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Salivary level of antimicrobial protein chromogranin A in relation to the salivary flow rate and swallowing function

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Abstract

Background: Dysphagia is one of the problems in elderly persons in this aging society. The inhibition of the secretion of the antimicrobial proteins could lead to oral dryness and oral dryness could lead to swallowing problems. The aim of this study was to examine salivary levels of antimicrobial protein in relation to the salivary flow rate and swallowing function.

Methods: In all, 26 patients with subjective oral dryness taking some medicines were studied. The unstimulated salivary flow rate was examined by ejecting gathered saliva from the mouth into a test tube for 10 min. The chromogranin A level was determined by

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an enzyme-linked immunosorbent assay. Swallowing function was evaluated using the repetitive saliva-swallowing test. Correlations among the salivary chromogranin A level, salivary flow rate, and swallowing function were analyzed using Spearman's correlation coefficient by rank.

Results: Statistically significant correlations were found between the salivary chromogranin A level and the unstimulated salivary flow rate ($r = 0.735$, $P < 0.01$) and between the salivary chromogranin A level and swallowing function ($r = 0.459$, $P < 0.05$). There was also a correlation between the unstimulated salivary flow rate and swallowing function ($r = 0.585$, $P < 0.01$).

Conclusion: The results of this study suggested that the salivary antimicrobial protein chromogranin A level was related to both the salivary flow rate and swallowing function. This result suggested that the inhibition of the secretion of the antimicrobial proteins could lead to oral dryness and oral dryness could lead to swallowing problems.

Keywords: Chromogranin A; Salivary flow rate; Swallowing function.

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1. Introduction

In an aging society, many elderly patients complain of oral dryness. Oral dryness has been recognized in 12–39% of the noninstitutionalized elderly population ≥65 years of age (Thomson WM 2005). The causes of oral dryness include certain medicines, diabetes, Sjögren's syndrome, and head and neck radiotherapy (Márton et al. 2004, Toljanic et al. 1996). The most common cause is the use of particular medicines (diuretics, antidepressants, neuroleptics, cytostatics, antiparkinson drugs, antihypertensives, and antihistamines) (Navazesh et al. 1992, Porter et al. 2004, Sreebny 2000). More than 400 medicines, many in common use, induce oral dryness (Llena-Puy 2006). Oral dryness leads to symptoms that include subjective oral dryness, burning sensation in the mouth, difficulty speaking or eating, and oral mucosal pain. Some patients with oral dryness complain of altered taste perception or difficulty swallowing (Cho et al. 2010, Mese et al. 2007). Dysphagia is another problem in elderly persons that can cause aspiration pneumonia or malnutrition (Marik et al. 2003, Teasell et al. 1996). Some studies have reported a relation between oral dryness and difficulty swallowing (Caruso et al. 1989, Rhodus et al. 1995, Rhodus et al. 1995), whereas another study showed opposite results (Rogus-Pulia et al. 2011). There is no consensus on this subject.

Several tests to address swallowing function have been introduced. The repetitive saliva-swallowing test (RSST) (Tamura et al. 2002, Tsukano et al. 2012) and the modified water-swallowing test (Gottlieb et al. 1996), for example, are used to screen for swallowing dysfunction. RSST, an effective means of screening to assess swallowing function (Hongama et al. 2012), is performed by counting the frequency of swallows during 30 s. The use of the RSST to train patients to improve their swallowing capability has reportedly produced great improvement in their oral function, although no significant changes were observed in saliva secretion or total microorganisms (Sakayori et al. 2013).

The relations among the salivary antimicrobial protein level, salivary secretion, and swallowing function, which are important for maintaining the oral environment of elderly patients, have not been clarified. The hypotheses were that there is a relation between salivary secretion, swallowing function, and the salivary level of antimicrobial protein, which is lower in patients with defective salivary secretion and swallowing problems because the inhibition of the secretion of the antimicrobial proteins could lead to oral dryness (Sugiya 2010) and oral dryness could lead to swallowing problems. This study examined salivary

levels of antimicrobial protein in relation to the salivary flow rate and swallowing function.

2. Material and Methods

2.1 Experimental subjects

The subjects of this study were 26 patients with subjective oral dryness and taking some medicines (6 men, 20 women; mean age 73.3 ± 8.2 years). The kinds of the medicines known to cause oral dryness that were taken by these patients are shown in Table 1. Patients with Sjögren's syndrome or any other connective tissue disease and those with a history of head and neck radiotherapy were excluded from this study. The subjects were elderly patients who visited the Dry Mouth Clinic in the Nippon Dental University Niigata Hospital. The Ethics Committee of the Nippon Dental University School of Life Dentistry at Niigata approved the study (ECNG-R-120), and written informed consent was obtained from all subjects before starting the study.

2.2 Unstimulated whole saliva collection

After rinsing the mouth with water, unstimulated whole saliva from each subject was measured using test tubes (Sarstedt AG & Co., Nümbrecht, Germany). The gathered saliva in the mouth of the seated subjects was ejected into the test tube over a 10-min period. The amount of saliva was measured based on the scale on the test tube. The test tubes already contained 40 µl of a protease inhibitor cocktail (leupeptin 100 µM, trypsin inhibitor 75 µg/ml, 5 mM 4-amidinophenylmethane sulfonyl fluoride hydrochloride, 250 mM benzamidine, aprotinin 100 µg/ml) to prevent degradation of the salivary proteins before we could examine the salivary antimicrobial protein level. The saliva was collected between 9 to 11 a.m. The collected unstimulated whole saliva was centrifuged at 14,000×g for 15 min at 4°C to remove debris. The supernatant was kept at −20°C for further study.

2.3 Protein assay

The total protein concentration of the saliva was determined with a protein assay kit (Bio-Rad Laboratories) with bovine serum albumin as a standard.

2.4 Chromogranin A assays

The antimicrobial protein chromogranin A (CgA) level was assessed in saliva. CgA is an acidic glycoprotein that is stored and co-released by exocytosis with catecholamines from the adrenal medulla and sympathetic nerve endings (Nakane et al. 1998, O'Connor et al. 1984). CgA is procured by the submandibular glands and secreted into saliva by stimulation with norepinephrine and acetylcholine (Kanno et al. 2000, Saruta et al. 2005). CgA-derived antimicrobial peptides, vasostatin-1, and catestatin are stored in secretory chromatin

granules that are co-secreted with CgA and catecholamines (Lugardon et al. 2000, Radek et al. 2008). Hence, the CgA concentration reflects the concentration of these antimicrobial peptides in saliva (Mizuhashi et al. 2015).

The salivary CgA level was determined using an enzyme-linked immunosorbent assay performed according to the manufacturer's directions (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA). The kit can be used for saliva samples, and the antibody in the kit was raised against a peptide fragment (342–355) of the CgA protein. This assay is reproducible and highly stable based on the manufacturer's tests.

2.5 Swallowing function

The repetitive saliva-swallowing test (RSST) (Tamura et al. 2002, Tsukano et al. 2012) was performed by counting the frequency of swallows during a 30-s period. One investigator counted the swallowing movements by palpating the laryngeal prominence and elevations of the hyoid bone. The number of swallows was used for analysis.

2.6 Statistical analysis

Correlations between the CgA flow rate per minute and the unstimulated salivary flow rate, the CgA flow rate per mg protein and the unstimulated salivary flow rate, the CgA flow rate per minute and the swallowing function, and the unstimulated salivary flow rate and the swallowing function were analyzed using Spearman's correlation coefficient by rank.

3. Results

Table 2 shows the results of the correlation analysis. The correlation between the CgA flow rate per minute and the unstimulated salivary flow rate was statistically significant ($P < 0.01$), and the unstimulated salivary flow rate increased as the CgA flow rate per minute increased. The correlation between the CgA flow rate per mg protein and the unstimulated salivary flow rate was statistically significant ($P < 0.05$), and the unstimulated salivary flow rate increased as the CgA flow rate per mg protein increased. The correlation between the CgA flow rate and swallowing function was statistically significant ($P < 0.05$), and swallowing function increased as the CgA flow rate increased. There was also a statistically significant correlation between the unstimulated salivary flow rate and swallowing function ($P < 0.01$), and the swallowing function increased as the unstimulated salivary flow rate increased.

4. Discussion

The salivary antimicrobial protein CgA level in relation to the salivary flow rate and swallowing function was examined in

patients with subjective oral dryness taking medicines, which is the most frequent cause of oral dryness. Most commonly, it is due to diuretics, antidepressants, neuroleptics, cytostatics, antiparkinson drugs, antihypertensives, and antihistamines (Navazesh et al. 1992, Porter et al. 2004, Sreebny 2000). The medicines most commonly used by the subjects of this study were antidepressants and antihypertensive agents.

The results of the present study showed same tendency to agree with previous studies that examined the relation between oral dryness and difficult swallowing (Caruso et al. 1989, Rhodus et al. 1995, Rhodus et al. 1995). Our results confirmed that the lower unstimulated salivary flow rate demonstrate swallowing difficulty. It was suggested that maintaining salivary secretory function is important for good swallowing function.

We evaluated the salivary CgA level in patients with subjective oral dryness and found statistically significant correlations between the CgA flow rate and the unstimulated salivary flow rate as well as between the CgA flow rate and swallowing function. These results suggested that there are relation among the antimicrobial protein CgA level, the unstimulated salivary flow rate, and swallowing function. Concerning limitations of this study, although the order which was occurred first is unknown, the results of this study could consent to the hypotheses that the inhibition of the secretion of the antimicrobial proteins could lead to oral dryness and oral dryness could lead to swallowing problems. Future research should examine the salivary level of antimicrobial proteins in relation to the salivary flow rate and swallowing function on the different subjects such as elderly persons without oral dryness, patients with Sjögren's syndrome, and patients after head and neck radiotherapy**.**

This study made it clear that the salivary antimicrobial protein CgA level was related to both the salivary flow rate and swallowing function in patients with subjective oral dryness.

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Table 1 Medicines causing oral dryness that were taken by patients in this study

Brand name	Generic name
Acecol Tablets	Temocapril Hydrochloride
Actonel	Sodium Risedronate Hydrate
Adalat-cr	Nifedipine
Alcadol	Alfacalcidol
Alfarol	Alfacalcidol
Allegra	Fexofenadine Hydrochloride
Amlodipine	Amlodipine Besilate
Amoban	Zopiclone
Amoxan	moxapine
Benzalin	Nitrazepam
Blopress Tablets	Candesartan Cilexetil
Bonalon Tablet	Alendronate Sodium Hydrate
Buscopan Tablets	Scopolamine Butylbromide
Cardenalin Tablets	Doxazosin Mesilate
Cephadol Granules	Difenidol Hydrochloride
Cercine Tablets	Diazepam
Cerocral	Ifenprodil Tartrate
Clarithromycin Tablets	Clarithromycin
Claritin Tablets	Loratadine

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Depas Tablets	Etizolam
Diovan Tablets	Valsartan
Elpinan Tablets	Epinastine Hydrochloride
Enchinin	Tizanidine Hydrochloride
Gasmotin	Mosapride Citrate Hydrate
Gaster Tablets	Famotidine
Hythiol	L-Cysteine
Jzoloft Tablets	Sertraline Hydrochloride
Lasix	Furosemide
Lendormin D Tablets	Brotizolam
Limarmone	Limaprost Alfadex
Lipozart	Simvastatin
Loxonin Tablets	Loxoprofen Sodium Hydrate
Maglax Tablets	Magnesium Oxide
Mequitamin Tablets	Mequitazine
Micardis Tablets	Telmisartan
Mucodyne Tablets	L-Carbocisteine
Mucosta Tablets	Rebamipide
Myonal	Eperisone Hydrochloride
Myslee Tablets	Zolpidem Tartrate
Neurotropin Tablets	Vaccinia virus inoculation rabbit inflammation skin extract component preparation

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Noraguard Tablets	Propiverine Hydrochloride
Norvasc Tablets	Amlodipine Besilate
Onealfa Tablet	Alfacalcidol
Pariet	Rabeprazole Sodium
PL granulated medicine	Salicylamide
Pletaal OD Tablets	Cilostazol
Prednisolone Tablets	Prednisolone
Protecadin Tablet	Lafutidine
Raspjine Tablets	Azelastine Hydrochloride
Rhythmy	Rilmazafone Hydrochloride Hydrate
Ridaura Tablets	Auranofin
Selbex	Teprenone
Soleton	Zaltoprofen
Sunrythm Capsules	Pilsicainide Hydrochloride Hydrate
Takepron Capsules	Lansoprazole
Urief Tablets	Silodosin
Voltaren Tablets	Diclofenac Sodium

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Table 2 Results of correlation analysis

Correlation analysis	*r*	*P*
Flow rate of CgA (ng/min) and USF [1] (mL/min)	0.735	< 0.01
CgA (ng/mg protein) and USF [1] (mL/min)	0.393	< 0.05
Flow rate of CgA (ng/min) and SF [2] (times)	0.459	< 0.05
USF [1] (mL/min) and SF [2] (times)	0.585	< 0.01

[1]Unstimulated salivary flow rate

[2]Swallowing function

