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Original Article

Microbiota profiling of bronchial fluids of elderly patients with pulmonary carcinoma

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ABSTRACT

Objectives: The source of the bacteria involved in silent aspiration remains to be completely defined. This study aimed to obtain reliable evidence on silent aspiration of oral bacteria in elderly patients.

Methods: After obtaining informed consent, the cough and swallowing reflexes of patients were assessed. Bronchial fluids from patients undergoing lung resections were collected with a micro-sampling probe, and α -amylase activity of bronchial fluids was measured to estimate the degree of silent aspiration. The bronchial fluids were cultured aerobically and anaerobically on blood agar plates, and colonies were identified by 16S rRNA gene sequencing. Additionally, whole saliva bacterial amounts and composition were analyzed.

Results: Six patients (72.2 ± 5.8 years) exhibited an impaired swallowing reflex and 5 (75.4 ± 7.9 years) had a normal swallowing reflex, while all patients had a normal cough reflex. α -Amylase activity was detected in bronchial fluids of both the impaired and normal reflex groups. The amount of anaerobic bacteria in bronchial fluids in the impaired reflex group [$(3.0 \pm 3.5) \times 10^4$] was higher than in the normal reflex group [$(2.5 \pm 5.3) \times 10^4$], although the difference was not significant. *Actinomyces*, *Gemella*, *Streptococcus*, *Rothia*, *Mogibacterium*, and *Campylobacter* were the predominant bacterial species in bronchial fluids of the impaired reflex group, while *Streptococcus*, *Lactobacillus*, *Veillonella*, and *Actinomyces* were predominant in the normal reflex group.

Conclusions: Our results suggest that bacteria in bronchial fluids associated with silent aspiration are derived from saliva, and that the bronchial fluids of elderly patients with an impaired swallowing reflex may have a characteristic microbiota.

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1. Introduction

To date, pneumonia has a high mortality rate in Japanese adults; 90% of pneumonia-associated deaths are observed in elderly people (≥ 65 years). Therefore, it is important to prevent pneumonia in elderly people. Aspiration pneumonia, in particular in elderly patients, has been suggested to be a crucial issue. Indeed, silent aspiration was observed, using indium-111 chloride scanning, in approximately 70% of elderly patients during the hours of sleep [1]. Furthermore, oral bacteria have been found in bronchoalveolar lavage fluids of elderly patients with community-acquired pneumonia [2–4] and in intraoperative bronchial fluids of elderly patients

with pulmonary carcinoma [5], suggesting that silent aspiration of oral bacteria frequently occurs in elderly patients.

Yamasaki et al. [4] reported that oral bacteria, e.g., *Fusobacterium*, *Prevotella*, *Streptococcus*, *Neisseria*, and *Veillonella*, are frequently detected in bronchoalveolar lavage fluids of patients with community-acquired pneumonia. Hasegawa et al. [5] have found indigenous oral bacteria, i.e., *Streptococcus*, *Veillonella*, *Gemella*, *Porphyromonas*, *Olsenella*, and *Eikenella*, in intraoperative bronchial fluids. Based on these findings, it has been suggested that the bacteria involved in silent aspiration may come from the oral cavity, although definite proof for this hypothesis is currently lacking.

In order to obtain more reliable evidence on silent aspiration of oral bacteria in elderly patients, we measured α -amylase activity in bronchial fluids to estimate the degree of silent aspiration. We compared bacterial amounts and composition in intraoperative bronchial fluids and saliva of the patients. Because the protective

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Table 1
Clinical features of subjects in this study.

	Subjects	Age	Gender	CR score	SR (s)	Smoking history	Number of teeth present (maximum 28)	Number of decayed teeth (maximum 28)	Periodontal pockets (≥ 4 mm) (%)	Bleeding on probing (%)	Plaque control record (%)	Wear of denture	Systemic diseases
Impaired swallowing reflex	1	79	M	6	6.61	+	0	28	–	–	–	Yes	Esophageal cancer after surgery, pulmonary emphysema, shingles
	2	80	M	6	4.13	+	0	28	–	–	–	Yes	Lumbar disc herniation, rheumatoid arthritis, diabetes
	3	70	M	6	7.85	–	23	5	10.1	7.2	25.0	No	Anemia
	4	68	M	6	3.71	+	27	1	1.8	8.0	37.0	No	Parkinson's disease
	5	69	M	6	4.49	+	20	8	15.0	10.8	26.2	No	Chronic obstructive pulmonary disease, myocardial infarction
	6	67	M	6	4.84	+	25	3	13.3	16.0	57.0	No	Pulmonary emphysema, paroxysmal ventricular fibrillation, renal dysfunction
Mean \pm SD		72.2 \pm 5.8		6 \pm 0	5.27 \pm 1.61		15.8 \pm 12.5	12.2 \pm 12.5	10.1 \pm 5.9	10.5 \pm 4.0	36.3 \pm 14.8		
Normal swallowing reflex	7	80	F	6	2.36	–	24	4	3.4	4.1	16.6	No	Sarcoidosis
	8	62	F	6	2.67	+	0	28	–	–	–	Yes	Hypopharyngeal cancer, chronic obstructive pulmonary disease
	9	82	F	6	2.66	–	9	19	20.3	62.9	61.1	Yes	Angina pectoris, Meniere syndrome, myoma of the uterus, depression
	10	78	M	6	2.76	+	24	4	2.0	4.1	23.9	Yes	High blood pressure, idiopathic pulmonary fibrosis
	11	75	M	6	2.43	–	22	6	1.5	6.0	22.7	No	Paroxysmal atrial fibrillation, left kidney cancer
Mean \pm SD		75.4 \pm 7.9		6 \pm 0	2.58 \pm 0.17		15.8 \pm 10.8	12.2 \pm 10.8	6.8 \pm 9.0	19.3 \pm 29.1	31.1 \pm 20.3		

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