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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 (25^{th}, 75^{th} 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 (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 ($P < 0.05$).
[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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