Oral dryness caused by calcium blocker -Comparison with saliva of healthy elderly persons and patients with Sjögren's syndrome-

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Abstract

Background: In this aging society, many elderly dental patients have subjective dry mouth. The most common cause of oral dryness is the use of particular medicines, and calcium blocker is widely taken as the treatment of hypertension. The purpose of this study was to evaluate the saliva of oral dryness patients taking calcium blocker in comparison with that of the healthy elderly persons and the patients with Sjögren's syndrome.

Methods: Forty six subjects were enrolled

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in this study (fifteen patients taking calcium blocker, fifteen patients with Sjögren's syndrome, and sixteen healthy elderly persons). The unstimulated salivary flow rate was examined by ejecting gathered saliva from the mouth into a test tube for 10 min. Stimulated salivary flow rate was measured by gum-chewing with gathered saliva ejected into the test tube over a 10-min period. The total protein concentration of the saliva was determined with a protein assay kit. The differences in unstimulated salivary flow rate, protein concentration, and flow rate of protein among the three groups was analyzed with Kruskal Wallis test. The difference in stimulated saliva was analyzed with one-way analysis of variance.

Results: Unstimulated salivary flow rate and the flow rate of protein on unstimulated saliva was significantly lower on patients taking calcium blocker and patients with Sjögren's syndrome than that on healthy elderly persons ($P < 0.01$). Stimulated salivary flow rate was significantly lower on patients taking calcium blocker than that on healthy elderly persons ($P < 0.05$), and significantly lower on patients with Sjögren's syndrome than that on healthy elderly persons ($P < 0.01$).

Conclusion: The results of this study suggested that the condition of salivary gland in oral dryness patients is different from healthy elderly. Furthermore, it was considered that the submaxillary gland in patients taking calcium blocker would be affected by the calcium blocker.

Keywords: Oral dryness, Calcium blocker, Sjögren's syndrome

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1. Introduction

In this aging society, many elderly dental patients have subjective dry mouth. It is reported that the prevalence of oral dryness is between 0.9% and 64.8% (Orellana MF et al. 2006). 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 of oral dryness 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), and many dental patients take some of these medicines. There are more than 400 medicines that induce oral dryness (Llena-Puy 2006).

Calcium blocker is widely taken as the treatment of hypertension and calcium blocker causes dry mouth (Hattori T et al. 2007). However, the mechanism of calcium blocker to cause dry mouth has not been cleared enough (He XJ et al. 1989). Calcium blocker also causes gingival thickening. The mechanism of calcium blocker to cause gingival thickening doesn't have just one point of view (Salo T et al. 1990, Seymour RA et al.1997). The gingival thickening is induced by the growth of the gingival component cell. The existence of this phenomenon indicates that calcium blocker causes not only the inhibition of the calcium channel but also some transcriptive activity.

We aimed to make clear the characteristics of the oral dryness patients who take calcium blocker. The purpose of this study was to evaluate the saliva of oral dryness patients taking calcium blocker in comparison with that of the healthy elderly persons and the patients with Sjögren's syndrome.

2. Material and Methods

2.1 Experimental subjects

Forty six subjects were enrolled in this study. Fifteen patients taking calcium blocker (2 men, 13 women; mean age 69.0 ± 7.9 years) and fifteen patients with Sjögren's syndrome (15 women; mean age 64.5 ± 8.0 years) who attended the Dry Mouth Clinic in the Nippon Dental University Niigata Hospital, and sixteen healthy elderly persons (7 men, 9 women; mean age 71.3 ± 6.6 years) who attended the Nippon Dental University Niigita Hospital for periodic health examination were investigated. The study was performed with the approval of the Ethics Committee of the Nippon Dental University School of Life Dentistry at

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Niigata (ECNG-H-155) and written informed consent was obtained from all subjects before starting the study.

2.2 Unstimulated whole saliva collection

Unstimulated whole saliva from ten patients taking calcium blocker and ten patients with Sjögren's syndrome, and eleven healthy elderly persons 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 (Dawes C 1987). 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 (Mizuhashi F et al. 2016). 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 Stimulated whole saliva collection

Stimulated salivary flow rate was measured by gum-chewing (Navazesh M 1982). A gum (Freezone, Lotte Co., Tokyo, Japan) that does not stick to the denture was used in this study. Stimulated whole saliva was gathered from five patients taking calcium blocker and five patients with Sjögren's syndrome, and five healthy elderly persons. 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 to prevent degradation of the salivary proteins (Mizuhashi F et al. 2016). The collected stimulated 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.

2.4 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.

2.5 Statistical analysis

The differences in unstimulated salivary flow rate, protein concentration (unstimulated saliva), and flow rate of protein (unstimulated saliva) among

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patients taking calcium blocker, patients with Sjögren's syndrome, and healthy elderly persons was analyzed with Kruskal Wallis test. The differences in stimulated salivary flow rate, protein concentration (stimulated saliva), and flow rate of protein (stimulated saliva) among patients taking calcium blocker, patients with Sjögren's syndrome, and healthy elderly persons was analyzed with one-way analysis of variance.

3. Results

Table 1 shows the result of unstimulated salivary flow rate, protein concentration (unstimulated saliva) and flow rate of protein (unstimulated saliva) among patients taking calcium blocker, patients with Sjögren's syndrome, and healthy elderly persons. Unstimulated salivary flow rate was significantly lower on patients taking calcium blocker and patients with Sjögren's syndrome than that on healthy elderly persons ($P < 0.01$). The mean value of unstimulated salivary flow rate on patients taking calcium blocker was 0.57 mL/10min and that on patients with Sjögren's syndrome was 0.57 mL/10min, and unstimulated salivary flow rate was almost same value on patients taking calcium blocker and patients with Sjögren's syndrome. There was no statistically significant difference on the protein concentration on unstimulated saliva among patients taking calcium blocker, patients with Sjögren's syndrome, and healthy elderly persons. The flow rate of protein on unstimulated saliva was significantly lower on patients taking calcium blocker and patients with Sjögren's syndrome than that on healthy elderly persons ($P < 0.01$). The mean value of the protein concentration and the flow rate of protein on unstimulated saliva of patients taking calcium blocker and patients with Sjögren's syndrome was also almost same value.

Table 2 shows the result of stimulated salivary flow rate, protein concentration (stimulated saliva), and flow rate of protein (stimulated saliva) among patients taking calcium blocker, patients with Sjögren's syndrome, and healthy elderly persons. Stimulated salivary flow rate was significantly lower on patients taking calcium blocker than that on healthy elderly persons ($P < 0.05$), and significantly lower on patients with Sjögren's syndrome than that on healthy elderly persons ($P < 0.01$). The mean value of stimulated salivary flow rate on patients taking calcium blocker was 3.94 mL/10min and that on patients with Sjögren's syndrome was 2.95 mL/10min, and stimulated salivary flow rate on patients

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taking calcium blocker tended to be larger than that on patients with Sjögren's syndrome. There were no statistically significant differences on the protein concentration and the flow rate of protein on stimulated saliva among patients taking calcium blocker, patients with Sjögren's syndrome, and healthy elderly persons. The mean value of the protein concentration on patients taking calcium blocker tended to be smaller than that on patients with Sjögren's syndrome, and the mean value of the flow rate of protein on patients taking calcium blocker tended to be larger than that on patients with Sjögren's syndrome.

4. Discussion

The most common cause of oral dryness is the use of particular medicines, and calcium blocker is widely taken as the treatment of hypertension (Hattori T et al. 2007). However, the mechanism of calcium blocker to cause dry mouth has not been cleared enough. For the purpose to make it clear the characteristics of the oral dryness patients who take calcium blocker, this study examined the unstimulated saliva and stimulated saliva of oral dryness patients taking calcium blocker and compared them to that of the healthy elderly persons and the patients with Sjögren's syndrome.

Sjögren's syndrome is autoimmune disease, and most commonly afflicted are the salivary and lacrimal glands, leading to reduced secretion and dry mucous membranes (Fox RI et al. 2005). Salivary secretion depends on parasympathetic and sympathetic signaling, and parasympathetic activation lead to increased Ca^{2+} release from the endoplasmic reticulum, and then water flux out of the cell was led (Romanenko VG et al. 2007). This is depending in part on the intracellular free Ca^{2+} levels (Foskett JK et al. 1989). Calcium blocker is one of the antihypertensive that cause oral dryness (Hattori T et al. 2007). Though there are many kinds of medicines for the treatment of hypertension, 70% of the patients with hypertension are taking calcium blocker (Koike S 2006).

In this study, unstimulated saliva and stimulated saliva was investigated. Unstimulated salivary flow rate in healthy elderly persons in this study became larger in comparison with the previous study though the mean age of the healthy elderly persons in this study was higher than that of the previous study (Márton K et al. 2004). Unstimulated salivary flow rate in patients with Sjögren's syndrome in this study became smaller in comparison with the previous study (Márton K et al. 2004).

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Therefore, the condition of the patients with Sjögren's syndrome in this study would be severely condition. Djukić LJ et al. reported the effect of anti-hypertensives on salivary flow. The anti-hypertensives investigated in that study was ACE inhibitor, beta blocker, and diuretic agent. The unstimulated salivary flow rate in healthy elderly persons in this study became larger than that in the past study (Djukić LJ et al. 2015) though the mean age of the healthy elderly persons in this study was higher than that of the past study (Djukić LJ et al. 2015). The study of Djukić LJ et al. indicated that unstimulated salivary flow rate in ACE inhibitor group was almost same value with healthy elderly persons, and that in beta blocker and diuretic agent groups was about five sixth of healthy elderly persons. The results of this study showed that unstimulated salivary flow rate in patients taking calcium blocker was one-tenth of healthy elderly persons, and almost same value to the patients with Sjögren's syndrome. These results suggested that the impact on oral dryness of calcium blocker is significant in comparison with ACE inhibitor, beta blocker, and diuretic agent.

On the other hand, the results of this study indicated that stimulated salivary flow rate in patients taking calcium blocker tended to be larger value than that in patients with Sjögren's syndrome. There was not statistically significant difference on the protein concentration on unstimulated saliva among the patients taking calcium blocker, patients with Sjögren's syndrome, and healthy elderly persons, but the flow rate of protein on unstimulated saliva was significantly lower on patients taking calcium blocker and patients with Sjögren's syndrome than that on healthy elderly persons. In our previous study, protein concentration and flow rate of protein were different between healthy elderly and oral dryness patients (Mizuhashi F et al. 2015). Therefore, it was suggested that the condition of salivary gland in oral dryness patients is different from healthy elderly. Furthermore, it was considered that the submaxillary and sublingual glands in patients taking calcium blocker would be affected by the calcium blocker. It has been reported that unstimulated saliva is composed by parotid gland (28%), submaxillary and sublingual glands (68%), and minor salivary gland (4%), and stimulated saliva is composed by parotid gland (53%), submaxillary and sublingual glands (46%), and minor salivary gland (1%) (Proctor GB 2016). Therefore, it could be said that unstimulated salivary flow rate indicate the condition of the submaxillary and sublingual glands.

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Unstimulated salivary flow rate in patients taking calcium blocker was almost same value to that in patients with Sjögren's syndrome though stimulated salivary flow rate became different value between the two groups; therefore, the submaxillary and sublingual glands in patients taking calcium blocker could be affected by the calcium blocker. Some studies have been reported the salivary cytokine on salivary protein in Sjögren's syndrome (Kang EH et al. 2011, Enger TB et al. 2014). However, there are few reports investigating salivary components in patients taking calcium blocker. After this, the protein components secreted from submaxillary gland and parotid gland should be examined to make clear the effect of the calcium blocker to the salivary glands.

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References

1. Dawes C. Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. J Dent Res. 1987; 66:648-653. [PubMed: 3476629]
2. Djukić LJ, Roganović J, Brajović MD, Bokonjić D, Stojić D. The effects of anti-hypertensives and type 2 diabetes on salivary flow and total antioxidant capacity. Oral Dis. 2015; 21:619-625. [PubMed: 25689395]
3. Enger TB, Aure MH, Jensen JL, Galtung HK. Calcium signaling and cell volume regulation are altered in Sjögren's Syndrome. Acta Odontol Scand. 2014; 72:549-556. [PubMed: 24471729]
4. Foskett JK, Melvin JE. Activation of salivary secretion: coupling of cell volume and [Ca2+]i in single cells. Science. 1989; 244:1582-1585. [PubMed: 2500708]
5. Fox RI. Sjögren's syndrome. Lancet. 2005; 366:321-331. [PubMed: 16039337]
6. Hattori T, Wang PL. Calcium antagonists cause dry mouth by inhibiting resting saliva secretion. Life Sci. 2007; 81:683-690. [PubMed: 17688889]
7. He XJ, Wu XZ, Wellner RB, Baum BJ. Muscarinic receptor regulation of Ca2+ mobilization in a human salivary cell line. Pflugers Arch. 1989; 413:505-510. [PubMed: 2787018]
8. Kang EH, Lee YJ, Hyon JY, Yun PY, Song YW. Salivary cytokine profiles in primary Sjögren's syndrome differ from those in non-Sjögren sicca in terms of TNF-α levels and Th-1/Th-2 ratios. Clin Exp Rheumatol. 2011; 29:970-976. [PubMed: 22132900]
9. Koike S. Current condition and future of pharmacotherapy. Folia Pharmacol Jap. 2006; 127:393-398. (in Japanese)
10. Llena-Puy C. The role of saliva in maintaining oral health and as an aid to diagnosis. Med Oral Patol Oral Cir Bucal. 2006; 11:E449-455. [PubMed: 16878065]
11. Márton K, Boros I, Fejérdy P, Madléna M. Evaluation of unstimulated flow rates of whole and palatal saliva in healthy patients wearing complete dentures and in patients with Sjogren's syndrome. J Prosthet Dent. 2004; 91:577-581. [PubMed: 15211301]
12. Mizuhashi F, Koide K, Toya S, Takahashi M, Mizuhashi R, Shimomura H. Levels of the antimicrobial proteins lactoferrin and chromogranin in the saliva of individuals with oral dryness. J Prosthet Dent. 2015; 113:35-38. [PubMed: 25300178]

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13. Mizuhashi F, Koide K, Toya S, Nashida T. Salivary level of antimicrobial protein chromogranin A in relation to the salivary flow rate and swallowing function. Medical Research Archives. 2016; 4:1-13.
14. Navazesh M, Christensen CM. A comparison of whole mouth resting and stimulated salivary measurement procedures. J Dent Res. 1982; 61:1158-1162. [PubMed: 6956596]
15. Navazesh M, Christensen C, Brightman V. Clinical criteria for the diagnosis of salivary gland hypofunction. J Dent Res. 1992; 71:1363-1369. [PubMed: 1629451]
16. Orellana MF, Lagravère MO, Boychuk DG, Major PW, Flores-Mir C, Ortho C. Prevalence of xerostomia in population-based samples: a systematic review. J Public Health Dent. 2006; 66:152-158. [PubMed: 16711637]
17. Porter SR, Scully C, Hegarty AM. An update of the etiology and management of xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004; 97:28-46. [PubMed: 14716254]
18. Proctor GB. The physiology of salivary secretion. Periodontol 2000. 2016; 70:11-25. [PubMed: 26662479]
19. Romanenko VG, Nakamoto T, Srivastava A, Begenisich T, Melvin JE. Regulation of membrane potential and fluid secretion by Ca2+-activated K+ channels in mouse submandibular glands. J Physiol. 2007; 581:801-817. [PubMed: 17379640]
20. Salo T, Oikarinen KS, Oikarinen AI. Effect of phenytoin and nifedipine on collagen gene expression in human gingival fibroblasts. J Oral Pathol Med. 1990; 19:404-407. [PubMed: 2176691]
21. Seymour RA, Thomason JM, Nolan A. Oral lesions in organ transplant patients. J Oral Pathol Med. 1997; 26:297-304. [PubMed: 9250928]
22. Sreebny LM. Saliva in health and disease: an appraisal and update. Int Dent J. 2000; 50:140-161. [PubMed: 10967766]
23. Toljanic JA, Siddiqui AA, Patterson GL, Irwin ME. An evaluation of a dentifrice containing salivary peroxidase elements for the control of gingival disease in patients with irradiated head and neck cancer. J Prosthet Dent. 1996; 76:292-296. [PubMed: 8887803]

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Table 1: Unstimulated salivary flow rate, protein concentration (unstimulated saliva) and flow rate of protein (unstimulated saliva) among patients taking calcium blocker, patients with Sjögren's syndrome, and healthy elderly persons.

	HE[1]	CB[2]	SS[3]	*P*
USF[4] (mL/10min)	5.42±3.74[a,b]	0.57±0.22[a]	0.57±0.40[b]	< 0.01
PC[5] (μg/mL)	2459.7±2311.1	3676.0±2011.1	3926.7±2454.3	0.145
FP[6] (μg/min)	1596.0±2330.2[c,d]	184.5±85.9[c]	185.9±147.1[d]	< 0.01

[1] Healthy elderly persons

[2] Patients taking calcium blocker

[3] Patients with Sjögren's syndrome

[4]Unstimulated salivary flow rate

[5] Protein concentration

[6] Flow rate of protein

[a,b,c,d] $P < 0.01$

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Table 2: Stimulated salivary flow rate, protein concentration (stimulated saliva) and flow rate of protein (stimulated saliva) among patients taking calcium blocker, patients with Sjögren's syndrome, and healthy elderly persons.

	HE[1]	CB[2]	SS[3]	*P*
SF[4] (mL/10min)	7.80±2.16[a,b]	3.94±1.42[a]	2.95±1.30[b]	< 0.01
PC[5] (μg/mL)	2727.3±1009.2	3311.9±2006.0	4378.5±1908.3	0.264
FP[6] (μg/min)	2029.5±694.8	1231.6±720.9	984.0±580.2	0.109

[1] Healthy elderly persons

[2] Patients taking calcium blocker

[3] Patients with Sjögren's syndrome

[4] Stimulated salivary flow rate

[5] Protein concentration

[6] Flow rate of protein

[a] $P < 0.05$

[b] $P < 0.01$

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