisol and the presence of xerostomia in menopausal women and found no significant association, although clear diagnostic criteria for xerostomia were not described. Their findings were different from the present results, which showed significant differences between the dry mouth and control groups, which might be attributable to the smaller sample size (n = 30), demographic characteristics of the subjects (gender and age group), and unclear criteria for the diagnosis of dry mouth in their study. In a previous study, we studied the associations among cortisol, CgA, and xerostomia in community-dwelling elderly subjects, and found significant associations with the level of CgA in saliva and xerostomia, drug use, and decreased salivary flow rate.[20] In the present study, the levels of cortisol and CgA in the dry mouth subjects were increased compared with the both control subgroups, i.e., control patients and community-based volunteers. Along with our former results, the present findings provide evidence that dry mouth symptoms are related to the activities of the HPA and SAM systems.

Stronger associations were found between the dry mouth subjects and control subjects regarding concentration compared with output. This may have been because flow rate is reduced far more than the concurrent increase in biomarker concentrations, which was particularly seen in the dry mouth 2 subgroup. Therefore, a more accurate evaluation of these biomarkers might be possible if the values are expressed as concentrations. In the present study, the dry mouth 2 group had higher concentrations of cortisol and CgA. If a patient with perceived xerostomia and hyposalivation is considered to have a serious dry mouth condition, elevated levels of both biomarkers might confirm a serious

pathophysiologic status. Furthermore, our analyses showed that the effects of xerogenic drugs on salivary flow rate (unstimulated and stimulated) as well as levels of cortisol and CgA were not statistically significant with the dry mouth subjects (Table IV). It is interesting that the effects of xerogenic drugs on dry mouth symptoms were minimal in light of earlier reports that many cases of dry mouth are associated with medication.[6]

The measurement of biomarkers in saliva has many advantages, because it is stress free and noninvasive, and allows for frequent and rapid sampling, whereas diurnal rhythm, artificial changes due to food or drinking substances, and blood contamination are some of the disadvantages.

The present study has some limitations. First, saliva sampling should have been performed earlier in the morning for more accurate determination of the underlying physiologic condition. Consequently, it is possible that our results reflect to some extent the condition of the subjects in a clinic, who are affected by a variety of stress factors such as travel on the day of testing. Second, data regarding salivary levels of stress-related hormones were obtained only from subjects able to produce an adequate quantity of measurable saliva with stimulation. However, because the salivary hormone assay kits used in the present study are capable of measuring saliva quantities as low as 50 μL, measurements of the salivary biomarkers should be possible in most subjects, even those with severe hyposalivation. Because the composition of stimulated and unstimulated saliva may differ,[4] we intend to investigate whether different results are obtained when unstimulated saliva is analyzed. Third, whether the present biomarkers are useful as predictors for dry mouth remains unclear, because the design of the present study was cross-sectional. Further, since we did not assess the changes in CRH and the adrenocorticotropic hormone in the HPA axis system, the causes and effects of salivary biomarkers remain unclarified by the present results.

In conclusion, we found that subjects with dry mouth had increased salivary levels of cortisol and CgA compared with those without dry mouth, which suggests an association with the markers studied and symptoms of oral dryness and lower salivary flow. Additional studies of salivary biomarkers may lead to the development of a method of monitoring the levels of anxiety and depression in subjects suffering from dry mouth in the near future.

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REFERENCES

1. Sreebny LM, Valdini A. Xerostomia. Part I: Relationship to other oral symptoms and salivary gland hypofunction. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1988;66:451-8.
2. Österberg T, Landahl S, Hedegard B. Salivary flow, saliva, pH and buffering capacity in 70-year-old men and women. Correlation to dental health, dryness in the mouth, disease and drug treatment. J Oral Rehabil 1984;11:157-70.
3. Nagler RM, Hershkovich O. Relationships between age, drugs, oral sensorial complaints and salivary profile. Arch Oral Biol 2005;50:7-16.
4. Vissink A, Spijkervet FK, Van Nieuw Amerongen A. Aging and saliva: a review of the literature. Spec Care Dentist 1996;16: 95-103.
5. Sreebny LM. Saliva in health and disease: an appraisal and update. Int Dent J 2000;50:140-61.
6. Nederfors T. Xerostomia and hyposalivation. Adv Dent Res 2000;14:48-56.
7. Närhi TO. Prevalence of subjective feelings of dry mouth in the elderly. J Dent Res 1994;73:20-5.
8. Hay EM, Thomas E, Pal B, Hajeer A, Chambers H, Silman AJ. Weak association between subjective symptoms or and objective testing for dry eyes and dry mouth: results from a population based study. Ann Rheum Dis 1998;57:20-4.
9. Frutos R, Rodriguez S, Miralles-Jorda L, Machuca G. Oral manifestations and dental treatment in menopause. Med Oral 2002;7:26-35.
10. Ben Aryeh H, Gottlieb I, Ish-Shalom S, David A, Szargel H, Laufer D. Oral complaints related to menopause. Maturitas 1996;24:185-9.
11. Bergdahl M, Bergdahl J, Johansson I. Depressive symptoms in individuals with idiopathic subjective dry mouth. J Oral Pathol Med 1997;26:448-50.
12. Bergdahl M, Bergdahl J. Low unstimulated salivary flow and subjective oral dryness: association with medication, anxiety, depression, and stress. J Dent Res 2000;79:1652-8.
13. Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. JAMA 1992;267:1244-52.
14. Dickerson SS, Kemery ME. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Phychol Bull 2004;130:355-91.
15. Levine A, Zagoory-Sharon O, Feldman R, Lewis JG, Weller A. Measuring cortisol in human psychobiological studies. Physiol Behav 2007;90:43-53.
16. O'Connor DT, Bernstein KN. Radioimmunoassay of chromogranin A in plasma as a measure of exocytotic sympathoadrenal activity in normal subjects and patients with pheochromocytoma. N Engl J Med 1984;311:764-70.
17. Nakane H, Asami O, Yamada Y, Harada T, Matsui N, Kanno T, et al. Salivary chromogranin A as an index of psychosomatic stress response. Biomed Res 1998;19:401-6.
18. Saruta J, Tsukinoki K, Sasaguri K, Ishii H, Yasuda M, Osamura YR, et al. Expression and localization of chromogranin A gene and protein in human submandibular gland. Cells Tissues Organs 2005;180:237-44.
19. Dimsdale JE, O'Connor DT, Ziegler M, Mills P. Chromogranin A correlates with norepinephrine release rate. Life Sci 1992;51: 519-25.
20. Horikawa M, Ansai T, Yoshida A, Soh I, Awano S, Shigeyama C, et al. Association between salivary chromogranin A and xerostomia in eldarly subjects: a pilot study. Int J Oral Health 2006:27-31.
21. Ericsson Y, Hardwick L. Individual diagnosis, prognosis and counselling for caries prevention. Caries Res 1978;12(Suppl 1):94-102.
22. Moore PA, Guggenheimer J, Etzel KR, Weyant RJ, Orchard T. Type 1 diabetes mellitus, xerostomia, and salivary flow rates.

Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92:281-91.
23. Galard R, Gallart JM, Catalan R, Schwartz S, Arguello JM, Castellanos JM. Salivary cortisol levels and their correlation with plasma ACTH levels in depressed patients before and after the DST. Am J Psychiatry 1991;148:505-8.
24. Hill CM, Walker RV. Salivary cortisol determinations and self-rating scales in the assessment of stress in patients undergoing the extraction of wisdom teeth. Br Dent J 2001;191:513-5.
25. Nakane H, Asami O, Yamada Y, Ohira H. Effect of negative air ions on computer operation, anxiety and salivary chromogranin A–like immunoreactivity. Int J Psychophysiol 2002;46:85-9.
26. Noto Y, Sato T, Kudo M, Kurata K, Hirota K. The relationship between salivary biomarkers and state-trait anxiety inventory score under mental arithmetic stress: a pilot study. Anesth Analg 2005;101:1873-6.
27. Miyakawa M, Matsui T, Kishikawa H, Murayama R, Uchiyama I, Itoh T, et al. Salivary chromogranin A as a measure of stress response to noise. Noise Health 2006;8:108-13.
28. Rivera Gómez B, Hernández Vallejo G, Arriba de la Fuente L, López Cantor M, Diaz M, López-Pintor RM. The relationship between the levels of salivary cortisol and the presence of xerostomia in menopausal women. A preliminary study. Med Oral Patol Oral Cir Bucal 2006;11:E407-12.

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Salivary levels of hyaluronic acid in female patients with dry mouth compared with age-matched controls: a pilot study

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ABSTRACT

Little is known regarding the association between the level of hyaluronic acid (HA) in saliva and dry mouth status. The aim of this study was to evaluate the salivary levels of HA in female patients with dry mouth (perceived xerostomia and hyposalivation) and compare them with age-matched controls. We studied 46 females, and classified them into two groups based on perceived xerostomia and salivary flow rate, as well as a control group without symptoms. HA concentrations in unstimulated whole saliva were determined and a significant difference was found between the groups. The statistical association was stronger in patients (perceived xerostomia, normosalivation) administrated xerogenic drugs, while the HA levels in that group were significantly lower than those in the controls when converted to absolute amount of saliva per min. Within the limitations of the present study, patients with dry mouth had lower HA levels in saliva, which may serve as a marker of local dryness or oral mucosa lubrication.

The sense of oral dryness or xerostomia is a major complaint of a number of elderly individuals. Sreebny and Valdini reported that 29% of their subjects stated that they were regularly troubled by the feeling of oral dryness in questionnaires (10), and Österberg *et al.* reported that 16% of men and 25% of women complained of oral dryness in their investigation (6). As etiologic factors of oral dryness, in general, age, sex, various systemic diseases, and medication have been reported (5).

Hyaluronic acid (HA) is a glycosaminoglycan that is a constituent of the ground substance of the subcutaneous tissues and functions as a mediator of cell proliferation and wound healing, while it also plays a prominent part in tumorigenesis and embryogenesis. Its presence and possible role in saliva has been scarcely investigated, with only a few reports presented. For example, Pogel *et al.* measured HA levels in saliva from 10 healthy adult volunteers, and found that it may contribute to the healing properties of saliva, by assisting in protecting oral mucosa and adding to the lubricating properties of saliva (7). Also, Tishler *et al.* investigated HA levels in saliva of patients with Sjögren syndrome (SS) and suggested that salivary HA concentration may be of value in its diagnosis (11). However, to our knowledge, little is known regarding HA levels in non-SS patients with dry mouth, though it is often found in a high percentage of xerostomia cases.

The purpose of the present study was to measure the salivary levels of HA in female subjects with dry mouth, including those with xerostomia and hyposalivation, and compare the results with control subjects. We also investigated the effects of xerogenic drug administration on HA levels.

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MATERIALS AND METHODS

Ethics committee approval and informed consent. This study was approved by the Ethics Committee of Kyushu Dental College (No.04071007). Written informed consent was obtained from each subject after the aims and methodology of the study were explained.

Subject recruitment. We enlisted outpatients being treated for dry mouth at Kyushu Dental College Hospital in Fukuoka Prefecture, Japan. A total of 88 female subjects, including dry mouth patients and control subjects, participated in the study. In order to rule out the effects of sex as a confounder, all participants in our study were females. The exclusion criteria utilized were as follows: 1) presence of SS, any other connective tissue disease, or a history of radiotherapy or chemotherapy; and 2) lower than normal level of saliva flow rate or HA level too low to measure. As a result, we analyzed 46 female subjects (mean age, 55.5 years).

The Dry mouth group was composed of 32 patients whose chief complaint was dry mouth and those were further classified into two subgroups: 1) subjects with perceived xerostomia and hyposalivation (Dry mouth 1; n = 16); and 2) perceived xerostomia with normosalivation (Dry mouth 2; n = 16). Those with an unstimulated salivary flow rate of less than 0.25 mL/min were considered to have hyposalivation, according to previously reported criteria (2, 9). Answers regarding perceived xerostomia were elicited by the question "Does your mouth usually feel dry?", which is often utilized in surveys of subjective oral dryness (4). The symptoms were then queried and the following responses noted: "always", "sometimes", and "never". Subjects with perceived xerostomia were defined as having subjective oral dryness ("always" and "sometimes" answers). Patients with oral complaints other than perceived xerostomia, such as a burning sensation in the mouth and tasting disturbance (mean age, 59.5 years, n = 14) were placed into the Control group. The Dry mouth and Control groups were matched for age. Each subject was asked to respond to a survey consisting of questions related to general medical condition, medication usage, and current smoking status. Xerogenic drugs were considered to include antihypertensive agents, antihistamines, analgesics, diuretics, hypnotics, antidepressants, and anti-anxiety drugs.

Saliva sampling. Measurements of biomarkers in saliva have many advantages, as the method used is stress-free and non-invasive, and allows for frequent and rapid sampling. In contrast, diurnal rhythm, artificial changes due to food or drinking substances, and blood-contamination are some of the disadvantages. Saliva samples were collected from all subjects between 9 a. m. and 11 a. m. to minimize any circadian rhythm effects, after they had refrained from oral intake, tooth brushing, and smoking for at least 2 h prior to saliva collection. Subjects with complete or removable partial dentures kept them in their mouth during saliva collection. Each subject was first asked to swallow all saliva in the mouth, then unstimulated saliva was collected. Next, the subjects were asked to chew a tasteless piece of paraffin (1 g) for 5 min at a constant pace of 60 times per minute, which was monitored with an electric metronome, after which they were asked to expectorate whole saliva into a sterilized plastic tube. Collected samples were placed on ice immediately and the salivary flow rate (mL/min) was estimated by measuring the volume of saliva collected in the tube. Thereafter, the saliva samples were frozen at −30°C until further analysis.

Biomarker analyses. Determination of concentrations of HA in saliva (ng/mL) was performed by a commercial laboratory (SRL Inc., Tokyo, Japan). The test is based on the use of specific HA binding proteins isolated from bovine cartilage, with the lower limit of detection at 10 ng/mL. To determine output, HA levels were also measured as absolute amounts, *i.e.*, the amount secreted into the oral cavity per minute. To obtain the output value, the mean flow rate and concentration values were multiplied.

Statistical analysis. To assess differences between groups, a χ^2 test was used for categorized variables, and a Kruskal-Wallis test for continuous variables, because a normal distribution was not present according to the results of a Kolomogorov-Smirnov test. A Scheffe test and Steel-Dwass test of multiple groups were applied following the Kruskal-Wallis test. All statistical analyses were performed using the statistical software package SPSS (version 11.0 for Windows; SPSS Japan, Tokyo, Japan). The level of statistical significance was set at 0.05 for all of the analyses.

RESULTS

The demographic characteristics for the 32 dry mouth and 14 control subjects are presented in Ta-

ble 1. There were no significant differences among the groups regarding age, current smoking status, diabetes (drug-treated), hypertension (drug-treated), and xerogenic drug use. We compared salivary flow rate (unstimulated and stimulated), and salivary levels of HA among the dry mouth and control groups, with the results shown in Table 2. The unstimulated salivary flow rate was significantly lower in the Dry mouth 1 as compared with the Control group, whereas the stimulated salivary flow rate was not significantly different. In addition, there was a significant association among the 3 groups regarding HA concentration, but not for HA output, whereas multiple comparison analysis showed no significant associations among the dry mouth and control groups in both measurements.

Next, we compared the levels of HA in saliva among the subjects in Dry mouth 1 and 2 who did not receive xerogenic drugs and the Control group, with the results shown in Table 3. The unstimulated salivary flow rate was lower in the Dry mouth 1 as compared with the Control group. However, according to multiple comparison analysis, no statistical significances was seen among the dry mouth patients and controls regarding either HA concentration or output. Table 4 shows comparisons between Dry mouth 1 and 2 for subjects administrated xerogenic drugs. The unstimulated salivary flow rate was lowest in Dry mouth 1, while there was no significant difference between Dry mouth 2 and Control group regarding unstimulated salivary flow rate in multiple comparison analysis. The HA concentration in Dry mouth 2 was the lowest and multiple comparison analysis showed a marginally significant difference between those subjects and the controls. In addition, HA output in Dry mouth 2 subjects that

Table 1 *Demographic characteristics*

	Dry mouth 1	Dry mouth 2	Control	*P* value
Perceived xerostomia	Yes	Yes	No	
Number of subjects	16	16	14	
Age (in years)	51.0 (44.8, 60.8)	62.0 (45.8, 67.0)	60.0 (50.8, 67.0)	0.427[a]
Current smoking status	4 (25)	1 (6)	2 (14)	0.196[b]
Diabetes (drug-treated)	0 (0)	0 (0)	1 (7)	0.075[b]
Hypertension (drug-treated)	1 (20)	4 (25)	0 (0)	0.177[b]
Xerogenic drug administrated*	9 (56)	11 (69)	8 (57)	0.725[b]

Dry mouth 1: perceived xerostomia (+), unstimulated salivary flow rate < 0.25 mL/min; Dry mouth 2: perceived xerostomia (+), unstimulated salivary flow rate ≥ 0.25 mL/min; Control: patients with perceived xerostomia (−).
Data indicate the median (25^{th}, 75^{th} percentile) (for age) or the number of subjects (%).
[a]Kruskal-Wallis test, [b]chi-squared test.
*Antihypertensive agents, antihistamines, analgesics, duretics, hypnotics, antidepressants, and antianxiety drugs were included.

Table 2 *Salivary flow rate and levels of HA (n = 46)*

	Dry mouth 1	Dry mouth 2	Control	*P* value*
Number of subjects	16	16	14	
Unstimulated salivary flow rate (mL/min)	0.12 (0.10, 0.16)[a, b]	0.40 (0.34, 0.58)	0.30 (0.29, 0.43)	< 0.001
Stimulated salivary flow rate (mL/min)	0.70 (0.60, 0.95)	1.30 (0.75, 1.78)	1.20 (0.75, 1.53)	0.037
Concentration (ng/mL)	462.0 (74.0, 631.0)	26.5 (15.5, 108.8)	118.5 (31.5, 318.0)	0.004
Output (ng/min)	56.7 (7.9, 103.1)	13.5 (7.2, 28.3)	40.8 (12.9, 131.3)	0.177

HA, hyaluronic acid.
Data indicate the median (25^{th}, 75^{th} percentile).
*Kruscal-Wallis test.
[a]Versus Control, as determined using Scheffe test for multiple comparisons ($P < 0.05$).
[b]Versus Control, as determined using Steel-Dwass test for multiple comparisons ($P < 0.05$).

Table 3 *Salivary flow rate and levels of HA in subjects not administrated xerogenic drugs (n = 18)*

	Dry mouth 1	Dry mouth 2	Control	*P* value*
Number of subjects	7	5	6	
Unstimulated salivary flow rate (mL/min)	0.14 (0.10, 0.16)[a, b]	0.50 (0.38, 0.70)	0.28 (0.25, 0.35)	0.001
Stimulated salivary flow rate (mL/min)	0.80 (0.70, 1.50)	1.60 (0.69, 2.75)	1.20 (0.75, 1.30)	0.388
Concentration (ng/mL)	549.0 (305.0, 765.0)	27.0 (18.5, 280.5)	83.0 (15.0, 271.8)	0.055
Output (ng/min)	76.5 (30.5, 116.0)	13.2 (9.2, 184.8)	23.4 (6.6, 74.9)	0.503

HA, hyaluronic acid.
Data indicate the median (25th, 75th percentile).
*Kruscal-Wallis test.
[a]Versus Control, as determined using Steel-Dwass test for multiple comparisons ($P < 0.05$).
[b]Versus Control, as determined using Scheffe test for multiple comparisons ($P < 0.1$).

Table 4 *Salivary flow rate and levels of HA in subjects administrated xerogenic drugs (n = 28)*

	Dry mouth 1	Dry mouth 2	Control	*P* value*
Number of subjects	9	11	8	
Unstimulated salivary flow rate (mL/min)	0.10 (0.07, 0.18)[a, b]	0.40 (0.30, 0.50)	0.31 (0.30, 0.48)	< 0.001
Stimulated salivary flow rate (mL/min)	0.60 (0.60, 0.70)	1.20 (0.70, 1.70)	1.10 (0.72, 1.58)	0.041
Concentration (ng/mL)	378.0 (26.0, 599.0)	26.0 (14.0, 51.0)[c, d]	47.5 (44.8, 476.0)	0.029
Output (ng/min)	28.2 (2.2, 84.7)	13.8 (5.6, 17.3)[b, c]	47.5 (27.5, 154.8)	0.057

HA, hyaluronic acid.
Data indicate the median (25th, 75th percentile).
*Kruscal-Wallis test.
[a]Versus Control, as determined using Scheffe test for multiple comparisons ($P < 0.05$).
[b]Versus Control, as determined using Steel-Dwass test for multiple comparisons ($P < 0.05$).
[c]Versus Control, as determined using Scheffe test for multiple comparisons ($P < 0.1$).
[d]Versus Control, as determined using Steel-Dwass test for multiple comparisons ($P < 0.1$).

received xerogenic drugs was the lowest, while multiple comparison analysis showed a significant difference between those subjects and the Control group. Thus, the differences remained significant when the HA concentrations in Dry mouth 2 and the Control group were adjusted using the amounts of saliva obtained for testing. Further, HA output in Dry mouth 1 group subjects who received xerogenic drugs was also lower as compared with the Control group, though the difference was not significant.

DISCUSSION

In the present study, we investigated the association between HA levels in saliva and dry mouth status in outpatients, and found that decreased levels of HA were associated with symptoms of oral dryness, with a stronger association between subjects in the Dry mouth 2 group (*i.e.*, perceived xerostomia (+) and normosalivation) and the Control group regarding both concentration and HA output.

To date, only a single known study has been presented regarding the association between dry mouth status and HA (11), which focused on patients with

SS. However, since patients without that condition are more frequently encountered in clinical practice, we excluded patients with SS and focused on age-matched females, in order to minimize the effects of confounding factors in the etiology of dry mouth. Recently, Loeb *et al.* investigated HA as well as chondroitin sulfate levels in saliva sample from patients with glossodynia, or burning mouth syndrome, and reported that the HA concentrations were similar between the patients and normal subjects, whereas the concentration of chondroitin sulfate was decreased in the saliva of the patients (3).

The present Dry mouth 2 group had both a lower concentration and lower output of HA, and the association between those was stronger in subjects administrated xerogenic drugs (Table 4). If a patient with xerogenic drugs is considered to have a serious dry mouth condition, a decreased level of HA might reflect a serious pathophysiological status. However, the association between the Dry mouth 1 and Control groups did not reach statistical significance. One possible explanation may have been because salivary flow rate was reduced to a greater degree than the concurrent changes in HA concentration in those groups.

The possible biological role of HA in the pathophysiological aspects of dry mouth remains unclear. However, when salivary film was defined as the thickness of saliva layer calculated by dividing the volume of saliva collected on each filter-paper strip by the surface area of each region of the mouth (12), the film on oral mucosa of subjects with dry mouth was found to be thinner, for example less than 10 μm on the hard palate (12), as compared to 70–100 μm in normal subjects (1). Considering that HA plays a role in protecting and lubricating the oral mucosa, it is possible that decreased HA levels in saliva may lead to local dryness of that tissue. On the other hand, the origin of HA in saliva remains speculative. The HA in whole saliva may originate from the endogenous material, including the product of the salivary glands, as well as bacteria (7). Though HA in parotid saliva is at predominantly one molecular weight only, HA in whole saliva shows two molecular weight bands. It seems likely that the low-molecular-weight HA in whole saliva results from cleavage by the hyaluronidase of the bacteria (8). Further studies will be needed to clarify interactions of HA and hyaluronidase in human saliva.

The present study has some limitations. First, the number of subjects analyzed was limited. This was in part because data regarding salivary levels of HA were obtained only from those able to produce an adequate quantity of measurable saliva. The device used in this study required saliva quantities of at least 200 μL, thus measurements of HA in subjects with extremely severe hyposalivation could not be performed. In addition, we could not analyze HA levels lower than 10 ng/mL, the limit lower limit of detection. Forty-two (approximately 48%) of the 88 subjects originally tested had HA levels lower than 10 ng/mL of HA, while 55% of the subjects in Dry mouth 1 and 52% in Dry mouth 2 also had HA levels lower than 10 ng/mL. A more sensitive assay method is needed for more accurate analysis. Finally, whether HA level is useful as a predictor of dry mouth remains unclear, because the design of the present study was cross-sectional.

In conclusion, subjects with dry mouth seem to have decreased salivary levels of HA as compared to those without dry mouth, and that association might be attributed to an altered HA function of protecting and lubricating the oral mucosa. Additional studies of salivary glycosaminoglycans including HA may lead to the development of effective method for diagnosis and treatment monitoring of treatment for subjects suffering from dry mouth.

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REFERENCES

1. Collins LM and Dawes C (1987) The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. *J Dent Res* **66**, 1300–1302.
2. Ericsson Y and Hardwick L (1978) Individual diagnosis, prognosis and counselling for caries prevention. *Caries Res* **12 Suppl 1**, 94–102.
3. Loeb LM, Naffah-Mazzacoratti MG, Porcionatto MA, Martins JR, Kouyoumdjian M,Weckx LM and Nader HB (2008) Chondroitin sulfate and kallikrein in saliva: markers for glossodynia. *Int Immunopharmacol* **8**, 1056–1058.
4. Moore PA, Guggenheimer J, Etzel KR, Weyant RJ and Orchard T (2001) Type 1 diabetes mellitus, xerostomia, and salivary flow rates. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **92**, 281–291.
5. Nederfors T (2000) Xerostomia and hyposalivation. *Adv Dent Res* **14**, 48–56.
6. Österberg T, Landahl S and Hedegard B (1984) Salivary flow, saliva, pH and buffering capacity in 70-year-old men and women. Correlation to dental health, dryness in the mouth, disease and drug treatment. *J Oral Rehabil* **11**, 157–170.
7. Pogrel MA, Lowe MA and Stern R (1996) Hyaluronan (hy-

aluronic acid) in human saliva. *Arch Oral Biol* **41**, 667–671.
8. Pogrel MA, Low MA and Stern R (2003) Hyaluronan (hyaluronic acid) and its regulation in human saliva by hyaluronidase and its inhibitors. *J Oral Sci* **45**, 85–91.
9. Sreebny LM (2000) Saliva in health and disease: an appraisal and update. *Int Dent J* **50**, 140–161.
10. Sreebny LM and Valdini A (1988) Xerostomia. Part I: Relationship to other oral symptoms and salivary gland hypofunction. *Oral Surg Oral Med Oral Pathol* **66**, 451–458.
11. Tishler M, Yaron I, Shirazi I and Yaron M (1998) Salivary and serum hyaluronic acid concentrations in patients with Sjögren's syndrome. *Ann Rheum Dis* **57**, 506–508.
12. Wolff M and Kleinberg I (1998) Oral mucosal wetness in hypo- and normosalivators. *Arch Oral Biol* **43**, 455–462.

編集後記

高齢者に対する摂食・嚥下リハビリテーションは、介護関連施設でも理解が深まり、介護予防の観点からも徐々に充実してきた感がある。しかし、臨床の現場における使用器材などは、それを反映していない場合も見受けられる。

口腔ケアや口腔機能向上の客観的評価基準については、対象者の理解度や運動機能にも大きく左右されることから、これらに関係ない客観的な評価基準が必要である。

本研究事業は、口腔の環境や口腔機能の指標として、口腔内から分泌されて口腔機能を発揮するために不可欠である唾液を応用することで、口腔ケアや口腔機能向上のサービスに役立てることを目的に、平成 19 年度から 3 年計画で総合的研究を開始し、初年度は、現状の課題と問題点の解析と、基本的なデータの解析を中心に進め、2年目である今年度は、唾液と口腔機能や嚥下機能などとの臨床的なデータを収集することが出来た。最終年度では、これらの研究成果を基により具体的な数値設定や口腔機能や嚥下機能のリスク判定基準について研究を進める予定である。

本研究事業の成果が高齢者における口腔機能の向上に役立ち、誤嚥性肺炎の予防や健康水準の亢進に役立つことが出来れば、望外の喜びです。

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