Table 3 Isolation frequencies of bacteria and fungi found in plaque and pharynx samples

Bacteria and fungi	Requiring care		Not requiring care	
	plaque (n = 329)	pharynx (n = 253)	plaque (n = 464)	pharynx (n = 453)
Candida albicans	133 (40%)[a]	83 (39%)[a]	135 (30%)	101 (27%)
Enterobacter cloacae	53 (16%)[b]	27 (11%)	26 (6%)	27 (6%)
Pseudomonas spp	37 (11%)[b]	7 (3%)[b]	27 (6%)	35 (8%)
Klebsiella pneumoniae	31 (9%)	35 (14%)[b]	32 (7%)	40 (9%)
Xanthomonas maltophilia	26 (8%)[b]	7 (2%)	8 (2%)	17 (4%)
Klebsiella oxytoca	21 (6%)[b]	3 (1%)	6 (1%)	13 (3%)
Staphylococcus aureus (MSSA)	18 (5%)[b]	8 (2%)	8 (2%)	20 (4%)
Coagulase negative staphylococci	17 (5%)	8 (2%)	15 (3%)	7 (2%)
Serratia marcescens	12 (4%)	4 (2%)	5 (1%)	5 (1%)
Pseudomonas aeruginosa	11 (3%)[a]	19 (6%)	4 (<1%)	4 (<1%)
β Hemolytic streptococcus (type B)	9 (2%)[a]	4 (2%)	2 (<11%)	2 (<1%)
Acinetobacter calcoaceticus	8 (2%)[b]	6 (2%)	41 (9%)	30 (7%)
Candida parapsilosis	8 (2%)	4 (4%)	23 (5%)	17 (4%)
Staphylococcus aureus (MRSA)	8 (2%)[a]	5 (2%)	2 (<1%)	2 (<1%)

MSSA = Methicillin susceptible *S aureus* MRSA = methicillin resistant *S aureus* All data are number of subjects (percent)

[a] $p < 0.05$ (χ^2 test with continuing correction plaque and pharynx samples in requiring care vs not requiring care)

[b] $p < 0.01$ (χ^2 test with continuing correction plaque and pharynx samples in requiring care vs not requiring care)

were no significant differences in the isolation frequency of coagulase negative staphylococci *Serratia marcescens* and *Candida parapsilosis* found in dental plaque or pharynx samples between the 2 groups The other microbial strains were isolated less frequently (<1%) in dental plaque and pharynx samples from both groups To evaluate the correlation between bedridden status and microbial isolation in dental plaque samples (table 4) the patient group was divided into 4 subgroups based on bedridden status and the isolation frequencies were calculated *Pseudomonas* spp was significantly more often isolated ($p < 0.01$ vs not) from 16 (16%) of 98 slightly bedridden subjects and 15 (14%) of 106 moderately bedridden subjects *C albicans* and *S marcescens* were also isolated ($p < 0.05$ vs not) from 38 (50%) and 7 (9%) of 76 respectively completely bedridden subjects Slightly bedridden status was found to significantly decrease the frequency of isolation of *C parapsilosis* and *E cloacae* There was no significant correlation between bedridden status and the other strains

Correlations between Systemic Disease and Microbial Carriage in Oral Samples

A comparison of the frequency between isolated (*C albicans* or *K pneumoniae*) and not isolated dental plaque samples from those requiring care showed heart disease as the only significantly correlated underlying condition (36/151 (23 8%) and 23/175 (12 6%) $p < 0.05$) while hypertension was the only significantly correlated underlying condition related to samples from the pharynx (54/103 (52%) and 41/114 (36%) $p < 0.01$)

We next compared heart disease and the isolation of *C albicans* and *Pseudomonas* spp in dental plaque samples The frequency of *C albicans* or *Pseudomonas* spp was significantly higher in elderly subjects with heart disease than in the control subjects (37/164 (22 6%) vs 22/162 (13 4%) $p < 0.05$) There were no significant differences in the incidence of other diseases between infected and control group samples

Associations between various parameters (oral status denture and oral cleaning and isolation of bacteria and fungus in plaque) and tooth number in the elderly subjects requiring systemic care were also analyzed (table 5) Complete dentures were used in 98 (56%) of the 165 edentu

Table 4 Correlation between bacteria and fungi in dental plaque and bedridden status

Bacteria and fungi	Bedridden status			
	not (n = 31)	slightly (n = 98)	moderately (n = 106)	completely (n = 76)
Candida albicans	8 (26%)	38 (39%)	41 (39%)	38 (50%)
Enterobacter cloacae	7 (23%)	8 (8%)[a]	18 (17%)	11 (14%)
Pseudomonas spp	0 (0%)	16 (16%)[b]	15 (14%)[b]	6 (8%)
Klebsiella pneumoniae	5 (16%)	10 (10%)	4 (4%)	10 (13%)
Xanthomonas maltophilia	5 (16%)	8 (8%)	6 (6%)	2 (3%)
Klebsiella oxytoca	3 (10%)	4 (4%)	5 (5%)	10 (13%)
Staphylococcus aureus (MSSA)	1 (3%)	5 (5%)	2 (2%)	3 (4%)
Coagulase negative staphylococci	2 (6%)	2 (2%)	3 (3%)	6 (8%)
Serratia marcescens	0 (0%)	2 (2%)	1 (1%)	7 (9%)[a]
Pseudomonas aeruginosa	2 (6%)	3 (3%)	3 (3%)	3 (4%)
β Hemolytic streptococcus (type B)	0 (0%)	1 (1%)	4 (4%)	6 (8%)
Acinetobacter calcoaceticus	0 (0%)	3 (3%)	3 (3%)	2 (3%)
Candida parapsilosis	6 (19%)	3 (3%)[b]	1 (1%)[b]	0 (0%)[b]
Staphylococcus aureus (MRSA)	1 (3%)	1 (1%)	2 (2%)	3 (4%)

MSSA = Methicillin susceptible *S aureus* MRSA = methicillin resistant *S aureus* All data are number of subjects (percent) Bedridden status is described in detail in Subjects and Methods

[a] $p < 0.05$ (χ^2 test with continuing correction not vs slightly moderately and completely)

[b] $p < 0.01$ (χ^2 test with continuing correction not vs slightly moderately and completely)

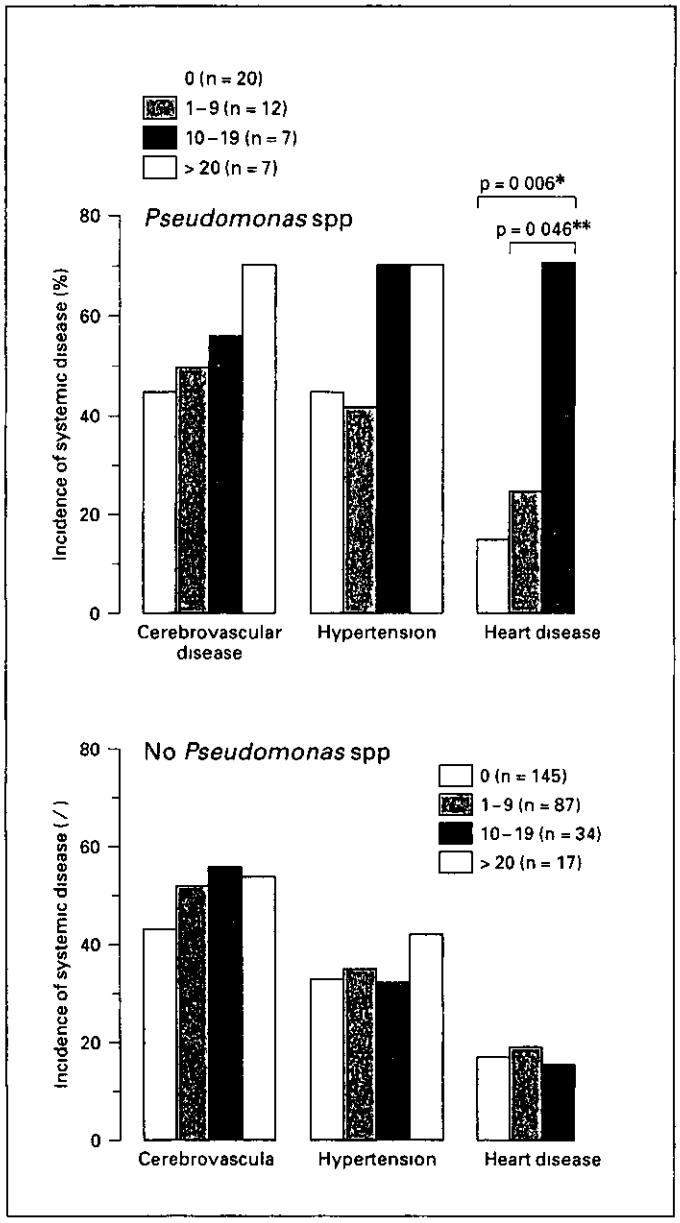
Table 5 Association between various parameters and tooth number in elderly requiring systemic care

Parameters	All subjects (n = 329)	Number of teeth			
		0 (n = 165)	1–9 (n = 99)	10–19 (n = 41)	>20 (n = 24)
Age years (mean ± SD)	83.9±7.5	86.5±6.2	83.1±7.7	78.9±8.8	79.3±8.1
Oral status					
Full denture	–	98 (56%)	37 (37%)	–	–
Partial denture	–	–	35 (35%)	12 (30%)	–
Periodontitis	–	–	60 (60%)	30 (73%)	11 (45%)[c]
Gingivitis	–	–	16 (16%)	10 (24%)	13 (55%)[b c]
Denture and oral cleaning					
Excellent	–	29 (18%)	14 (14%)	1 (2%)[a b]	0 (0%) [b]
Moderate	–	120 (73%)	36 (36%)[a]	15 (37%)[a]	2 (8%)[a c]
Poor	–	16 (9%)	49 (49%)[a]	25 (61%)[a]	22 (92%)[a b]
Bacteria and fungus					
Candida albicans	133 (40%)	59 (36%)	46 (47%)	13 (32%)	15 (63%)[a]
Enterobacter cloacae	53 (16%)	27 (17%)	12 (12%)	8 (20%)	5 (21%)
Pseudomonas spp	47 (12%)	20 (12%)	12 (12%)	6 (15%)	8 (33%)[a]
Klebsiella pneumoniae	31 (9%)	15 (9%)	8 (8%)	6 (15%)	2 (8%)[b]
Xanthomonas maltophilia	25 (8%)	13 (8%)	5 (5%)	2 (5%)	5 (21%)[b]
Klebsiella oxytoca	21 (6%)	11 (7%)	3 (3%)	4 (10%)	3 (13%)
Staphylococcus aureus (MSSA)	18 (5%)	2 (1%)	9 (9%)	4 (10%)	3 (13%)

[a] $p < 0.05$ χ test vs 0

[b] $p < 0.05$ χ test vs 1–9

[c] $p < 0.05$ χ test vs 10–19



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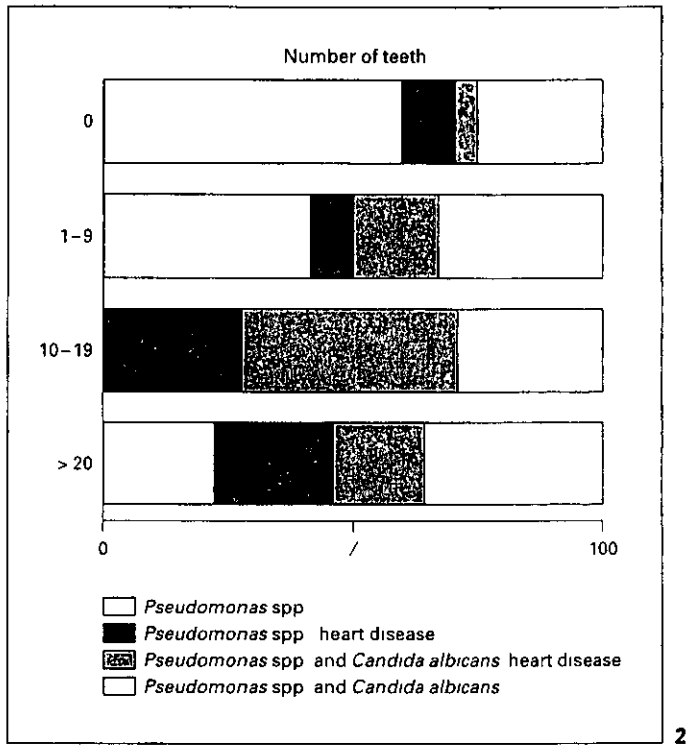

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Fig 1 Relationship between the number of teeth and prevalence of systemic disease in *Pseudomonas* spp positive elderly The incidence of cerebrovascular disease hypertension and heart disease between patients with and without *Pseudomonas* spp in plaque were compared among those with 0 1–9 10–19 and >20 teeth using a χ^2 test (0 vs 1–9 10–19 or >20 1–9 vs 10–19 or >20 teeth and 10–19 vs >20 teeth) Significances ** $p < 0.05$ * $p < 0.01$

Fig 2 Relationship between the number of teeth and prevalence of heart disease in *Pseudomonas* spp and/or *C albicans* positive elderly Groups infected with *Pseudomonas* spp alone without and with heart disease and with *Pseudomonas* spp and *C albicans* without and with heart disease were compared to patients with 0 1–9 10–19 or >20 teeth

lous subjects and complete or partial dentures were used in 37 (37%) and 35 (35%) respectively of 99 elderly subjects with 1–9 teeth in whom periodontitis was found in 60% and gingivitis in 16% The incidence of periodontitis in elderly subjects with 10–19 teeth (73%) was significantly higher than in those with 20 or more (45%) In contrast the incidence of gingivitis in subjects with 10–19 teeth (24%) was significantly lower than in those with 20 or more (55%) Oral hygiene (denture and oral cleaning) tended to worsen as the number of teeth increased in elderly requiring systemic care

An analysis of the relationship between plaque microbial count and the number of remaining teeth revealed that *C albicans Pseudomonas* spp, and methicillin susceptible *Staphylococcus aureus* were detected more frequently in subjects with 20 or more teeth than in edentulous subjects (table 4) In general denture wearing habits had an influence on the prevalence of *C albicans* in the oral cavity In the present study *C albicans* was also detected more frequently in edentulous subjects with complete dentures (46%) than without (21%) Therefore the prevalence of *C albicans* was

influenced by the usage of complete dentures in edentulous subjects

We also analyzed the relationship between the number of teeth and the prevalence of systemic disease *Pseudomonas* spp -positive elderly people with 10–19 teeth had heart disease significantly more frequently (approximately 71%) than those with 0 teeth (13% $p = 0.006$) or 1–9 teeth (25% $p = 0.046$) as shown in figure 1 There were no significant differences in the incidence of other diseases between infected and noninfected group samples Further the incidence of heart disease in those with 20 or more teeth was lower than in those with 10–19 teeth (fig 1) Subjects with 10–19 teeth and accumulations of both *Pseudomonas* spp and *C albicans* in plaque also showed a significant risk for heart disease (fig 2) Subjects with 20 or more teeth and accumulation of *Pseudomonas* spp and *C albicans* in plaque however did not show a significant risk for heart disease (fig 1 2)

Discussion

The isolation frequencies of *C albicans* some Enterobacteriaceae Pseudomonadaceae and *S aureus* (methicillin susceptible and methicillin resistant) in plaque samples and *C albicans* and *Xanthomonas maltophilia* in pharynx samples were significantly higher in the elderly requiring care (table 3) An increased prevalence of these bacteria was associated with the requirement for care since the elderly in nursing homes cannot care for themselves and as a result generally have poor oral condition [3] Previous studies have suggested that the oral carriage of bacteria causing pneumonia such as *K pneumoniae Pseudomonas* spp and *Staphylococcus* spp is low in healthy subjects and higher in immunodeficient and myelo suppressed subjects [20–22] and also in patients with severe periodontal disease [23 24] The significantly higher prevalence of these bacteria in the elderly requiring care is of interest as they may be at greater risk of developing systemic disease such as pneumonia and heart disease The proportion of those who were bedridden and with one underlying medical condition accounted for 89% of all the elderly subjects in nursing homes (table 1) It is possible that individuals who are completely bedridden tend to suffer a deterioration of host defense and poor oral hygiene Our results also demonstrated that the frequency of *C albicans Pseudomonas* spp and *S marcescens* was significantly higher in bedridden than control subjects (table 4) Therefore it is suggested that the bedridden condition encourages infection or accumulation of *C albicans Pseudomonas* spp and *S marcescens* in plaque and thus is a risk factor for respiratory tract infection by aspiration Bedridden subjects were also found to be subject to a progressive loss of protective reflexes which is also a factor with aspiration pneumonia in the elderly [25]

C albicans Pseudomonas spp and methicillin susceptible *S aureus* were detected more frequently in subjects with 20 or more teeth than in edentulous subjects (table 5) Care of the oral cavity is difficult for elderly people with many teeth and it is more difficult for a care provider to clean teeth in a patient than to clean removable dentures Edentulous elderly people and those with 1–9 teeth often use total or partial dentures making it easier for the care provider to assist with oral hygiene This was supported by our results and probably explains why the pathogenic microorganism detection rate increased with the number of teeth in the oral cavity (table 5) We also found that *C albicans* and *Pseudomonas* spp positive elderly people with 10–19 teeth had heart disease significantly more frequently than those with 0 or 1–9 teeth as shown in figures 1 and 2 However the incidence of heart disease in those with 20 or more teeth was lower than in those with 10–19 teeth It also seems possible that pathogenic bacteria can invade the body via inflamed gingiva in people with relatively many remaining teeth and periodontitis In edentulous people heart disease is less likely to develop because there is no route of body invasion for bacteria responsible for heart disease via the oral cavity In addition it has been reported that *Porphyromonas gingivalis* a putative pathogenic microorganism associated with periodontal diseases may be involved in the pathogenesis of coronary heart disease [26 27] and that *Pseudomonas* spp was detected around the gingiva of patients with periodontal diseases [23]

Our results demonstrate a mildly significant association of multiple biofilm formation by *Pseudomonas* spp and *K pneumoniae* and/or *C albicans* with heart disease However these findings were limited to elderly requiring systemic care and with poor oral hygiene because there were few numbers of systemic disease patients in control subjects Further this connection may well have been a result of more than one cross correlation with other pathogens that induce heart disease During the development of biofilm on the teeth pathogenic strains are incorporated into the biofilm in association with oral bacteria Together they are likely to be key species for biofilm formation that infect various body sites such as the heart through the blood stream or the lower respiratory tract during aspiration

Dental plaque is defined as a diverse microbial community found on the tooth surface which is embedded with a matrix of polymers of bacterial and salivary origins [28] In the elderly requiring care biochemical gradients develop that enable the coexistence of a number of species including opportunistic organisms that form a bacterial community during the process of colonization Opportunistic organisms are those that rarely if ever lead to disease in immunocompetent people however they can cause serious disease in older people with underlying systemic problems To identify these high risk individuals the surveillance of potential indicators such as *Pseudomonas* spp and *K pneumoniae* that induce harmful oral biofilm may be a necessary health care procedure for elderly adults requiring systemic care Furthermore such biofilm should be removed through improved methods of oral hygiene the use of chemical agents and by access to a professional such as a dental hygienist Some studies have found that good oral hygiene habits and professional care can reduce the number of oral cavity microorganisms and that use of an antimicrobial agent and physical cleaning for removal of oral biofilm can reduce the prevalence of bacteria [29 30] From the present results, we suggest that the first step should be to remove the dental biofilm thus disrupting microbial adhesion and biofilm formation, otherwise chemotherapeutic and antimicrobial agents will not be able to function effectively

Infection and colonization of *Pseudomonas* spp *K pneumoniae* and *C albicans* on the tooth surfaces of elderly people who require care can serve as indicators of the accumulation of various microorganisms as well as risk factors for the development of systemic diseases such as heart disease Recently Yoneyama et al [31] suggested that oral care might be useful in preventing pneumonia in older patients in nursing homes Therefore attention to oral hygiene including professional care may diminish the risk of systemic disease In addition identification of high risk patients education of staff in nursing homes hand washing and the use of disposable gloves are also proposed to help diminish the risk Taken together such procedures may be beneficial for the continued health of elderly people

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Relationship between the number of remaining teeth and physical activity in community-dwelling elderly

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Abstract

The relationship between oral health and general health in the elderly has been much studied. However, further studies focussing on the influence of oral health on the quality of life (QOL) among the elderly are required. The goal of this study was to evaluate the relationship between oral health and physical or cultural activities. Subjects were 101 community-dwelling elderly persons who were functionally independent (mean age 70.3). Oral health status was evaluated according to the number of remaining teeth and the number of functional teeth. Physical and cultural activities were evaluated from self-reported information. The relationship between oral health and physical or cultural activities was examined by logistic regression analysis. About 60% of subjects took part in cultural activities and less than half actively exercised (leisure sports 33.6%, travel 42.6%). Persons with 20 or more remaining teeth were more active in leisure sports (Odds ratio (OR) = 4.86, 95% confidence interval (CI) = 1.34–17.38) and travel (OR = 5.42, 95% CI = 1.63–18.08) than those with fewer than 20 remaining teeth. These results suggest that the number of remaining teeth is associated with physical activity in elderly persons.

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Keywords Oral health Remaining teeth Physical activity Functionally independent elderly

1 Introduction

The influence of impaired oral health through tooth loss in the elderly has been noticed There have been several reports on the relationship between oral health and functional status in the elderly Miura et al (1997) reported that chewing ability was related to levels of ADL in elderly persons Osterberg et al (1990) reported a relationship between functional impairment in the oral cavity and reduction of physical activity in the elderly We reported a close relationship between oral health and levels of biological and phychosocial functioning in bedridden elderly (Hanada and Tada 2001) These studies suggest that oral health is closely associated with the functional status of functionally dependent elderly persons

The influence of oral health on the quality of life (QOL) of the functionally independent elderly has been drawing considerable attention Decreased chewing ability was found to affect eating habits (Chauncey et al , 1984, Osterberg and Steen 1982, Drummond et al 1988, Petersen and Noitov 1989, Hand et al 1991), and the improvement of eating habits was associated with an improvement of QOL and maintenance of health in old age (Hansen, 1983 Ferrucci et al , 2000 Wahlqvist and Savage 2000) In addition to eating the oral cavity has various functions, such as speech and contributing to facial appearance Impairment of oral health is thought to cause loss of motivation in keeping company with others or playing active part in social and community life Moderate physical and cultural activities in daily life are important for maintaining and improving QOL especially for the elderly (Iida et al 2000 Schechtman and Ory, 2001) Tooth loss may also decrease willingness to participate in physical and cultural activities

The aim of this study was to evaluate the association between oral health status and participation in physical or cultural activities in the functionally independent elderly

2 Subjects and methods

2 1 Subjects

Participants in this study were 101 (48 men and 53 women) community dwelling elderly residents of Mihama Ward who were recruited in the health promotion facility for elderly persons in Mihama Ward Chiba All were functionally independent and often attended this facility The age distribution was as follows 60–69 years 58 (57 4%) 70–79 years 24 (23 8%) 80 years or over 19 (18 8%) Mean age was 70 3±8 2 years No dentists had been involved specifically in the care of the participants before the study We explained the purpose of this study to participants

before the study began and obtained verbal consent to participate The present study reports on data collected in October 1999 The questionnaire was administrated and the oral examination was performed in the health promotion facility for elderly persons in Mihama Ward, Chiba

2 2 Methods

2 2 1 Oral examination

We asked the Chiba City Dental Association, which has 390 registered dentists (membership rate 84%) to recruit dentists to perform the oral examinations Three dentists were selected to participate Their clinical experience was at the same level and they performed examinations with reasonable consistency using commonly established standards We informed them in advance of the purpose and procedures of this study and the examination criteria Oral examinations were performed according to WHO oral examination procedures (WHO, 1997) Variables of oral health status were the number of remaining teeth and the number of functional teeth The number of remaining teeth = number of sound teeth + number of treated teeth + number of decayed teeth, while the number of functional teeth = number of remaining teeth + number of prosthetic teeth

2 2 2 Evaluation of physical and cultural activities

We assessed the physical activity for using two items leisure sports and travel Cultural activities include writing a kind of Japanese traditional poem and playing Japanese chess and singing The information on these items was obtained by a questionnaire administered by an experienced interviewer Subjects were asked to answer questions about their activities for the past year with responses to each item being either 'active' or 'inactive' Criteria for a response of either active or 'inactive' are shown in Table 1

2 2 3 Statistical analysis

The differences in the number of remaining teeth between genders and among age groups were analyzed using the χ^2 test Logistic regression models were used to clarify the contributing factors for each physical and cultural activity The dependent variables were the evaluation of each physical and cultural activity item (active inactive) Gender age and the number of remaining teeth were used as independent variables Age was classified into three groups, 60–69, 70–79 and 80 years or over

Table 1
The criteria of evaluation in physical and cultural activities

	Active	Inactive
Leisure sports	Participates 1 day or more per week	Participates less than 1 day per week
Travel	Once or more than once per year	Less than once per year
Cultural activity	Participates 1 day or more per week	Participates less than 1 day per week

The number of remaining teeth was classified into two groups less than 20 (39 6%) and 20 or more (60 4%) This classification was based on a study that reported that persons with 20 or more remaining teeth had significantly higher ability to masticate than persons with less than 20 remaining teeth (Takehara and Honda 2000) Differences at the 0 05 level were considered statistically significant SPSS for Windows (version 10 0) was used for all statistical analyses

3 Results

Table 2 shows the state of participation in leisure sports 'travel' and cultural activity according to gender and age group About 60% of subjects were estimated to be actively engaged in cultural activities while less than half were active in travel and leisure sports (leisure sports, 33 6%, travel, 42 6%) There was no significant difference in each item between genders In terms of age groups persons aged 60–69 had the highest rate of activity for all three items Subjects aged 70–79 and those aged 80 or over had about the same active rate for cultural activity, but those over the age of 80 had considerably lower rates for participation in leisure sports and travel There were significant differences in each item among age groups (P value leisure sports 0 003 travel 0 003 cultural activity 0 022)

The mean number (SD) of remaining teeth was 17 9 (10 3) The number of remaining teeth according to gender and age group is shown in Table 3 More than half of both male and female subjects had 20 or more remaining teeth The rate of edentulous in women was about three times higher than in men However there was no significant difference between genders With regard to age groups, the younger groups had more remaining teeth ($P = 0\ 000$)

We examined the state of physical and cultural activities with respect to the number of remaining teeth About half of the subjects with 20 or more remaining teeth were active in leisure sports and travel (leisure sports 49 2% travel 62 3%) In contrast most subjects with fewer than 20 remaining teeth were inactive in leisure sports (active rate 10–19, 7 7%, 1–9, 25 0% 0, 6 7%) and travel (active rate 10–19 23 1%, 1–9, 8 3% 0, 6 7%) With regard to cultural activity about two-thirds of subjects with 20 or more or 10–19 remaining teeth were active (20 or more, 69 2%, 10–19 67 2%) Subjects with 1–9 remaining teeth and edentulous subjects exhibited less cultural activity (1–9 50% 0, 33 3%)

Logistic regression analyses were performed to test whether oral health status variables are predictive factors for each physical and cultural activity items(Table 4) The odds of practicing leisure sports among those with 20 or more teeth were 4 9 times greater than the odds of those with fewer than 20 In addition the interaction between remaining teeth and traveling had a significantly increased OR of 5 4 (95% CI = 1 63 18 08) The number of remaining teeth was not significantly associated with cultural activity (OR = 1 16 95% CI = 0 39 3 45)

In subjects with fewer than 20 remaining teeth the mean number of remaining teeth of was 6 3, however the mean number of functional teeth among those subjects was 26 2 And 90% of them had more than 25 functional teeth Because 92 5% of

Table 2
Number and percentage of participants in physical and cultural activities

	Leisure sports		P	Travel		P	Cultural activity		P
	Active	Inactive		Active	Inactive		Active	Inactive	
Age group									
60–69	27 (46 6)	31 (53 4)		33 (56 9)	25 (43 1)		42 (72 4)	16 (27 6)	
70–79	8 (33 3)	16 (66 7)	0 003	8 (33 3)	16 (66 7)	0 003	10 (41 7)	14 (58 3)	0 022
80+	0 (0 0)	19 (100)		2 (10 5)	17 (89 5)		9 (47 4)	10 (52 6)	
Gender									
Men (mean age 68 9)	19 (39 6)	29 (60 4)	0 217	20 (41 7)	28 (58 3)	0 511	30 (62 5)	17 (35 4)	0 270
Women (mean age 71 5)	16 (30 2)	37 (69 8)		23 (43 4)	30 (58 6)		30 (56 6)	23 (43 4)	

Number (%)

Table 3
Number of remaining teeth according to gender and age group

	Number of teeth				
	0	1–9	10–19	20+	*P*
Age group					
60–69	0 (0 0)	3 (5 4)	6 (10 7)	49 (83 9)	
70–79	2 (1 3)	6 (25 0)	5 (20 8)	11 (43 8)	0 000
80+	13 (66 7)	3 (16 7)	2 (11 1)	1 (5 6)	
Gender					
Men (mean age 68 9)	3 (6 4)	5 (10 6)	8 (17 0)	32 (68 0)	0 096
Women (mean age 71 5)	12 (21 2)	7 (13 5)	5 (9 6)	29 (55 8)	

Number (/)

those wore dentures this accounted for a high number of functional teeth among them Nonetheless only 12 5% of these subjects were active in leisure sports or travel

4 Discussion

In the present study an association was found between the number of remaining teeth and physical activity The interpretation of the results is complex because several factors may explain these associations Osterberg et al (1995) reported that the number of remaining teeth was significantly associated with physical ability such as body extension and body flexion and in another report they described the relationship between the number of remaining teeth and muscle strength in the elderly (Osterberg et al 1990) Physical ability is considered to be associated with participation in physical activity Tooth loss have been reported to affect eating habit of elderly (Chauncey et al 1984 Warren et al 2002) Eating habit might relate to participation in physical activity because eating habit is known to have considerable influence on motivation and energy in daily life Furthermore health attitude and psychological factors might explain our results

Although most persons with fewer than 20 remaining teeth had more than 20 functional teeth they participated less in physical activity than persons with 20 or more remaining teeth This suggests that prosthesis treatment did not promote participation in physical activity We reported previously that the mean number of functional teeth was associated with physical function (Hanada and Tada 2001) It is expected that prostheses would help retain physical function to some extent Therefore elderly who have lost their teeth are necessary to be motivated to participate in physical activity

About half of the subjects with fewer than 20 remaining teeth were active in cultural activity suggesting that cultural activity was not significantly related to the number of remaining teeth possibly because cultural activities do not require

Table 4
Multivariable analysis of potential predictive factors for leisure sports travel and cultural activity

	OR (CI)	*P* value
Leisure sports		
Gender		
Men	1 00	
Women	0 87 (0 35–2 20)	0 772
Age group		
60–69	1 00	
70–79	1 00 (0 32–3 08)	0 995
80+		
Remaining teeth		
0–19	1 00	
20+	4 86 (1 34–17 38)	0 016
Travel		
Gender		
Men	1 00	
Women	1 62 (0 65–4 06)	0 301
Age group		
60–69	1 00	
70–79	0 42 (0 20–1 94)	0 627
80+	0 17 (0 04–1 76)	0 276
Remaining teeth		
0–19	1 00	
20+	5 42 (1 63–18 08)	0 006
Cultural activity		
Gender		
Men	1 00	
Women	0 83 (0 35–1 98)	0 674
Age group		
60–69	1 00	
70–79	0 29 (0 10–0 85)	0 024
80+	0 41 (0 10–1 67)	0 214
Remaining teeth		
0–19	1 00	
20+	1 16 (0 39–3 45)	0 786

OR and 95% CI cannot be calculated in this category because no subjects in this category were active in leisure sports

considerable movement It has been reported that tooth loss affects brain activity (Kondo et al 1995 Noridemam et al 1996 Kato et al 1997) It is also considered desirable for elderly people who have lost teeth to participate in cultural activity for maintain their brain activity

To maintain QOL of the elderly they must participate in social activities in various ways (Lawton et al 1999) Participation in physical activity is important for maintaining QOL Our study suggests that retention of teeth is associated with participation in physical activity in the elderly However the influence of oral health including the number of teeth on QOL has not been fully studied To increase our

knowledge in this field, further epidemiological studies investigating a causal relationship between oral health and QOL should be performed

It has been reported that elderly with poor oral health status has difficulty in eating and decreased eating pleasure (Lamy et al., 1999, Warren et al 2002) Mojon et al (1999)reported that functional status was a significant factor for nutritional deficiency in functionally dependent and semi dependent elderly Furthermore the number of remaining teeth in the oral cavity influence the oral health because of the change of oral condition Senpuku et al (in press) reported that the bedridden elderly with many remaining teeth had higher detection rate of pathogenic bacteria species than those with many replaced teeth On the other hand the relationship between the number of remaining teeth and nutritional status and oral bacteria flora in functionally independent elderly have been noticed In the future such that studies have worth to be performed in the functionally independent elderly

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Different frequencies of *Streptococcus anginosus* infection in oral cancer and esophageal cancer

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Multiple cancers frequently occur in the upper aerodigestive tract The high incidence rate of multiple carcinomas in this region is often explained in terms of involvement of the same underlying risk factors It has been reported that the oral bacterium *Streptococcus anginosus* (*S anginosus*) is associated with esophageal gastric and pharyngeal cancer tissues In this study a highly specific quantification method for *S anginosus* DNA using real time PCR was established We employed this assay to determine whether *S anginosus* is also associated with oral cancer tissues This precise quantification method revealed different degrees of infection with *S anginosus* in esophageal cancer and oral cancer We assayed 10 ng of genomic DNA from cancer tissues and found that eight of 18 samples (44%) from the esophagus contained a detectable level (>10 fg) of *S anginosus* DNA whereas this was the case for only five of 38 samples (13/) of oral cancer The quantity of *S anginosus* DNA in the esophageal cancer tissues was significantly higher than in oral cancer The maximum amount of *S anginosus* DNA was approximately ten times higher in esophageal than in oral cancer tissues In addition none of the five different oral cancer sites (floor of the mouth mandibular gingival maxillary gingival buccal mucosal and tongue) showed significant signs of *S anginosus* infection On the other hand most non cancerous tissues of the esophagus and tongue showed an undetectable level of *S anginosus* These results suggest that *S anginosus* is associated with esophageal cancer but is not closely related with oral cancer (Cancer Sci 2003 94 492–496)

Bacterial and viral infections are important factors in cancer development [1] It has been reported that *Helicobacter pylori* (*H pylori*) is associated with gastritis gastric atrophy and gastric cancer [4] The presence of microorganisms in several kinds of human cancers was recently investigated and *Streptococcus anginosus* (*S anginosus*) DNA fragments were frequently found in DNA samples from esophageal cancer tissues gastric cancer tissues and dysplasia of the esophagus [5,6] Viable *S anginosus* was also recovered from esophageal cancer tissues (unpublished data) These results suggest that *S anginosus* infection occurs at an early stage of esophageal cancer and is related to esophageal and gastric carcinogenesis *S anginosus* is classified as an oral bacterium and can be isolated from several parts of the body such as the oral cavity gastrointestinal tract and genitourinary tract It is often associated with pyogenic infections including endocarditis [7–9] *S anginosus* DNA has also been found in head and neck squamous cell carcinomas[10] it was found much less frequently in non cancerous tissues of the esophagus and was absent from the colon lung bladder renal and cervical cancer tissues [6] Therefore it was suggested that *S anginosus* DNA is associated with cancers in the upper digestive tract although the involvement of *S anginosus* infection in the carcinogenic process has not been clarified

It is generally accepted that the upper aerodigestive tract is a region in which multiple primary cancers occur at a high rate Squamous cell carcinoma of the oral cavity is often accompanied by other squamous cell carcinomas of the aerodigestive tract such as oropharyngeal cancers or esophageal cancers [11–13] The high incidence of multiple carcinomas in this region is often explained by the concept of field cancerization which is based on the hypothesis that exposure to carcinogenic agents leads to independent carcinogenesis in epithelial cells at different sites in this region Although little is known about this hypothetical etiology many epidemiological studies have indicated some possible etiological factors such as alcohol use [13,14]

We investigated the presence of *S anginosus* DNA in squamous carcinoma tissues of the oral cavity in this study and made comparisons with the esophagus *S anginosus* infection in cancer tissues of the upper digestive tract and the concept of field cancerization led us to consider *S anginosus* to be one possible risk factor for cancer development and also led us to propose that *S anginosus* infection occurs in oral cancer tissues as well as other sites in the upper digestive tract Presently neither the biochemical criteria nor PCR methods for identification of streptococci are sufficiently specific and reliable because various oral *Streptococci* that are biochemically and phylogenetically similar to *S anginosus* are dominant in the oral cavity [15,16] and these species sometimes interfere with detection of *S anginosus* Therefore in the present study a quantitative real time PCR coupled with *Taq*Man chemistry a highly sensitive and specific approach [17] was developed to assess *S anginosus* status Using this assay *S anginosus* DNA was accurately identified and quantified in oral and esophageal cancer tissues The numerical values obtained were considered in relation to cancer development in the digestive tract

Materials and Methods

Tumor samples Eighteen esophageal carcinoma tissues and 6 non cancerous tissues were obtained from patients at the National Cancer Center Hospital (Tokyo) Nineteen lingua carcinoma tissues 5 mandibular and 4 maxillary gingival carcinoma tissues 5 buccal carcinoma tissues and 7 non cancerous tissues of the tongue were obtained from patients at Yokohama City University Hospital (Yokohama) All of the surgical specimens were stained with Lugol s solution They were then washed with PBS several times to remove surface adherent bacteria then frozen immediately in liquid nitrogen and stored at −80 C until use Informed consent was obtained from all the patients This study was approved by the ethical committees of the National Cancer Center and Yokohama City University

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Bacterial strains *S anginosus* ATCC 33397 *S intermedius* ATCC 27335 *S constellatus* ATCC 27823 *S mutans* LM 7 *S sobrinus* AHT *S sanguis* ATCC 10556 *S gordonii* ATCC 10558 *S mutis* ATCC 6249 and *S salivarius* ATCC 9759 were cultured and centrifuged The resulting pellet was treated with 20 mg/ml lysozyme before DNA extraction

DNA extraction Genomic DNA was isolated from tissue samples and bacteria by a standard phenol chloroform method or a fil tration method DNA content was determined spectiophotomet rically

Primers and PCR amplification 16S rDNA sequences from 11 spe cies which cover the dominant oral streptococcal species groups [15] were obtained from the GenBank database[18] and aligned by the computer program Clustal W[19] *S anginosus* ATCC 33397T was chosen from several *S anginosus* strains for the alignment since this strain and the ATCC 33397T like strains are closely associated with infection [20] Primers for am plification of 16S rDNA of *S anginosus* were designed based on the sequence of *S anginosus* ATCC 33397T in alignment Sequences of primers were as follows forward primers F0 (5 GAACGGGTGAGTAACGCGTAGGTA 3) F1 (5 CAAG TAGGACGCACAGTTTA 3) F2 (5 AAGTAGGACGCA CAGTTTAT 3) F3 (5 CGTAGCTTGCTACACCATAG 3) F4 (5 GTAGCTTGCTACACCATAGA 3) reverse primers R0 (5 AAGCATCTAACATGTGTTACATAC 3) R1 (5 AG CATCTAACATGTGTTACATA 3) R2 (5 AAGCATCTAA CATGTGTTACAT 3) R3 (5 CAAGCATCTAACATGTG TTAC 3) F0 and R0 are the same as St1 and St3 respec tively in our previous report [6] PCR for amplification of 16S rDNA of *S anginosus* was performed in a total volume of 25 μl containing 2 μM of each primer and 50 fg to 50 ng of template DNA The thermal cycling conditions were 35 cycles of denaturation at 94°C for 30 s annealing at 65 C for 30 s with a decrease of 0 2 C per cycle and extension at 72 C for 30 s

Quantitative real time PCR Quantification of *S anginosus* 16S rDNA was performed using real time PCR based on *Taq*Man chemistry (ABI Prism 7700 Sequence Detection System Ap plied Biosystems Foster City CA) The F1 and R3 primers were chosen for use in the quantification and the *Taq*Man probe (5 AGCTTGCTACACCATAGACTGTGAGTTGCGA 3) was designed by the Primer Express 1 0 software package (Applied Biosystems) to perfectly complement the 16S rDNA gene of *S anginosus* downstream of the forward primer The *Taq*Man probe was labeled at the 5 end with reporter dye (6 FAM) and at the 3 end with quencher dye (TAMRA) The re action mixture in a total volume of 25 μl contained *Taq*Man Universal PCR Master Mix (Ampli*Taq* Gold Amperase uracil *N* glycosylase Applied Biosystems) 200 nM of each primer a 120 nM probe and 5 μl of DNA solution The thermal cycling conditions were set to activate AmpErase uracil *N* glycosylase at 50°C for 2 min and activate Ampli*Taq* Gold at 95°C for 10 min followed by 45 cycles of denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min Human DNA was quantified by real time PCR with *Taq*Man β actin control re agent (Applied Biosystems) according to the manufacturer s in structions

Statistical analysis Differences in the quantity of *S anginosus* DNA in the DNA samples from cancer tissues were statistically analyzed using the Mann Whitney *U* test

Results

Specificity and sensitivity of the primers for detecting *S anginosus* DNA Fig 1 shows part of an alignment of the 16S rDNA of 11 species of *Streptococcus* and regions of the primers for detect ing *S anginosus* Four forward and three reverse primers that were designed based on the sequence of *S anginosus* ATCC 33397T in the variable region are shown above the alignment (F1–4 R1–3) F0 and R0 are previously reported primers [6] All combinations of the 5 forward and 4 reverse primers were tested for specificity and sensitivity for detecting *S anginosus* Using 5 pg of *S anginosus* DNA and primers F1 and R3 en abled strong amplification of a 160 bp DNA fragment which is the expected size considering the position of the primers (Fig 2A) However only a faint band was detected for *S salivarius* even when using 50 000 pg of DNA as a template (Fig 2A) A similar result with slightly weaker bands was obtained from *S*

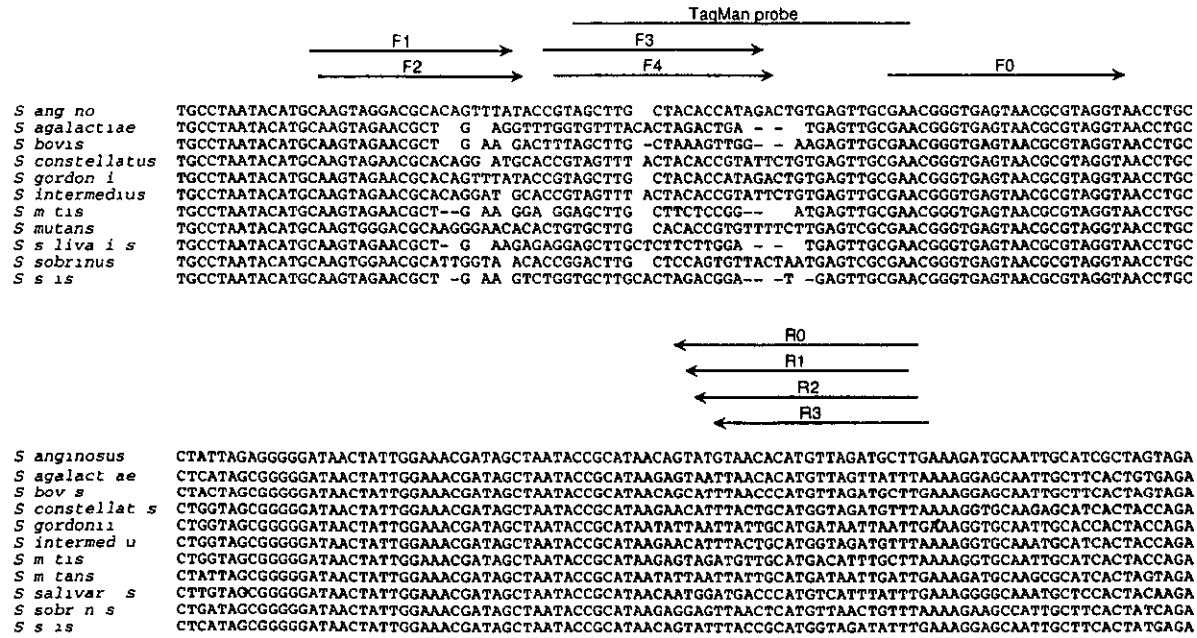

Fig 1 Primers and *Taq*Man probe for specific detection of *S anginosus* that were designed based on an alignment of the 5 part of 16S rDNA from 11 species of *Streptococcus* Arrows and the bar indicate primers and the *Taq*Man probe respectively Stars represent identical nucleotides

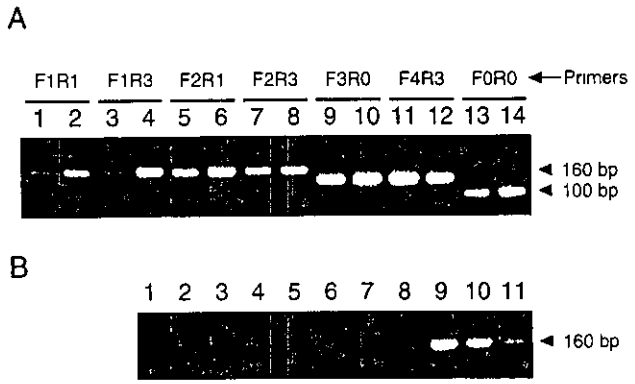

Fig 2 Test of PCR primer sets for specific detection of *S anginosus* DNA A 5 pg of *S anginosus* DNA (lanes 2 4 6 8 10 12 14) and 50 000 pg of *S salivarius* DNA (lanes 1 3 5 7 9 11 13) were amplified with the primer sets shown above the bars B 50 pg of DNA from *S salivarius* (lane 1) *S mutans* (lane 2) *S sobrinus* (lane 3) *S sanguis* (lane 4) *S gordonii* (lane 5) *S mitis* (lane 6) *S constellatus* (lane 7) *S intermedius* (lane 8) or *S anginosus* (lane 9) and 5 pg and 0 5 pg of *S anginosus* DNA (lane 10 and lane 11) amplified with the primer set F1 and R3

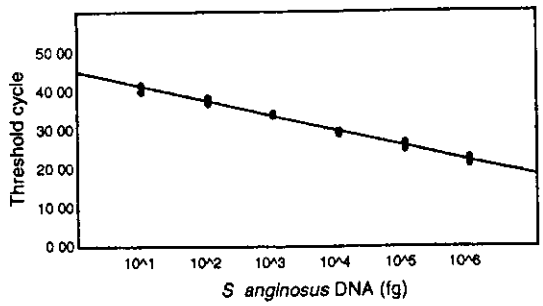

Fig 3 Standard curve for *Taq*Man PCR 10^1 to 10^6 fg of DNA of *S ginosus* were assayed by real time PCR The threshold cycle numb which corresponds to the PCR cycle number at which the fluorescer signal exceeded the detection threshold was plotted against e standard DNA Correlation coefficient=0 989

anginosus DNA using the F1 and R1 primers (Fig 2A) In contrast PCR using the remaining combinations of primers demonstrated that rDNA fragments are equally amplified in *S anginosus* and *S salivarius* samples when a concentration of *S salivarius* DNA 10 000 times greater was used (Fig 2A) This suggests that large amounts of *Streptococcus* might yield bands similar to that of *S anginosus* in PCR analysis even when using specific primers for *S anginosus* We also examined amplification of a DNA fragment in other streptococcal strains using the F1 and R3 and the F1 and R1 primer sets but observed no strong bands with either (Fig 2B) Therefore we concluded that F1 and R3 as well as F1 and R1 are highly specific and sensitive primers for detecting *S anginosus*

Real time PCR for quantifying *S anginosus* DNA Absolute quantification of DNA is based on generating a standard curve with external standards To this end 10^0 to 10^6 fg of *S anginosus* DNA in a series of 10 fold dilutions was assayed by real time PCR The standard curve was created by plotting the C_t number the cycle number at which the fluorescence signal crossed the detection threshold against each DNA concentration tested spectrophotometrically The amounts of DNA plotted against the C_t values were linear over the range of 10^1 to 10^6 fg ($r>0$ 989 Fig 3) indicating that *S anginosus* DNA can be properly quantified within this range The quantities of *S anginosus* DNA in test samples were then derived from the C_t numbers and the standard curve The specificity of this assay for *S anginosus* was determined using other *Streptococcus* species including those which have the greatest similarities with *S anginosus* in the regions of the primers and probe based on the GenBank data base When 10^6 fg of genomic DNA from the seven *Streptococcus* species were assayed the calculated values derived from the C_t numbers were lower than the detectable level 10^1 fg (data not shown) These results demonstrate that this system properly quantifies not only *S anginosus* but also other species

Quantities of *S anginosus* DNA in tumor tissue samples The amount of *S anginosus* DNA in 38 oral and 18 esophageal cancer tissues was assessed by real time PCR (Table 1) Ten nanograms of genomic DNA from the esophageal cancer tissues were assayed Eight of 18 samples (44%) contained detectable levels of *S anginosus* DNA Five of these yielded over 10 fg and the maximum found was $1\,9\times10^3$ fg Assuming that 1 cell of *S anginosus* contains about 5 fg of genomic DNA and 1 human cell contains about 0 006 ng of genomic DNA it was estimated that approximately 0 2 cell of *S anginosus* was present per 1 human cell at the highest level In contrast only 5 of oral cancer tissue samples (13%) showed detectable levels of *anginosus* DNA and only 1 derived from the tongue had over 10^2 fg One other sample of lingua cancer contained a detectable level of *S anginosus* DNA Two of 5 samples from the floor of the mouth and 1 of 5 from the buccal mucosa yielded detectable levels of *S anginosus* DNA Neither mandibular nor maxillary gingival cancer samples contained a detectable level of *S anginosus* DNA Statistical analysis indicated that the difference between the quantities of *S anginosus* DNA in esophageal and oral cancer tissues was significant ($P<0.0$ assuming that the samples with undetectable levels of *S anginosus* DNA were all at the same level 10 fg

Quantities of *S anginosus* DNA in non cancerous tissue samples Quantities of *S anginosus* DNA were determined in DNA samples from non cancerous tissues Five out of 6 esophageal and all 7 lingua normal tissues showed an undetectable level of *anginosus* DNA (Table 2)

Quantities of human DNA in tumor tissue samples Quantities human DNA were determined in DNA samples from tissues real time PCR Each sample yielded a similar value to whole genomic DNA measured spectrophotometrically (data not shown) These results indicated that the genomic DNA isolated from tissue samples was mostly human DNA

Discussion

We determined the amounts of *S anginosus* in oral and esophageal cancer tissues by quantitative real time PCR The method that we established enables very sensitive and specific detection of *S anginosus* DNA The level of sensitivity is 10 fg of *S ginosus* DNA and the other phylogenetically closely related species presently classified did not interfere with the quantification

The conventional PCR method was employed in detecting *anginosus* DNA in esophageal gastric and head and neck cancer tissues [6 10] although its accuracy is not as good as real time PCR It was also reported that gingival smears from cancer patients contain *S anginosus* [10] In addition epithelial tissues the oral cavity and the esophagus are exposed to similar kinds of oral bacteria through the saliva Therefore we expected that *S anginosus* would be present to the same degree in oral cancer tissues as in other upper digestive tract cancers However quantitative real time PCR revealed a low frequency and small amounts of *S anginosus* DNA in oral cancer tissues Genomic DNA from tissue samples was mostly human DNA When ng of genomic DNA from the tissue samples was assayed maximum value of *S anginosus* DNA in esophageal cancer

Table 1 *S anginosus* load in esophageal and oral cancer tissues

Oral cancer				Esophageal cancer	
Case	*S anginosus* DNA[1)] (fg/10 ng total DNA)	Case	*S anginosus* DNA[1 2 3)] (fg/10 ng total DNA)	Case	*S anginosus* DNA[1 2)] (fg/10 ng total DNA)
Floor of the mouth		Tongue		Esophagus	
1	ND	20	ND	39	ND
2	ND	21	ND	40	9 5 (1 3)×10¹
3	ND	22	ND	41	ND
4	1 5 (0 3)×10¹	23	ND	42	3 0 (2 0)×10
5	1 1 (0 2)×10¹	24	ND	43	1 3 (0 2)×10²
		25	ND	44	ND
Mandibular gingival		26	1 9 (0 7)×10¹	45	ND
6	ND	27	ND	46	ND
7	ND	28	ND	47	ND
8	ND	29	ND	48	1 9 (0 9)×10¹
9	ND	30	2 0 (0 6)×10²	49	2 6 (0 5)×10²
10	ND	31	ND	50	ND
		32	ND	51	2 7 (0 7)×10²
Maxillary gingival		33	ND	52	1 5 (0 8)×10²
11	ND	34	ND	53	ND
12	ND	35	ND	54	ND
13	ND	36	ND	55	ND
14	ND	37	ND	56	1 9 (0 6)×10³
		38	ND		
Buccal mucosa					
15	ND				
16	ND				
17	3 0 (1 3)×10¹				
18	ND				
19	ND				

1) Mean number of triplicate experiments and standard deviation in parenthesis
2) 10 ng DNA extracted from tissue samples were used
3) ND <1 0×10¹

sues was 1 9 (0 6)×10³ fg but in oral cancer tissues it was ten times lower [2 0 (0 6)×10 fg] The quantity of *S anginosus* DNA was found to be significantly lower in oral cancer tissues than in esophageal cancer tissues ($P<0$ 01) The frequency of *S anginosus* DNA also differed in that the percentage of subjects with a detectable level of *S anginosus* DNA (>10 fg) was about three times lower for oral cancer tissues (13%) than for esophageal cancer tissues (44%) In addition five different oral cancer sites were examined and none yielded high frequencies of *S anginosus* infection or contained large amounts of DNA Two of 5 cancer tissues from the floor of the mouth did contain detectable levels of *S anginosus* DNA but the values were not as high as in esophageal cancer On the other hand both the normal esophageal and the normal oral tissue samples gave similar results i e undetectable or only a low level of *S anginosus* DNA

In our previous report[6)] 14 of 15 (93%) esophageal cancers showed positive In another report[10)] 4 of 10 (40%) head and neck cancers showed positive when they used our original primers while 100% of head and neck cancers showed positive with other primers for detecting *S anginosus* In our previous report 100 ng of cancer tissue DNA was used for 35 cycles of PCR In this study only 10 ng of DNA was used *S anginosus* DNA was then quantified within the range of linear amplification This explains why there is a discrepancy between our present results and our previous report

With the present method further study such as comparison of *S anginosus* DNA content of cancer tissues of different parts of the body or comparison of *S anginosus* DNA content between ulcerated (usually with inflammation) and non ulcerated portions of cancer tissues should be performed so that the pathophysiology of *S anginosus* in cancer development may be better understood Investigating bacteria other than *S anginosus* involved in cancers of the upper digestive tracts is also important A real time PCR system can not be used to investigate the roles of various kinds of bacterial species simultaneously because suitable specific primers or probes are not available Therefore we have to perform PCR using universal primers to detect a variety of bacteria and then sequence DNA clones in the tissue samples Unfortunately a comprehensive detection system has still not been developed Thus we can not state whether or not other bacterial species may also be involved in esophageal and oral cancer However it appears that *S anginosus* increases specifically in esophageal cancer tissues and we speculate that there is a strong association of this bacterium with esophageal cancer

Table 2 *S anginosus* load in normal tissues of esophagus and tongue

Tongue		Esophagus	
Case	*S anginosus* DNA[1 2 3)] (fg/10 ng total DNA)	Case	*S anginosus* DNA[1 2 3)] (fg/10 ng total DNA)
57	ND	64	ND
58	1 5 (0 7)×10¹	65	ND
59	ND	66	ND
60	ND	67	ND
61	ND	68	ND
62	ND	69	ND
63	ND		

1) Mean number of triplicate experiments and standard deviation in parenthesis
2) 10 ng DNA extracted from tissue samples were used
3) ND <1 0×10¹

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