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Professional oral care reduces influenza infection in elderly

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Abstract

Influenza is a major cause of respiratory infection and has a high mortality rate in the elderly. Neuraminidase (NA) on the surface of the influenza virus and bacterial trypsin-like proteases (TLP) play key roles in influenza virus infections. We investigated the effects of oral care on influenza, evaluating in particular the activities of NA and TLP in saliva, as they may contribute to an increased risk of infection with influenza. One hundred ninety elderly patients who visited day care service facilities once a week were randomly assigned to either a professional oral care group or to an own oral care group as the control group. Nine individuals in the control group and one person in the professional oral care group were diagnosed with influenza during the follow-up period. The relative risk of developing influenza while under professional oral care compared to that in the control group was 0.1 (95% CI 0.01-0.81, $p = 0.008$). Significant decreases in numbers of salivary anaerobic bacterial CFUs, and NA and TLP levels were observed in the professional oral care group compared to that in the control group ($p < 0.01$). This study suggests that maintenance of oral hygiene is effective in the prevention of influenza in the elderly.

1. Introduction

Influenza is a serious infectious respiratory disease in the elderly (Nicholson et al., 1997), and the prevention of an annual pandemic of influenza is a matter of urgency (Nicholson et al., 2003). Persons aged over 65 years are at particular risk from influenza, and the infection has a high mortality rate in this age group (Simonsen et al., 1998). A recent study has suggested a relationship between oral care and aspiration pneumonia in the elderly (Yoneyama et al., 1999, 2002). Professional oral care has been found to reduce both cryptogenic fever (Yoneyama et al., 1996; Adachi et al., 2002) and aspiration pneumonia, along with reducing number of oral bacteria (Abe et al., 2001). Impairment of the swallowing reflex in the elderly has been shown to increase the risk of aspiration, providing greater opportunity for oral bacteria to enter the lower respiratory tract with the saliva (Kikuchi et al., 1994). Compromised host defense mechanisms and aspiration both increase the risk of aspiration pneumonia, especially in the elderly (Kikuchi et al., 1994). Deterioration in oral hygiene may lead to an increase in levels of oral bacteria and bacterial enzymes. Bacterial enzymes may injure the oral mucosa and possibly accelerate the onset of viral and bacterial infections (Scannapieco, 1999). It has been reported that bacterial proteases increase the risk of infection with the influenza virus, thus triggering a further increase

in the incidence of pneumonia (Tashiro et al., 1987a, b; Scheiblauer et al., 1992).

Hemagglutinin (HA) and neuraminidase (NA) on the surface of the virus play important roles in infection by and multiplication of the influenza virus.   NA cleaves sialic acid at the cell's viral binding site, which may lead to an increased risk of viral infection.   NA inhibitors are an important class of influenza drug that prevents both diffusion of the virus and viral activity, and their safety has been confirmed (Kim et al., 1997; Gubareva et al., 2000).   Trypsin-like proteases (TLP) produced by bacteria (e.g. *Staphylococcus aureus, Pseudomonas aeruginosa* and pneumococcus) also play a significant role in activating infection by mediating influenza virus HA modification (Tashiro et al., 1987a, b; Scheiblauer et al., 1992).   These bacteria reside and multiply in the oral cavity, especially in the elderly (Abe et al., 2001).   It has also been reported that NA and TLP are produced by oral bacteria (Leach and Hynes, 1967; Laughon et al., 1982; Loesche et al., 1987).   An increase in oral bacteria due to poor oral hygiene increases the activity of both NA and TLP in the oral cavity, and this may increase the risk of infection by and multiplication of the influenza virus.

In this study, we investigated the effects of professional oral care on influenza infection in the elderly by evaluating the total number of colony forming units (CFUs) of anaerobic bacteria and NA and TLP activities in saliva.

2. Patients and methods

2.1. Subjects

The participants consisted of elderly people who lived in their own residences in Tokyo, and who visited day care service facilities once a week. Fully-informed written and verbal consent was obtained from all 216 patients and their families. All patients were randomly assigned to either a professional oral care group or to an own oral care control group who were not to receive professional oral care. However, 26 patients dropped out of the study as a result of hospitalization or difficulties in visiting the day care center during the follow-up period. Of the remaining 190 patients, 98 patients (45 men, 53 women, mean age 82 ± 8 years) received professional oral care and 92 patients (41 men, 51 women, mean age 84 ± 6 years) did not (Table 1). The patients in the control group were informed that they could also receive professional oral care if they so wished. However, none of the controls requested it.

2.2. Intervention

The professional oral care group had their teeth and gingivae cleaned once a week by a dental hygienist using toothbrushes, dental floss and tongue brushes. They also

received weekly guidance on personal oral care.   The control group carried out oral care by themselves, without supervision.

Before the study, all patients received a physical examination, oral examination and saliva test.   Activities of daily living (ADL) were also evaluated using the modified Barthel Index (Mahoney and Barthel, 1965).   The intervention period was 6 months from September 1st, 2003 to March 30th, 2004.

2.3. Saliva tests

Two saliva tests were performed in all patients, once at baseline study and once at the 6-month follow-up, except in those that showed a lack of saliva and/or poor physical condition.   There were problems obtaining a sufficient amount of saliva from elderly subjects due to associated problems of aging such as xerostomia.   Of the 92 patients in the control group, total CFUs were counted in 41 patients, and activity of NA and TLP was evaluated in 45 patients and 44 patients, respectively.   Of the 98 patients in the intervention group, total CFUs were counted in 57 patients, and activity of NA and TLP was evaluated in 54 patients and 55 patients, respectively.   Saliva samples were obtained 2 hours after breakfast.   Participants took no food or drink after breakfast until saliva sampling.   This sampling required that the participant spit into a sterile cup

under passive supervision.

This method was adopted to avoid any sampling bias which might arise from differences in the area of the oral cavity sampled or differences in the sampling procedure adopted. Each saliva sample (10 µl) was used for a total CFUs count, as well as NA and TLP activity tests.

2.4. Total CFUs of anaerobic bacteria

Ten µl samples of saliva were diluted with phosphate-buffered saline (PBS, pH 7.4) in a 10-fold gradient to $1:10^{-8}$, and 100 µl of each dilution was then inoculated onto Trypticase soy agar plates (Becton Dickinson, Cockeysville, MD, USA) supplemented with hemin (5 µg·ml^{-1}), menadione (0.5 µg·ml^{-1}) and 10% defibrinated horse blood in duplicate. These inoculated plates were then incubated at 37°C for a week under anaerobic conditions (10% CO_2, 10% H_2, and 80% N_2). The total numbers of anaerobic bacteria were counted and expressed as CFUs (CFUs/ml).

2.5. NA activity test

Presence of NA in the saliva was measured with an Amplex Red Neuraminidase Assay Kit (Molecular Probes, Eugene, OR). The saliva was mixed with a reagent and

allowed to react for 30 minutes at 37°C. This was then followed by measurement of

neuraminidase activity at 514 nm of excitation light and 610 nm of fluorescence using a

FluoroImager (Amersham Bioscience, Piscataway, NJ). All measurements were

quantified based on a 0-20 mU/ml neuraminidase activity curve.

2.6. TLP activity test

Salivary TLP activity was measured with a Perio Check (Sunstar, Takatsuki, Japan).

The saliva was mixed with the Perio Check reagent and incubated at 37°C for 15

minutes. This was followed by measurement of optical density of enzyme activity

using a 630 nm absorbance photometer.

2.7. Influenza diagnosis and vaccination

We examined all the patients who developed influenza during the intervention period.

Influenza was diagnosed using a rapid antigen detection test-QuickVue kit (Quidel, San

Diego, CA) for patients with influenza-like illnesses (ILI), which were specified as

being characterized by coughing and a temperature of at least 37.8°C (Monto et al.,

2000). Patients who exhibited ILI symptoms, but who were diagnosed as not having

influenza, were categorized as having developed the common cold.

Vaccination against influenza had been performed in 36.7% of the oral care group and 42.4% of the control group. All care givers (including doctors, nurses and dental hygienists) were vaccinated against influenza on September 1st, 2003.

2.8. Statistical analysis

All comparisons between the professional oral care and the control group were made at baseline and at after 6 months. A Wilcoxon's rank sum test was performed in both groups to determine any differences in number of CFUs and NA and TLP values between before and after the intervention period. The relationship between professional oral care and development of influenza was evaluated with Fisher's exact test. All statistical analyses were carried out using SAS ver. 8.02 software and significance was established as $p < 0.05$.

3. Results

There were no significant differences in the baseline physical characteristics of the participants between the two groups (Table 1). Neither was there any significant difference in terms of influenza vaccination. Total CFUs of bacteria, along with NA and TLP activities, all showed a significant reduction in the patients who had received

professional oral care (Fig. 1, 2, and 3, respectively). In contrast, the control group showed no significant difference in any category.

Thirty patients developed ILI during the intervention period. Ten of these patients were diagnosed as being influenza positive, while the other 20 patients were diagnosed as having the common cold (Table 2). Among the patients who were infected with influenza, four out of nine from the control group and one from the professional oral care group had been vaccinated. Nine (9.8%) in the control group and one (1.0%) in the professional oral care group were diagnosed as having influenza. There was no significant difference in the rate of vaccination between the professional oral care group and the control group, although the rate of influenza infection was significantly different. None of the dental hygienists who provided oral care developed influenza. The relative risk of developing influenza while under professional oral care compared to not being under professional oral care was 0.1 (95% CI 0.01-0.81, $p = 0.008$).

4. Discussion

This study suggests that receiving active weekly professional oral care and oral health guidance from a dental hygienist reduces both the number of oral bacteria and the activities of NA and TLP in saliva, resulting in a reduction in the risk of infection from

influenza.　Thorough professional oral care was carried out by a dental hygienist using a toothbrush and an interdental brush.　This resulted in a reduction in oral bacteria and salivary enzyme activity.　On the other hand, routine oral care carried out by the participants themselves or care givers had no influence on these parameters.　This suggests that oral care without professional assistance is insufficient to maintain good oral hygiene and prevent respiratory infections.　The effect of a reduction in NA activity may be similar to that of NA inhibitors in preventing influenza.　Moreover, a decrease in TLP levels may attenuate virus HA modification.　These improvements in the state of oral hygiene may be effective in decreasing the susceptibility of the upper respiratory tract to infectious influenza.　Gross et al. (1995) reported that the influenza vaccine prevented 50% of influenza development in elderly people and emphasized the importance of vaccination.　However, influenza outbreaks occur every year throughout the world and still have a high mortality rate.　It has been suggested that vaccination alone is insufficient to control influenza in nursing homes (Monto et al., 2004).　New advanced research and effective prevention and treatment methods for influenza are still required (Nicholson et al., 2003).　In this study, the immunization rate in the professional oral care group was 36.7%, and that in the control group was 42.4%, indicating that there was no significant difference between these two groups.　However,

compared to the controls, the professional oral care group showed a significantly lower rate of infection. The relative risk of developing influenza while under professional oral care compared with nonprofessional oral care was 0.1. Furthermore, there were more common cold patients in the control group than in the professional oral care group. This suggests that, not only influenza but also the common cold may be influenced by status of oral hygiene. However, this preliminary study needs to be followed up using a larger sample of patients. Further study is also needed on how the TLP of specific oral bacteria mediate modification of viral HA.

This study showed that professional oral care reduced the levels of oral bacteria and enzymatic activity, and that this lowered the risk of infection with the influenza virus. This suggests that adequate oral care may be one approach to reducing the incidence of influenza in the elderly.

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Table 1. Comparisons between professional oral care and control groups*

Characteristic	Professional oral care (n = 98)	Control (n = 92)	p =
Gender (Male/ Female)	27/71	25/67	0.2
Age, yr	82 ± 8	84 ± 6	0.5
Number of remaining teeth,	8.3 ± 8.6	7.3 ± 9.3	0.6
ADL Score	81.2 ± 23.3	75.1 ± 27.7	0.8
Number of patients with cerebrovascular events (%)	25 (25.5)	9 (9.8)	0.1
Number of patients with dementia (%)	11 (11.2)	8 (8.7)	0.9

*Values given as mean ± SD or No. (%), unless otherwise indicated.

Table 2. Influenza vaccination and incidence of influenza

	Professional oral care (n = 98)	Control (n = 92)
Influenza vaccine (%)	39 (42.4)	36 (39.1)
Influenza (%)	1 (1.0)	9 (9.8) †
Common cold（%）	8 (8.2)	12 (13.0)

*Values are No. (%).   †Fisher's exact test; p=0.008

Figure Legends

Fig. 1: Total CFUs at baseline and at after 6 months in professional oral care group vs.

control group.

*Wilcoxon's rank sum test; p < 0.05.

Fig. 2: NA activity at baseline and at after 6 months in professional oral care group vs.

control group.

 * Wilcoxon's rank sum test; p < 0.05.

Fig. 3: TLP activity at baseline and at after 6 months in professional oral care group vs.
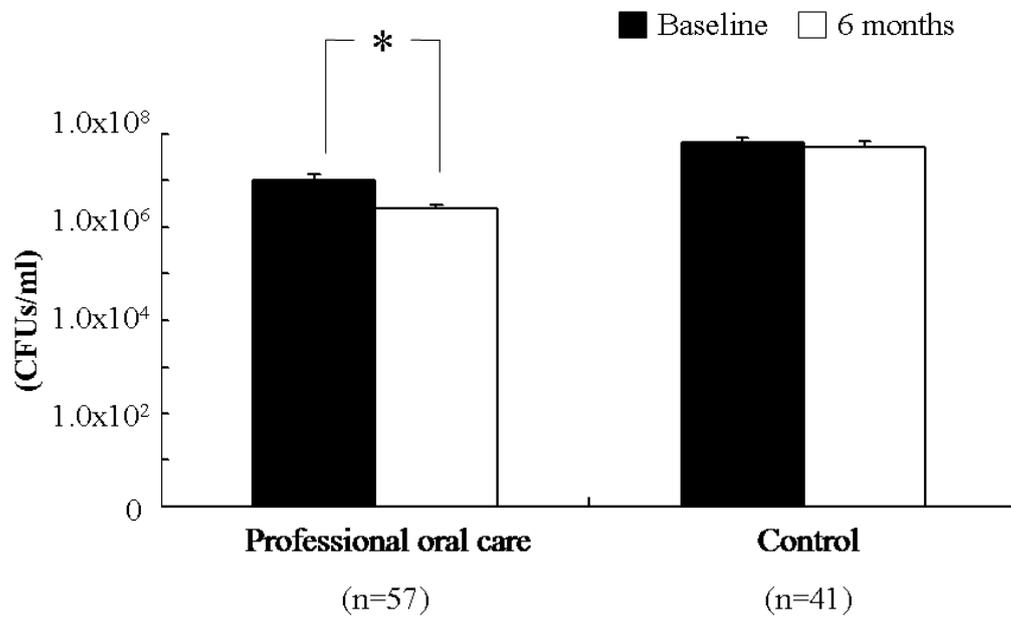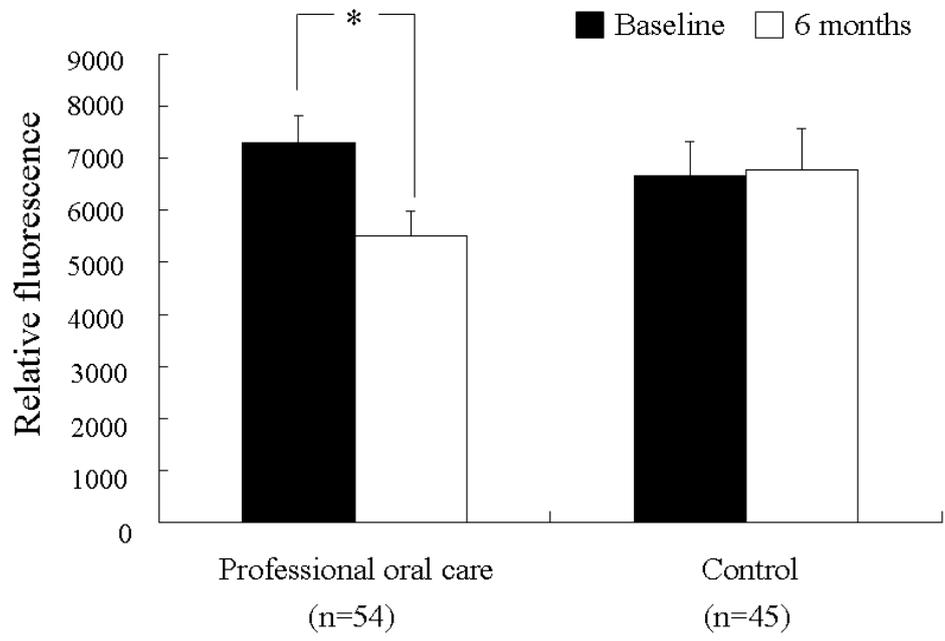
control group.
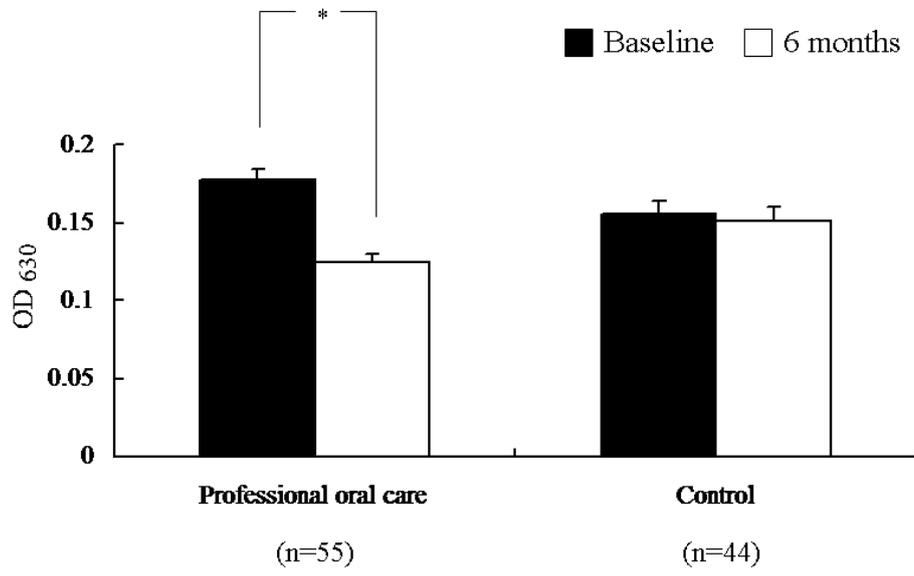
* Wilcoxon's rank sum test; p < 0.05.

Fig. 1

Fig. 2

Fig. 3