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Original Article

Comparison of the inhibitory effects of tooth brushing and povidone-iodine mouthwash on salivary bacterial counts in patients undergoing surgery for malignant solid tumors other than head and neck cancers

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Abstract *Background/purpose:* Infectious complications after invasive surgeries may originate from oral sources through either hematogenous infection or direct exposure to salivary bacteria. Perioperative oral management aims to remove oral foci of infection. However, the type of oral care that can reduce the salivary bacterial load remains unclear. This study aimed to identify factors influencing salivary bacterial counts during the perioperative period in patients with malignant tumors and to evaluate the effectiveness of tooth brushing and povidone-iodine mouthwash in reducing oral bacterial counts.

Materials and methods: Patients aged ≥18 years who underwent surgery under general anesthesia for malignant solid tumors other than head and neck cancer were included. Participants were randomly assigned to the brushing or povidone-iodine mouthwash groups. Factors such as sex, age, primary disease, preoperative blood-test results, oral functional metrics, and salivary bacterial counts were analyzed. Saliva samples were collected before and on the day after surgery, both before and after oral-care interventions. The total bacterial and streptococcal counts were determined using real-time polymerase chain reaction (PCR), and delayed real-time PCR was used to determine the viable bacterial count.

Results: Postoperatively, the salivary bacterial counts increased slightly. Significant factors affecting postoperative bacterial counts included high preoperative counts and postoperative

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fasting status. Brushing increased salivary bacterial counts, whereas the povidone-iodine mouthwash decreased them.
Conclusion: Poor preoperative oral hygiene and postoperative fasting were significantly associated with increased salivary bacterial counts. The povidone-iodine mouthwash reduced postoperative bacterial counts, indicating its effectiveness as a perioperative oral-care method.

Introduction

Infectious complications after invasive surgeries, such as malignant tumor surgery, cardiac surgery, and organ transplantation, may be caused by oral sources via two possible mechanisms: hematogenous infection from an infected lesion in the oral cavity to a remote site or direct exposure to salivary bacteria. An example of the former is surgical site infection (SSI) after heart valve or joint replacement surgery. The latter includes SSI after head and neck cancer surgery, upper gastrointestinal cancer surgery, and aspiration pneumonia after surgery for esophageal cancer, lung cancer, and other types of surgery that decrease swallowing function and sputum output.

The main goals of perioperative oral management are to remove oral foci of infection, such as periapical lesions and severe periodontal disease, and to reduce the salivary bacterial load. However, the type of oral care that can reduce the salivary bacterial load remains unclear. Several studies have reported that dental plaque and periodontal disease are associated with aspiration pneumonia and that plaque removal and periodontal treatment are important for prevention of aspiration pneumonia.[1–4] Therefore, oral care, mainly brushing, is widely practiced for perioperative patients and older adults requiring nursing care. However, brushing may increase the salivary bacterial load by dispersing bacteria from the plaque that was firmly adhered to the tooth surfaces into the oral cavity.[5]

Clarifying the changes in the salivary flora during the perioperative period and the factors that affect them are necessary for establishing effective oral care methods to decrease the salivary bacterial load and to prevent infectious complications in perioperative patients. This study aimed to determine the factors that affect the salivary bacterial load during the perioperative period in patients with malignant tumors and to determine whether tooth brushing and mouthwashes are effective in reducing the same.

Materials and methods

Patients

Patients aged ≥18 years who underwent surgery under general anesthesia for malignant solid tumors other than head and neck or hematological cancers between August 3, 2023 and December 31, 2023 at Nagasaki University Hospital were enrolled. Patients who were intubated after surgery, those who could not gargle, and those with iodine allergy or abnormal thyroid function were excluded.

Allocation and intervention

Participants were randomly assigned in a 1:1 ratio to the brushing and povidone-iodine mouthwash groups. The allocation factor was the cancer site (esophageal or lung cancer, which is more likely to cause dysphagia than other cancers). In the povidone-iodine mouthwash group, participants rinsed for 30 s using Isodine® Gargle solution 7 %, which was adjusted to the concentration described in the package insert. In the brushing group, dentists removed dental plaque using toothbrushes and interdental brushes and wiped the teeth using a sponge brush.

Variables

The following information was collected: sex, age, smoking history, body mass index, disease name, preoperative blood test data (leukocyte count and serum C-reactive protein, albumin, and creatinine levels), level of independence in daily living of disabled older adults, surgical procedure, surgical time, surgical site, pre- and postoperative eating status, number of remaining teeth, denture use, periodontal pocket depth, O' Leary's Plaque Control Record,[6] Tongue Coating Index,[7] occlusal force, masticatory efficiency, and tongue pressure. The level of independence in daily living was classified according to the criteria of the Ministry of Health, Labor and Welfare[8] as follows: Grade J: Independent in daily living and going out on their own; Grade A: Independent in indoor living but does not go out without assistance; Grade B: Needs some assistance in indoor living and stays in bed mainly during the day but keeps sitting position; Grade C: Spends all day in bed and needs assistance in excretion, eating, and changing clothes. Grades J and A were defined as mostly independent, and Grades B and C as requiring nursing care.[9] Occlusal force was measured using the Dental Prescale II (GC Co, Ltd., Tokyo, Japan),[10] and <500 N was considered decreased. Masticatory efficiency was measured using the Glucosensor GS-II (GC Co., Ltd.),[11] and <100 mg/dl was defined as decreased. Tongue pressure was measured using the JMS tongue pressure measuring device (JMS Co. Ltd., Hiroshima, Japan), and <25 kPa was considered decreased.[12] For denture wearers, oral function tests were performed with dentures.

Measurement of salivary bacteria counts

A Corning tube was used to collect 1 mL of saliva directly from the patient. Preoperative saliva samples were collected on the day before surgery before and after the oral-care intervention. The total bacteria and streptococci in the saliva were measured using real-time polymerase chain reaction (PCR). The DNA extraction method, primers, artificial DNA, and reaction conditions used were as previously reported by Tsuda et al.[13] Postoperative salivary bacterial loads were determined using delayed real-time PCR, a novel method which we developed to measure only viable bacteria,[14] because real-time PCR amplifies and counts not only viable bacteria but also the DNA of bacteria inactivated by povidone-iodine.

Statistical analysis

All statistical analyses were performed using SPSS ver. 26.0 (Japan IBM Co., Ltd., Tokyo, Japan). The relationship between each variable and the number of salivary bacteria was analyzed. One-way analysis of variance was used to analyze categorical variables, and Spearman's rank correlation coefficient was used to analyze continuous variables. Multivariate analysis was performed using multiple regression analysis, including significant factors in the univariate analysis and several other factors of interest. Statistical significance was defined as a two-tailed P-value <0.05.

Ethics

This study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Biological Research Involving Human Subjects of the Ministry of Health, Labour and Welfare of Japan. This study was approved by the Institutional Review Board of the University (CRB23-002-1, August 1, 2023). Written informed consent was obtained from all participants. The study protocol was registered in the Japan Registry of Clinical Trials (jRCT) on August 1, 2023 (jRCT1071230048).

Results

Patient characteristics

Patient characteristics are shown in Table 1. This study included 28 men and 32 women, with a mean age of 68.9 years. Primary diseases were gastrointestinal cancer (n = 19), lung cancer (n = 11), prostate cancer (n = 10), breast cancer (n = 6), and others (n = 14). The average

Table 1 Patient characteristics.

Variable		Number of patients/mean ± SD		*P*-value
		Brushing group	PV-I group	
Sex	Male	12	16	0.438
	Female	18	14	
Age	Years	69.6 ± 11.9	68.1 ± 11.9	0.612
Body mass index		23.7 ± 3.77	23.4 ± 2.72	0.745
Smoking	(−)	19	15	0.435
	(+)	11	15	
Leukocytes	$\times 10^3/\mu$	5.60 ± 1.73	5.80 ± 1.46	0.701
Albumin	g/dL	3.81 ± 0.482	3.97 ± 0.564	0.339
Creatinine	mg/dL	1.06 ± 0.764	0.808 ± 0.377	0.119
Bedridden level	Almost independent (J, A)	24	27	0.472
	Requires care (B, C)	6	3	
Fasting before surgery	(−)	23	27	0.299
	(+)	7	3	
Number of teeth include denture	≥20	25	28	0.424
	<20	5	2	
Plaque control record	≤20 %	9	14	0.288
	>20 %	21	16	
Periodontal pocket ≥4 mm	(−)	21	20	1.000
	(+)	6	6	
Tongue coating index	<50 %	23	26	0.506
	≥50 %	7	4	
Occlusal force	≥500 N	12	12	1.000
	<500 N	18	18	
Masticatory efficiency	≥100 mg/dl	20	23	0.567
	<100 mg	10	7	
Tongue pressure	≥25 kPa	23	24	0.748
	<25 kPa	7	5	
Total		30	30	

Abbreviation: SD: Standard deviation.

number of remaining teeth was 22.6, and 12 patients had <20 teeth, including dentures. Regarding oral function decline, 36, 17, and 12 patients exhibited decreased occlusal force, masticatory efficiency, and tongue pressure, respectively. Background factors were not significantly different between the brushing and povidone-iodine mouthwash groups.

Factors related to the preoperative salivary bacterial count

The mean logarithm of the salivary bacterial count on the day before surgery was 6.49. Univariate analysis revealed that the salivary bacterial count was higher in patients with fewer teeth; however, the difference was not statistically significant ($P = 0.053$). The salivary bacterial count was slightly higher in patients with impaired oral functions such as occlusal force, masticatory efficiency, and tongue pressure; however, the difference between the two groups was not statistically significant (Table 2). In this study, no factors were significantly associated with the preoperative salivary bacterial count.

Factors related to the postoperative salivary bacterial count

The salivary bacterial count on the day after surgery was 6.55 ± 1.35 in all patients, which was slightly higher than the preoperative count. Univariate analysis showed that preoperative plaque control record >20 % ($P = 0.014$) and fasting status after surgery ($P = 0.005$) were significantly associated with the postoperative bacterial count (Table 3). Furthermore, these two factors were remained significant in the multivariate analysis (Table 4). If the preoperative bacterial count was 100 %, it was reduced to 90.2 % ($P = 0.028$) in patients who were on oral feeding the day after surgery. In contrast, the bacteria count increased to 107.7 % ($P = 0.018$) in patients who were not on an oral diet the day after surgery (Fig. 1). All patients received intravenous antimicrobials on the day after surgery.

Effects of brushing and povidone-iodine mouthwash on salivary bacteria

Salivary bacterial counts before and after the oral-care intervention on the day after surgery were compared. Brushing increased the number of salivary bacteria by 232 %, whereas the povidone-iodine mouthwash significantly decreased the number of bacteria by 25.2 % (Fig. 2).

Discussion

Complications such as SSI and aspiration pneumonia can occur after cancer surgery, and salivary bacteria are a

Table 2 Factors related to the number of bacteria in saliva before surgery (Univariate analysis).

Variable		mean ± SD	*P*-value
i) Categorical variable			
Sex	Male	6.43 ± 0.781	0.502
	Female	6.54 ± 0.482	
Smoking	(−)	6.46 ± 0.540	0.637
	(+)	6.54 ± 0.752	
Bedridden level	Almost independent (J, A)	6.52 ± 0.616	0.468
	Requires care (B, C)	6.35 ± 0.768	
Fasting before surgery	(−)	6.51 ± 0.677	0.707
	(+)	6.42 ± 0.392	
Number of teeth	≥20	6.43 ± 0.600	0.053
	<20	6.93 ± 0.783	
Plaque control record	≤20	6.42 ± 0.610	0.472
	>20	6.54 ± 0.656	
Periodontal pocket ≥ 4 mm	(−)	6.51 ± 0.708	0.739
	(+)	6.44 ± 0.341	
Tongue coating index	<50 %	6.48 ± 0.639	0.712
	≥50 %	6.56 ± 0.649	
Occlusal force	≥500 N	6.43 ± 0.600	0.524
	<500 N	6.54 ± 0.664	
Masticatory efficiency	≥100 mg/dl	6.44 ± 0.612	0.289
	<100 mg	6.63 ± 0.693	
Tongue pressure	≥25 kPa	6.46 ± 0.597	0.443
	<25 kPa	6.62 ± 0.808	
ii) Continuous variable	**Pearson's correlation coefficient**		***P*-value**
Age	−0.067		0.611
Body mass index	−0.124		0.343
Leukocytes ($\times 10^3/\mu$)	0.044		0.740
Albumin	−0.011		0.946
Creatinine	0.188		0.157

Table 3 Factors related to the number of bacteria in saliva after surgery (Univariate analysis).

Variable		mean ± SD	*P*-value
i) Categorical variable			
Sex	Male	6.73 ± 1.31	0.347
	Female	6.39 ± 1.38	
Smoking	(−)	6.40 ± 1.50	0.344
	(+)	6.74 ± 1.13	
Bedridden level	Almost independent (J, A)	6.58 ± 1.43	0.724
	Requires care (B, C)	6.4 ± 0.800	
Fasting before surgery	(−)	6.60 ± 1.36	0.545
	(+)	6.31 ± 1.32	
Number of teeth	≥20	6.50 ± 1.36	0.415
	<20	6.94 ± 1.27	
Plaque control record	≤20	6.01 ± 1.26	0.014
	>20	6.88 ± 1.31	
Periodontal pocket ≥4 mm	(−)	6.69 ± 1.50	0.217
	(+)	6.13 ± 0.715	
Tongue coating index	<50 %	6.53 ± 1.32	0.794
	≥50 %	6.65 ± 1.54	
Occlusal force	≥500 N	6.60 ± 1.35	0.796
	<500 N	6.51 ± 1.37	
Masticatory efficiency	≥100 mg/dl	6.46 ± 1.41	0.427
	<100 mg	6.77 ± 1.21	
Tongue pressure	≥25 kPa	6.56 ± 1.35	0.765
	<25 kPa	6.69 ± 1.29	
Fasting after surgery	(−)	5.91 ± 1.24	0.005
	(+)	6.92 ± 1.29	

ii) Continuous variable	**Pearson's correlation coefficient**	***P*-value**
Age	−0.031	0.817
Body mass index	0.154	0.239
Leukocytes ($\times 10^3/\mu$)	−0.076	0.564
Albumin	−0.181	0.251
Creatinine	−0.065	0.630
Operation time (Minutes)	0.047	0.723

known cause. In this study, salivary bacterial counts were significantly increased in patients with poor preoperative oral hygiene and in those who fasted after surgery.

Previous studies have suggested that plaque and periodontal pockets are reservoirs of bacteria that cause aspiration pneumonia. However, the frequency of SSI after cancer surgery in edentulous patients is comparable to that in dentulous patients.[15] Moreover, the incidence of aspiration pneumonia in older adults in need of care is comparable between edentulous and dentulous patients,[16] indicating that dental plaque and periodontal disease are not the primary causes of aspiration pneumonia. Furthermore, oral care is as effective in edentulous patients as in dentulous patients,[16] and preoperative oral care is effective in preventing SSI and pneumonia regardless of the presence of teeth.

In this study, an increase in the salivary bacterial load was observed in patients who fasted postoperatively. Sakamoto et al. also reported an increase in the salivary bacteria load in patients fasting after surgery.[17,18] Oral feeding stimulates saliva secretion owing to tongue movements, which decreases salivary bacteria as the food mass passes through the oral cavity. Because salivary bacteria are thought to be associated with aspiration pneumonia, oral feeding may decrease the risk of pneumonia. Guidelines for accelerating postsurgical recovery recommend

Table 4 Factors related to the number of bacteria in saliva after surgery (Multivariate analysis).

Variable		Unstandardized coef.		Std. coef.	95 % CI	*P*-value
		B	SE	β		
Plaque control record	>20/≤20	0.829	0.322	0.301	0.185−1.474	0.013
Fasting after surgery	(+)/(−)	0.968	0.325	0.349	0.318−1.619	0.004

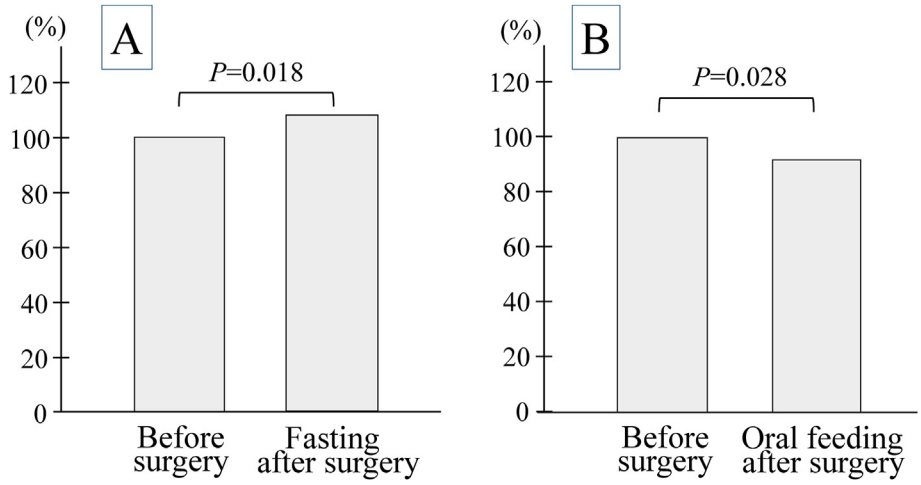

Figure 1 The number of bacteria in saliva before vs. after surgery. Bacterial count was reduced in patients who were on oral feeding the day after surgery, and it increased in patients who were not on an oral diet the day after surgery.

oral feeding in the perioperative period as a measure to shorten the hospital stay and reduce complications.[19] However, patients without postoperative oral feeding may require adjunctive measures such as frequent rinsing using a mouthwash.

Oral function may affect physical function because masticatory dysfunction and dysphagia are directly related to malnutrition and ultimately lead to the deterioration of general health. In addition, deteriorated oral function is associated with systemic sarcopenia.[20] The tongue plays an important role in feeding and swallowing by forming a bolus in the oral cavity and sending it to the pharynx. Saliva, which is stimulated by mastication and tongue movement, has a self-cleaning effect, and a decrease in tongue pressure may reduce the self-cleaning effect on the oral cavity, leading to an increase in salivary bacteria. Previous studies have reported that decreased tongue pressure is significantly associated with increased salivary bacteria.[18,21] However, these studies included older adults requiring nursing care, and several participants had significantly decreased tongue pressure. In contrast, this study included patients undergoing surgery under general anesthesia, and few participants had extremely decreased tongue pressure. In this study, no significant correlation between deteriorated oral function and salivary bacterial counts was observed. This could be attributed to the small number of patients with reduced tongue function in this study.

In Japan, pre- and postoperative oral care has been covered by the public medical insurance system since 2012; perioperative oral management is widely practiced, and effective oral care methods are being sought to prevent postoperative complications.[22–25] The results of this study showed that tooth brushing without a mouthwash increased the number of bacteria in the saliva, which is thought to be due to the dispersion of plaque bacterial from the tooth surfaces into the oral cavity. Previous studies have shown that mechanical brushing is not effective in preventing ventilator-associated pneumonia.[26–28] Hayashida et al. reported that bacterial counts in the oropharyngeal fluids did not decrease after tooth brushing but significantly

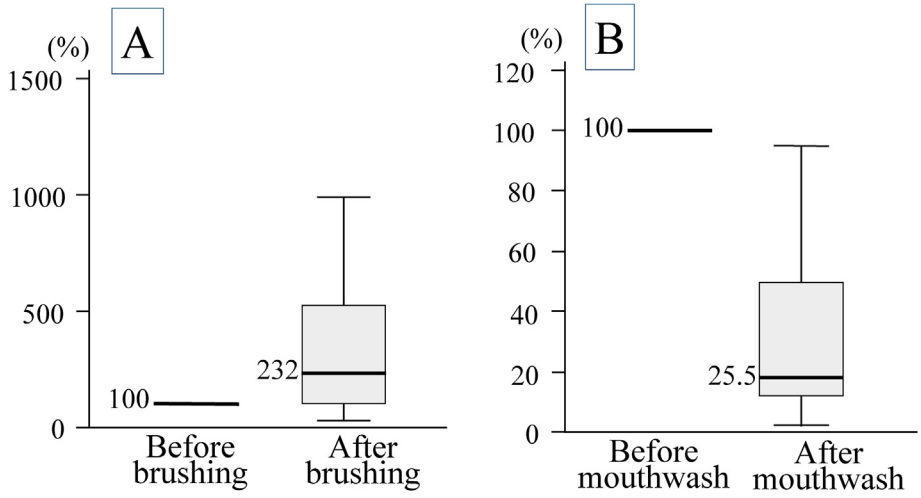

Figure 2 The number of bacteria in saliva before vs. after intervention. Brushing increased the number of salivary bacteria, whereas the povidone-iodine mouthwash significantly decreased the number of bacteria.

decreased after gargling with water.[29] Chlorhexidine 0.12 %, which is used overseas for ventilator-associated pneumonia prophylaxis, has been banned in Japan owing to reports of anaphylactic shock. Povidone-iodine has antibacterial activity similar to that of chlorhexidine and is approved for oral use in Japan. Tsuda et al. reported that povidone-iodine does not promote the growth of antimicrobial-resistant bacteria, such as methicillin-resistant staphylococcus aureus or fungi, and can reduce oral bacterial load without disturbing the balance of the oral flora.[13] In this study, postoperative salivary bacterial counts were significantly reduced with povidone-iodine mouthwash, indicating the effectiveness of povidone-iodine mouthwash.

Patients undergoing surgery under general anesthesia may experience difficulty in gargling after surgery. Therefore, careful oral care is necessary for these patients. When gargling is not possible, brushing can be combined with alternative methods. However, Funahara et al. reported that salivary bacterial counts increased after brushing, even when oral gels were used to prevent the spread of dental plaque after brushing or when sponge brushes were used to cleanse the mouth.[5] Based on these findings, in patients unable to gargle postoperatively, oral cleansing with povidone-iodine is preferred to toothbrushing for pneumonia prevention.

This study had several limitations. The generalizability of the results is unclear because of the small number of patients with relatively similar ages and backgrounds. Furthermore, owing to the small number of patients with deteriorated oral function, we were unable to show an association between oral function and the salivary bacterial load. However, this study clearly demonstrated that a povidone-iodine mouthwash is more effective than brushing for reducing the salivary bacterial load. In the future, we intend to increase the number of cases and conduct similar studies on patients with various conditions.

In summary, poor preoperative oral hygiene and postoperative fasting after surgery were significantly associated with increased salivary bacterial counts after surgery. Brushing in the absence of a mouthwash increased the salivary bacterial load, whereas the povidone-iodine mouthwash decreased it, indicating that povidone-iodine mouthwash is an effective postoperative oral-care method.

Declaration of competing interest

The authors declare no conflict of interest. This research received no external funding.

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