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Original Article

Effects of Oral Care on Development of Oral Mucositis and Microorganisms in Patients with Esophageal Cancer

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SUMMARY: We evaluated the effects of special oral care using a toothbrush with combined irrigation and suctioning functions, along with povidone-iodine to treat oral bacteria and mucositis, in esophageal cancer patients undergoing chemoradiotherapy. In the special care group, oral hygiene was performed 3 days a week after dinner. Bacteria in saliva and plague samples were measured at various sampling points after chemoradiotherapy. The incidence of mucositis was significantly reduced in the special care group in comparison with the control group. Total streptococci were significantly decreased in the opportunistic pathogens-positive and lower-level mutans streptococci control group during chemoradiotherapy, but they were not reduced in the opportunistic pathogens-negative and higher-level mutans streptococci control groups or in the special care group. Our results showed that a special oral care regimen enabled the total population of streptococci microflora to remain stable, was negatively correlated with opportunistic pathogens and positively correlated with mutans streptococci infection, and prevented the development of mucositis.

INTRODUCTION

Chemoradiotherapy is frequently used as a non-surgical treatment and has been shown to be more effective than radiotherapy alone in patients with esophageal cancer (1-5). 5-fluorouracil (5-FU) and platinum derivatives (cisplatin [CDDP] and nedaplatin) are frequently used drugs in chemoradiotherapy for cancer patients (6,7), because they act synergistically with radiation to control the outgrowth of cancer cells and treat inactive cells. However, both these drugs lack specificity for cancer cells, and thus have toxic effects on rapidly dividing normal cells as well. Therefore, normal cells can also be damaged by chemoradiotherapy, which induces side effects that include vomiting, diarrhea, esophagitis, renal dysfunction, pneumonia, and oral mucositis (7).

Oral mucositis, which is related to mucosal inflammation associated with the presence of oral microorganisms, frequently causes serious problems for cancer patients during and after chemoradiotherapy. Oral mucositis is also considered to be a significant risk factor for systemic infection, because it is induced orally by various organisms, after which some of the pathogens become established and translocated into blood vessels. The relative risk of septicemia has been reported to be 4 times greater in neutropenic patients with oral mucositis than in those without the condition (8,9). Therefore, it is important to establish clinical procedures to prevent oral mucositis in order to maintain the health of esophageal patients treated with chemoradiotherapy, and several procedures for reducing the risk of its development as well as the establishment of pathogenic bacteria have been reported (10-13). However, no established therapy for oral mucositis is currently recognized, because the condition develops under the influence of complex factors, including mucosal interaction with oral microorganisms, while the systemic condition is related to immunoreactions and dietary balance (14-16).

Oral hygienic procedures are generally used to remove biofilm from tooth and mucosal surfaces for the purpose of creating a healthy environment in the oral cavity. As a special technique to regulate the development of oral mucositis, we developed an oral hygiene method that utilizes brushing combined with irrigation with povidone-iodine and suctioning. This technique was previously proposed as able to remove oral microorganisms, microorganism-infected epithelial cells, and other foreign particles after brushing for a short time. Oral streptococci are the principal commensal bacteria making up normal biofilms and play a role in resistance to colonization by invading pathogens in the oral cavity (17), while mutans streptococci are oral biofilm bacteria that function as pathogens in the development of dental caries (18-20). In spite of the increased interest in the effects of oral care on oral mucositis, little information is available on the essential effects of oral care on microorganisms in patients with oral mucositis. In the present study, we investigated the effects of a special oral care regimen on oral mucositis as well as the removal of oral streptococci, including mutans streptococci and opportunistic pathogens, in esophageal cancer patients undergoing chemoradiotherapy. Our results clearly show the potential role of this oral hygiene method for the regulation of oral mucositis and microorganisms.

PATIENTS AND METHODS

Subjects: Forty patients (35 males and 5 females, mean age 66.2 ± 7.9 and 58.0 ± 6.3 years, respectively; all patients, 65.2 ± 8.1 years) being treated at Shizuoka Cancer Center in

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Shizuoka, Japan, participated. Edentulous patients not using dentures were excluded. Prior to the study, the aims and details of the experiment were explained, and consent was obtained from all subjects before registration. The study was conducted according to the ethical guidelines of Shizuoka Cancer Center and Tokyo Medical and Dental School in accordance with the Helsinki declaration. Dental examinations were conducted under artificial white light by trained dentists. Missing teeth and filled teeth scores were recorded along with findings of dental caries according to the WHO criteria (21). All of the subjects were newly diagnosed with squamous cell cancer (SCC) of the esophagus and treated with chemoradiotherapy at Shizuoka Cancer Center between October 2003 and January 2005, and met the following inclusion criteria: (i) Eastern Cooperative Oncology Group performance status of 0 to 2, (ii) the oral cavity was not included in the target volume of chemoradiotherapy, (iii) there was no history of chemotherapy or radiation therapy for treatment of cancer, (iv) supragingival scaling and instruction regarding proper plaque control had not been received for at least 3 months, and (v) there were no ulcers or sores in the oral cavity. Subjects were randomly divided into 2 groups: a non-special care group (n = 20) and a special care group (n = 20).

Treatment schedule: All patients were treated with chemoradiotherapy. In the non-special oral care (non-special care) group, 5-FU + CDDP and 5-FU + nedaplatin were used as antineoplastic drugs for 14 and 6 patients, respectively, along with radiotherapy. In the special oral care (special care) group, 5-FU alone, 5-FU + CDDP, and 5-FU + nedaplatin were used for 1, 15, and 4 patients, respectively, along with radiotherapy. Other details of the chemoradiotherapy are given in Table 1.

Human saliva and plaque collection: Both whole saliva and plaque were collected at 2 - 3 h after breakfast. After patients had chewed paraffin gum for 5 min to stimulate saliva production, saliva samples were collected on cotton swabs, placed in transport fluid (0.4% agar, 0.15% thioglycolate/phosphate buffered saline) and sent to Bio Medical Laboratory (BML) (Tokyo, Japan) to determine the numbers of mutans streptococci, lactobacilli, total streptococci, and *Porphyromonas gingivalis*. Saliva samples were collected in plastic tubes and stored at −80°C. The plaque assessments were performed on 6 surfaces of every tooth (four-line angle, mid-buccal and mid-lingual) before sampling. The supragingival plaque samples were collected from the posteroanterior buccal surfaces of the upper right second premolar and first molar using a sterile cotton swab, after confirmation of the presence of more than 80% plaque in all experimental cases, for measurement of opportunistic pathogens. For edentulous patients who used a complete denture, samples were collected from the same regions of the upper right second premolar and first molar of the complete denture. Patients without any of the above-mentioned teeth provided samples from the opposite sides or other remaining teeth. Saliva and supragingival plaque samples from each patient were obtained at the Shizuoka Cancer Center at four different time points; at the first visit prior to oral cleaning (baseline), after oral cleaning and prior to chemoradiotherapy (before chemoradiotherapy), while chemoradiotherapy was being given (during chemoradiotherapy, around 12 days after starting chemoradiotherapy), and just prior to discharge (before discharge). Patients were instructed not to use mouthwash for 2 h prior to the sample collection.

Table 1. The characteristics of patients in two groups

Characteristic	non-care (n = 20)	oral care (n = 20)
Male	18	17
Female	2	3
Age, years		
Range	56 - 79	49 - 81
Mean age (SD)	68.3 (7.0)	62.6 (8.4)
Complication		
Hypertension	4	4
Diabetes	2	3
Smoking		
Regularly, occasionally	1	5
Previously	13	13
Never	6	2
Performance status		
0	14	17
1	4	2
2	2	1
UICC TNM stage		
T1	1	2
T2	0	0
T3	11	10
T4	7	8
not clear	1	0
Primary tumor site[1)]		
Cervical	3	4
Thorcic	18	19
Abdominal	1	1
not clear	1	0
Treatment		
5-FU + RT	0	1
5-FU + CDDP + RT	14	15
5-FU + nedaplatin + RT	6	4
Chemotherapy (mg)		
Mean of total dose of 5-FU	5,735.5	5,680
Mean of total dose of CDDP	118	114.5
Mean of total dose of nedaplatin	106.7	129.3
Radiation therapy (Gy)		
Mean of total dose	33.7	32.1
Number of teeth[2)]	21.1	17.8
Number of missing teeth	10.8	13.4
Number of filled teeth	6.15	4.9
Denture		
Yes	8	7
No	12	13

[1)]: including double cancer.
[2)]: including third-year molar tooth.
RT, radiotherapy.

Oral care: At the first visit, all patients received regular dental and medical health checks, and were initially treated according to the protocol for oral cleaning, which included supragingival scaling of all teeth with an ultrasonic scaler (Puchipiezo; Yoshida Dental Trade Distribution Co., Tokyo, Japan), and received guidance regarding oral hygiene, including dental brushing by a dentist. In the non-special care group, the patients performed dental brushing with a dental brush (Microdent; Hakusui Trading Co., Osaka, Japan) after meals by themselves. In the special care group, in addition to the oral hygiene protocol for the non-special group, the patients also underwent oral care with a dental brush by a dentist in combination with irrigation and suctioning (Fig. 1A: e-brush®, Santo Medical Instruments Mfg. Co., Ltd., Tokyo, Japan) for 15 min 3 days per week for 2 to 4 weeks between 19:00 and 20:00, after dinner. At that time, 20 ml of 0.5% povidone-

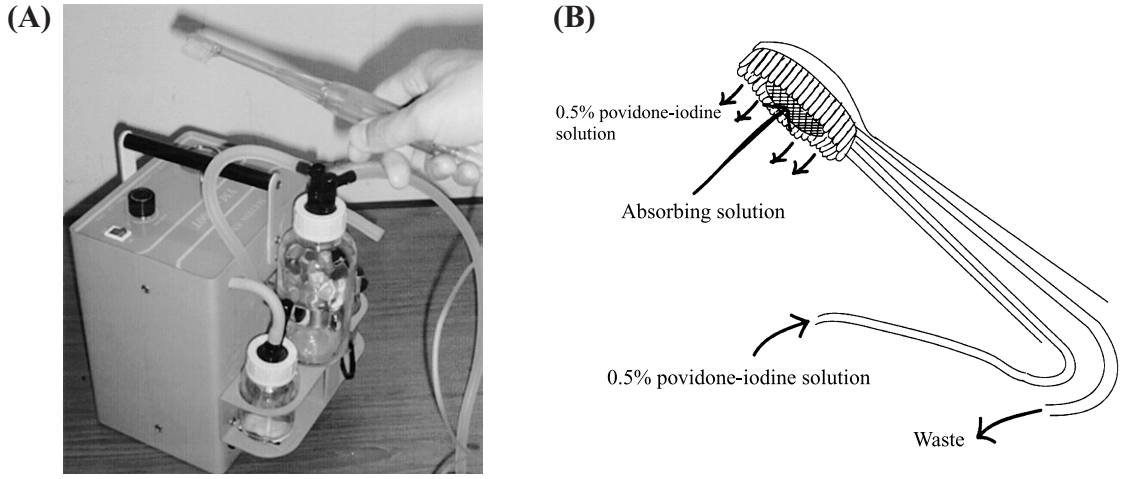

Fig. 1. Dental brush (e-brush®) with combined irrigation and suctioning functions.
(A) Flow of 0.5% povidone-iodine. (B) Removal of waste products.

iodine solution (Isodine-Gargle; Meiji Seika, Tokyo, Japan) was placed into the connecting tube of the e-brush® and ejected from the tips of the brush hairs for circulation in the mouth, after which the waste materials were suctioned into the center hole of the e-brush® head (Fig. 1B). Brushing using an inter-dental brush (DENT.EX inter-dental brush/SS size; Lion, Tokyo, Japan) and treatment with povidone-iodine was also performed on the sides of the teeth. In addition, the e-brush® was used to clean the tongue and mucosal surfaces. Finally, patients rinsed with a 0.5% povidone-iodine solution mouthwash to clean the oral cavity.

Bacterial counting: Bacterial counts of total streptococci, mutans streptococci, lactobacillus and opportunistic pathogens were performed using the procedures published previously by the BML (22-26).

P. gingivalis was detected quantitatively in the saliva samples using Invader® technology (27,28). A primary probe (wild-type signal probe) and Invader® oligonucleotides (Invader® probe) were used to detect *P. gingivalis* with an Invader® assay.

Oral mucositis: Oral mucositis was diagnosed by a dentist with experience in oral surgery and gerodontology during hospitalization on Monday, Wednesday, and Friday of each week, using criteria defined by the Japan Clinical Oncology Group. These criteria are based on the following National Cancer Institute Common Toxicity Criteria, version 3: grade 0, none; grade 1, erythema of the mucosa; grade 2, patchy ulceration or pseudomembrane; grade 3, confluent ulcerations or pseudomembranes, and bleeding with minor trauma; grade 4, tissue necrosis, significant spontaneous bleeding, and life-threatening consequences (29). All of the subjects with an onset of clinically manifested mucositis were scored as having mucositis of grade 2 or higher.

Grouping strategies: Non-special care patients were divided into 2 groups: an opportunistic pathogens-positive (n = 8) and an opportunistic pathogens-negative group (n = 12) based on the detection of opportunistic pathogens after the start of chemoradiotherapy. The detected opportunistic pathogens were *Pseudomonas aeruginosa*, methicillin-sensitive *Staphylococcus aureus* (MSSA), *Klebsiella pneumoniae*, β-streptococci, *Candida* sp. and *Serratia* sp.

Mutans streptococci are principal microorganisms associated with the development of oral biofilm and known to incorporate other bacteria in the biofilm. The non-special and special care groups were divided into 2 subgroups based on the number of mutans streptococci in saliva: a higher-level mutans streptococci group (>5 × 10^3 CFU/ml) and lower-level mutans streptococci group (<5 × 10^3 CFU/ml) at baseline. The concentration of mutans streptococci >5 × 10^3 CFU/ml was significant level in the experimental identification by BML (24).

Statistical analysis: All data were analyzed using the Statistical Package for Social Science (SPSS) software package, version 11.0. The incidence of oral mucositis in the 2 groups was calculated using Fisher's exact test, and comparisons of the CFU of total streptococci in each group among the different sampling periods were made using a paired t test, while comparisons among groups the CFU of total streptococci at each period were performed using Mann-Whitney's U-test. A P value of less than 0.05 was considered significant.

RESULTS

The results for dental and systemic status, along with the medical treatments for cancer and other parameters in the present patients are listed in Table 1. Oral mucositis was diagnosed for the period of chemoradiotherapy, and the grades are shown for the non-special (n = 11) and special (n = 4) care patients. There were no significant differences between the 2 groups in any of the parameters, except for the incidence of oral mucositis, which was significantly lower in the special care group (4/20, 20%) than in the non-special care group (11/20, 55%) (P = 0.048).

To analyze the hygienic effects of the present oral treatment method, we determined the quantities of total streptococci and mutans streptococci in saliva between the 2 groups at 4 time points: baseline, before chemoradiotherapy, during chemoradiotherapy, and before discharge. Further, the relationships between the numbers of streptococci and opportunistic pathogens as well as mutans streptococci in oral microflora were examined. The levels of total streptococci and mutans streptococci in 1 ml of saliva were not significantly different among the time periods in either group (Fig. 2A, B). Further, total bacteria, as well as the numbers of lactobacillus and *P. gingivalis*, did not vary among the sampling points in either group (data not shown). After starting chemoradiotherapy, the proportion of subjects with newly detected opportunistic pathogens was higher in the non-special care group (8/19, 42.2%) than in the special care group

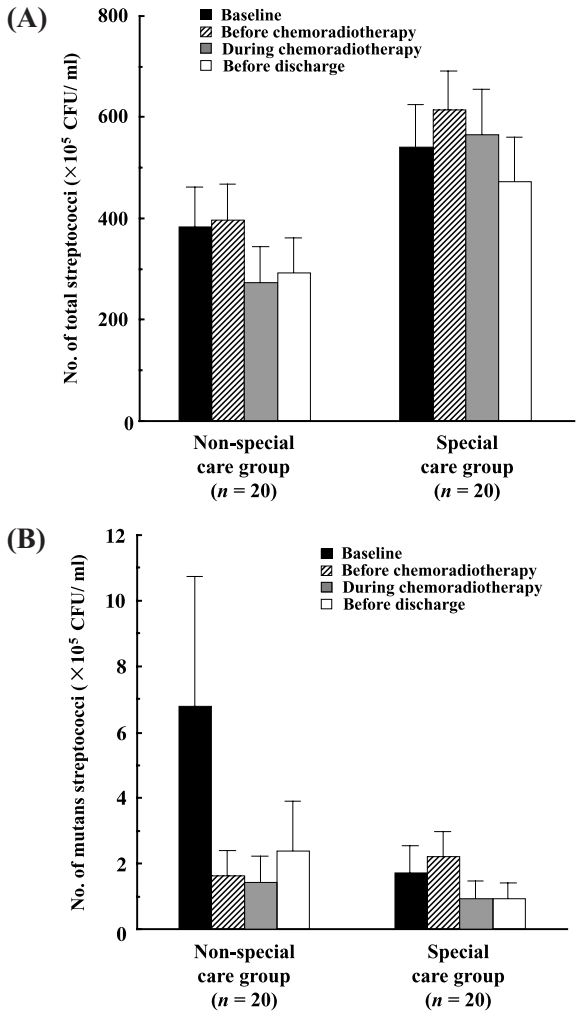

Fig. 2. Total numbers of streptococci organisms at the sampling time points in the non-special care and special care groups. Shown are the mean total CFU/ml of total streptococci (A) and mutants streptococci (B) at the baseline, before chemoradiotherapy, during chemoradiotherapy, and before discharge examinations for the special care and non-special care groups. The results are expressed as the mean ± SE for 20 human subjects in each group.

(2/15, 13.3%), except for subjects with opportunistic pathogens detected at baseline, though there was not a significant difference in the ratio of opportunistic pathogens between the groups. To clarify the relationship between opportunistic pathogens infection and total streptococci number, subjects in the non-special care group were subdivided into an opportunistic pathogens-positive and opportunistic pathogens-negative group. Patients with opportunistic pathogens infection showed a significant decrease in the total number of streptococci at the during chemoradiotherapy time point ($P = 0.019$) in comparison with the before chemoradiotherapy time point (Fig. 3).

In the non-special care patients classified as having a lower level of mutans streptococci, the total number of streptococci organisms was significantly lower during chemoradiotherapy than at baseline ($P = 0.034$), but this was not the case in the special-care patients (Fig. 4). In contrast, the total number of streptococci organisms did not vary among the sampling points in those classified as having a higher level of mutans streptococci, while the special oral care group did not show significant changes in the total number of streptococci among the sampling points irrespective of mutans streptococci classification (data not shown). Further, there were no significant differences in microbiological data between patients treated with 5-FU + CDDP + RT (radiotherapy) and patients treated with nedaplatin + RT, or between patients with and without dentures in either group (data not shown).

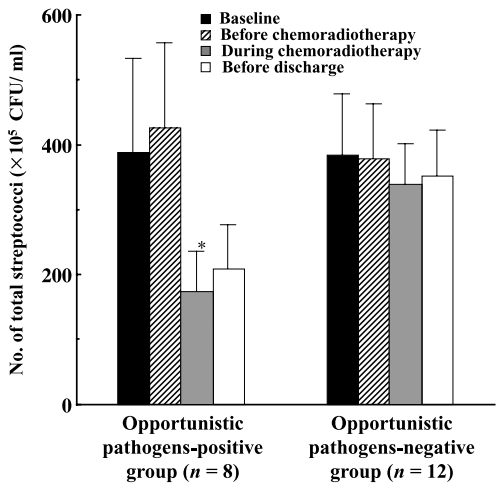

Fig. 3. Total numbers of streptococci organisms at the sampling time points in the opportunistic pathogens-positive and -negative patients in the non-special care group. Shown are the mean total CFU/ml of total streptococci at the baseline, before chemoradiotherapy, during chemoradiotherapy, and before discharge examinations in the opportunistic pathogens-positive ($n = 8$) and -negative ($n = 12$) patients in the non-special care group. The results are expressed as the mean ± SE. Asterisks denote significantly different relative antibody levels (before chemoradiotherapy versus during chemoradiotherapy; *: $P < 0.05$).

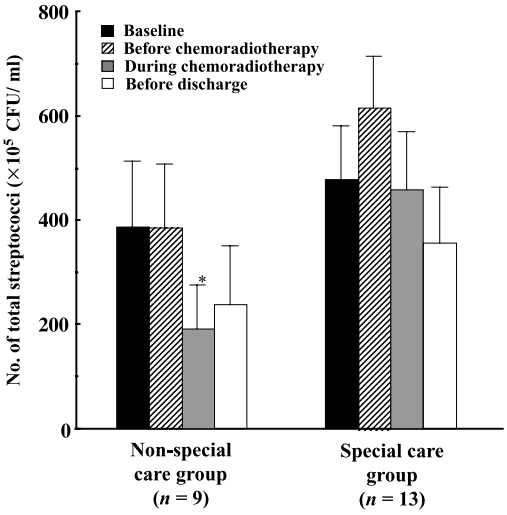

Fig. 4. Total numbers of streptococci organisms at the sampling time points in patients classified as lower-level mutans streptococci in non-special care and special care groups. The mean total CFU/ml of total streptococci at the baseline, before chemoradiotherapy, during chemoradiotherapy, and before discharge examinations in patients classified as lower-level mutans streptococci in the non-special care ($n = 9$) and special care ($n = 13$) groups. The results are expressed as the mean ± SE. Asterisks denote significantly different relative antibody levels (baseline versus during chemoradiotherapy; *: $P < 0.05$).

DISCUSSION

We investigated the effects of a special oral-care regimen that included use of an e-brush® with povidone-iodine irrigation for the treatment of oral mucositis and oral micro-

organisms in patients with esophageal cancer undergoing chemoradiotherapy. Patients in the non-special care group with opportunistic pathogens in biofilm samples taken from tooth surfaces showed a significant decrease in the total number of streptococci in saliva during chemoradiotherapy. However, there were no significant differences in the total number of streptococci at any of the sampling periods in the special care group. We considered that an alteration of the oral environment, such as a decrease in the numbers of oral streptococci, would lead to the appearance of opportunistic pathogens and oral mucositis. The present special oral care regimen had significant effects for inhibiting oral mucositis and inducing stable microflora composed of oral streptococci.

Administration of antineoplastic drugs down-regulates both systemic and oral mucosal immunity, and leads to changes in the oral environment, including a decrease in saliva volume and modification of saliva constituents. Salivary proteins and glycoproteins are present in multiple active formations within the human oral cavity, a number of which are selectively adsorbed on tooth enamel. Proline-rich proteins and statherin change their conformation upon binding to enamel (30), thus unmasking the receptors involved in the adhesion of streptococci and *Actinomyces* spp. (31-33). This ability of oral microorganisms to bind immobilized salivary proteins is of considerable ecological significance. In addition to the above influences, acute changes by radiotherapy are observed in the oral mucosa (erythema pseudomembrane-covered ulceration), salivary glands (hyposalivation, changed salivary composition), and taste buds (decreased acuity) (34). Therefore, chemoradiotherapy may induce an alteration of the oral environment, resulting in a loss of balance between commensal bacterium flora and opportunistic pathogens. It was previously reported that oral streptococci have an antagonistic effect toward many bacterial species (35-37). Further, they are producers of lactic acid and other organic acids, which have been shown to have antimicrobial activities (38), and also inhibit methicillin-resistant *S. aureus* (MRSA) colonization in the oral cavities of newborns (39) and MRSA growth in vitro (40). In a recent epidemiological report, the detection of oral commensal bacteria such as α-streptococci had an inverse correlation with the detection of opportunistic pathogens such as MRSA in inpatients who were not treated with a special oral regimen (41). Therefore, a decrease in the total number of streptococci organisms provides an opportunity for infection of the oral cavity by opportunistic pathgens. We consider that the incidence of oral mucositis caused by chemoradiotherapy may be associated with a loss of balance in the oral commensal bacterium flora and the appearance of opportunistic pathogens.

In the present study, we utilized a specially designed toothbrush that provided irrigation with povidone-iodine and suctioning capabilities. We expected that the brush would remove and suction-out various bacteria on tooth and mucosal surfaces in the oral cavity, and also kill or inhibit the growth of oral microorganisms through the irrigation with povidone-iodine, which has shown bactericidal activities toward opportunistic pathogens such as *Enterobacteriaceae* and *Pseudomonaceae*, as well as oral streptococci in vitro (42). However, such activities have not been observed for bacteria such as mutans streptococci, because they are resistant to various antimicrobial agents including povidone-iodine (43,44). In the present study, patients in the special care group had similar total levels of streptococci organisms at baseline, before chemoradiotherapy, and after chemoradiotherapy time points. In a previous report on a more rigorous treatment using 0.2% chlorhexidine and the physical removal of biofilm in the oral cavity, the number of total streptococci recovered shortly after the last treatment (45). Therefore, we considered that brushing with the e-brush® cleaned the tooth and mucosal surfaces, and then allowed re-establishment and growth by fresh commensal bacteria such as total streptococci, which disturbed the infection by opportunistic pathogens, in the oral cavity of patients undergoing chemoradiotherapy.

In both groups, the total number of streptococci tended to be controlled when mutans streptococci organisms were also present, indicating that mutans streptococci may play an important role in ensuring that the flora are composed primarily of streptococcal bacteria. Generally, mutans streptococci are thought to play a central role in the development of biofilm and dental caries, as those organisms produce insoluble glucans, which incorporate other bacteria, and have been shown to form mature biofilm in a quorum-sensing system (46,47). Therefore, mutans streptococci may be one of the contributors to the stability of the streptococcal bacteria population, which helps to inhibit the infection of the oral cavity by opportunistic pathogens. The total number of streptococci organisms remained stable in the presence of a high level of mutans streptococci, but not when the numbers of mutans streptococci were decreased. However, the unstable population was restored to the stablility by the oral-care regimen in the lower-level mutans streptococci group.

In conclusion, the high level of physical and chemical cleaning provided by the present technique enabled the total population of streptococci microflora to remain stable, and inhibited infection of the oral cavity by opportunistic pathogens, thereby inhibiting oral mucositis during and after chemoradiotherapy. Our results provide important information regarding the prevention of side effects for cancer patients, as well as improvement in their quality of life and lowering the length of hospitalization and medical costs by improving oral hygiene.

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