Prevalence of oral *Capnocytophaga* species and their association with dental plaque accumulation and periodontal inflammation in middle-aged and older people

HIDEO SHIGEISHI[1], NATSUKI HAMADA[1], YOSHINO KANEYASU[1], YOSHIE NIITANI[2], TOSHINOBU TAKEMOTO[2] and KOUJI OHTA[1]

Departments of [1]Public Oral Health and [2]Oral Health Management, Program of Oral Health Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima 734-8553, Japan

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Abstract. *Capnocytophaga* species are commonly found in human oral microbiome. The aim of the present study was to understand the association of the prevalence of oral *Capnocytophaga* species with oral hygiene and periodontal inflammation. A total of 136 patients (median age 72 years) who visited the Hiroshima University Hospital (Hiroshima, Japan) between April 2021 and June 2023 were enrolled. Swab samples were obtained from the tongue surface. DNA from *Capnocytophaga* species (*C. ochracea* and *C. sputigena*) was detected by real-time PCR analysis. Dental plaque accumulation was observed to assess the oral hygiene condition of participants. Additionally, clinical periodontal inflammation was assessed with periodontal inflamed surface area (PISA) scores. Clinical confounding factors such as age, sex, lifestyle-related disease, remaining teeth and denture wearing between *Capnocytophaga* species-positive and -negative groups were adjusted with a propensity score matching method. Mann-Whitney U and χ^2 or Fisher's exact test were employed for statistical analysis. The prevalence rate was 67.6% for oral *C. ochracea* and 83.1% for *C. sputigena*. *C. ochracea*-positive participants showed significantly higher plaque control record scores (an indicator of dental plaque accumulation) than *C. ochracea*-negative participants (P=0.03). Additionally, *C. ochracea*/*C. sputigena* dual-positive participants exhibited significantly higher plaque control record and PISA scores than non-dual-positive participants (P=0.01 and P=0.04, respectively). Propensity score matching was conducted in the *C. ochracea*/*C. sputigena* dual-positive group and the non-dual-positive group for adjustment of clinical factors, resulting in 51 matched patient pairs. *C. ochracea*/*C. sputigena* dual-positive participants had significantly higher plaque control record scores than non-dual-positive participants (P=0.02). The present results suggest that the prevalence of both oral *C. ochracea* and *C. sputigena* is associated with poor oral hygiene in middle-aged and older people.

Correspondence to: Dr Hideo Shigeishi, Department of Public Oral Health, Program of Oral Health Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami, Hiroshima 734-8553, Japan
E-mail: shige@hiroshima-u.ac.jp

Key words: Capnocytophaga, oral hygiene, dental plaque, periodontitis, real-time PCR, dentistry, periodontology

Introduction

Periodontitis is a common infectious disease caused by periodontopathic bacteria with dysbiosis of the oral microbiota (1) and is associated with systemic diseases such as diabetes, cardiovascular disease and adverse pregnancy outcomes (2). For prevention of periodontitis, daily toothbrushing , flossing between teeth, promotion of oral hygiene, professional dental care and regular replacement of toothbrushes to avoid bristle splaying and softening are required (2-5). Among periodontopathic bacteria, 'red complex' bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* are major pathogens for chronic periodontitis (6). The periodontal inflamed surface area (PISA) score indicates the severity of clinical periodontal inflammation (7). The prevalence of both *T. forsythia* and *T. denticola* has been associated with increased PISA values in older Japanese people (8), suggesting that these bacteria are associated with the severity of periodontitis.

Capnocytophaga species such as *C. ochracea* and *C. sputigena* are classified as 'green complex' bacteria and are involved in early colonization of the dental biofilm (6). It has been reported that many young children exhibit a high prevalence of oral *Capnocytophaga* species with or without gingivitis (9). *Capnocytophaga* species are primarily found in the subgingival plaque of patients with periodontitis (10). Furthermore, *Capnocytophaga* species are abundantly found in oral squamous cell carcinoma tissue, suggesting that *Capnocytophaga* may contribute to the development of oral cancer as well as the pathology of periodontitis (11). However, the association between *Capnocytophaga* species and oral health status indicators such as oral hygiene and periodontal disease has not been fully understood in middle-aged and

older people. Therefore, the present study investigated the prevalence of two oral *Capnocytophaga* species, *C. ochracea* and *C. sputigena*, and their association with oral hygiene and periodontitis in middle-aged and older Japanese people.

Materials and methods

Participants. A total of 136 (43 male, 93 females; median age, 72 years, range 36-91 years) patients attending the Hiroshima University Hospital (Hiroshima, Japan) for supportive periodontal therapy from 2021 April to 2023 June were included. Patients aged ≥20 years attending the Hiroshima University Hospital were included. Patients with cancer receiving chemotherapy or radiotherapy and patients with severe immunodeficiency disease were excluded. Ethical approval for the present cross-sectional study was obtained from the Ethics Committee of Hiroshima University (approval no. E-1115). All participants signed informed consent before participating in the study. Clinical characteristics of participants (age, sex, lifestyle-related diseases, denture wearing and number of remaining teeth) were recorded from their medical records.

Specimen collection and DNA isolation. The specific anatomical structure of the tongue surface may contribute to the characteristic oral microbiome composition that includes periodontopathic anaerobic bacteria. Therefore, tongue surface bacteria were collected (12). Samples were collected by swabbing the tongue surface using sterilized disposable swab applicators (Orcellex® Brush; Rovers Medical Devices) 10 times, as previously described (12). Immediately after swabbing, the swab brush was soaked in lysis buffer (S1-Lysis Buffer; cat. no. A29790, Invitrogen; Thermo Fisher Scientific, Inc.) in a 15 ml plastic tube (Greiner Bio-One). Total DNA was isolated from the swab samples using PureLink™ Microbiome DNA Purification kit (Invitrogen; Thermo Fisher Scientific, Inc.) as previously described (13).

Oral examination. In accordance with our previous studies (13,14), dental plaque accumulation was assessed by calculating plaque control record scores. Dental plaque was stained using a microbrush and plaque disclosing solution (PROSPEC®; GC Co., Ltd.), as previously described (14). The participants then rinsed with water and the presence of stained dental plaque was recorded at six areas (mesiobuccal, mid-buccal, distobuccal, mesiolingual, mid-lingual and distolingual sites) of individual teeth (13). Plaque control record score (%) was calculated as number of tooth surfaces with stained dental plaque divided by the total number of tooth surfaces. Periodontal pocket depth and bleeding on probing (BOP) was examined in accordance with a previous study (15). PISA and periodontal epithelial surface area (PESA) scores were calculated in accordance with a modification of Nesse's methods as reported by a previous study (16).

Real-time PCR analysis. The presence of bacterial DNA was examined using a real-time PCR device (Thermal Cycler Dice® Real Time System III; Takara Bio, Inc.). The THUNDERBIRD SYBR qPCR mix (Toyobo Life Science) and specific primer sets (Hokkaido System Science Co., Ltd.) were used for the detection of *C. ochracea* and *C. sputigena* DNA. Universal primer for bacterial 16S rRNA gene (Hokkaido System Science Co., Ltd.) was used as a reference primer (8). The PCR amplification protocol was as follows: Initial denaturation at 95˚C for 2 min, followed by 40 cycles of 95˚C for 30 sec, 58˚C for 30 sec and 72˚C for 30 sec, and a final step of 72˚C for 2 min. The following previously described PCR primer sets (8,9) were used: *C. ochracea* forward, 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse, 5'-GATGCCGTCCCTATATACTATGGGG-3' (accession no. FJ577260) (9); *C. sputigena* forward, 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse, 5'-GATGCCGCTCCTATATACCATTAGG-3' (accession no. NR_113564) (9) and universal primer for bacterial 16S rRNA gene forward, 5'-CGCTAGTAATCGTGGATCAGAATG-3' and reverse, 5'-TGTGACGGGCGGTGTGTA-3' (8). Two independent experiments were performed.

Statistical analysis. Data were analyzed with IBM SPSS Statistics, version 24.0 (IBM Corp.). The χ^2 or Fisher's exact test were used to analyze associations between two categorical variables. The Mann-Whitney U test was used to compare medians between two dependent groups. Data are presented as median (interquartile range). Adjustment and matching of clinical confounding factors between two groups were conducted by propensity score matching. Propensity scores were estimated by logistic regression using seven clinical factors (age, sex, hypertension, diabetes, dyslipidemia, remaining teeth and denture use). A caliper size was set at 0.25 of the standard deviation of the propensity score. G*Power (version 3.1.9.4, Heinrich-Heine-Universität Düsseldorf) was used to determine the sample size required for the Wilcoxon signed-rank test following propensity score matching. The Wilcoxon signed-rank test was used to compare plaque control record, PISA and PESA scores between two groups. Sample size calculation with an effect size of 0.5, significance level of 0.05 and statistical power of 0.8 (minimum acceptable level) (17) revealed that at least 35 paired participants were required. Additionally, post hoc statistical power analysis of the Wilcoxon signed-rank test was performed from the total sample size, with an effect size of 0.5 and significance level of 0.05 using G*Power. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Prevalence of oral C. ochracea and C. sputigena. The prevalence of oral *C. ochracea* and *C. sputigena* was 67.6 and 83.1%, respectively. The associations between *Capnocytophaga* and clinical characteristics are summarized in Table I. A significant association between *C. ochracea* prevalence and sex was found. No significant associations were found between the prevalence of *C. ochracea* and clinical factors such as age, systemic disease, number of remaining teeth or denture use. There was no significant association between *C. sputigena* prevalence and age, sex, systemic disease, the number of remaining teeth or denture use.

Association of Capnocytophaga species with oral hygiene and periodontal condition. *C. ochracea*-positive participants recorded higher plaque control record scores (21.0%) than *C. ochracea*-negative participants (14.0%; Fig. 1A). A

Table I. Association between *Capnocytophaga* species and clinical characteristics.

Characteristic	*C. ochracea*			*C. sputigena*		
	Negative (n=44)	Positive (n=92)	P-value	Negative (n=23)	Positive (n=113)	P-value
Median age, years (IQR)	73.5 (13.5)	72.0 (14.0)	0.27[a]	72.0 (18.0)	72.0 (14.5)	0.75[a]
Age, years (%)						
30-39 (n=2)	1 (2.3)	1 (1.1)	0.16[b]	0 (0.0)	2 (1.8)	0.98[b]
40-49 (n=7)	1 (2.3)	6 (6.5)		1 (4.3)	6 (5.3)	
50-59 (n=12)	0 (0.0)	12 (13)		2 (8.7)	10 (8.8)	
60-69 (n=33)	12 (27.3)	21 (22.8)		5 (21.7)	28 (24.8)	
70-79 (n=50)	19 (43.2)	31 (33.7)		9 (39.1)	41 (36.3)	
80-89 (n=30)	11 (25.0)	19 (20.7)		6 (26.1)	24 (21.2)	
90-99 (n=2)	0 (0.0)	2 (2.2)		0 (0.0)	2 (1.8)	
Sex (%)						
Male (n=43)	7 (15.9)	36 (39.1)	0.01[c]	7 (30.4)	36 (31.9)	>0.99[c]
Female (n=93)	37 (84.1)	56 (60.9)		16 (69.6)	77 (68.1)	
Hypertension (n=30)	10 (22.7%)	20 (21.7%)	>0.99[c]	5 (21.7%)	25 (22.1%)	>0.99[c]
Diabetes (n=19)	6 (13.6%)	13 (14.1%)	>0.99[c]	4 (17.4%)	15 (13.3%)	0.74[c]
Dyslipidemia (n=25)	8 (18.2%)	17 (18.5%)	>0.99[c]	5 (21.7%)	20 (17.7%)	0.77[c]
Median number of teeth (IQR)	25.5 (7.6)	25.0 (7.8)	0.78[a]	23.0 (10.0)	25.0 (7.5)	0.12[a]
Denture use (n=54)	18 (40.9%)	36 (39.1%)	0.85[c]	12 (52.2%)	42 (37.2%)	0.24[c]

IQR, interquartile range; *C.*, *Capnocytophaga*. [a]Mann-Whitney U; [b]χ^2; [c]Fisher's exact test.

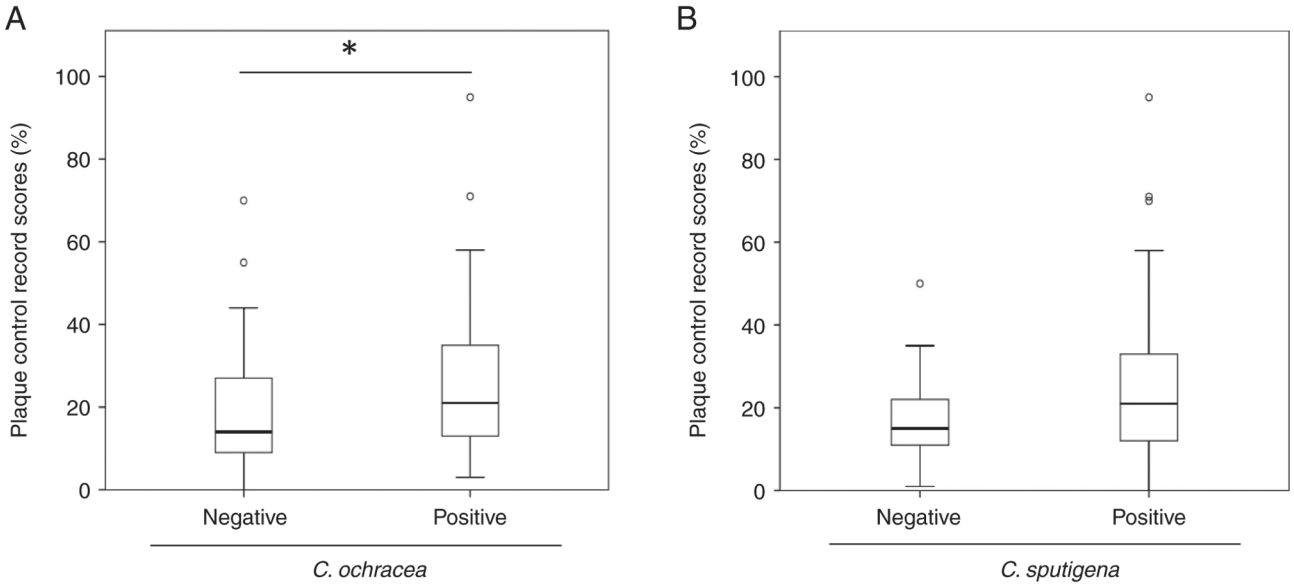

Figure 1. Plaque control record score in *Capnocytophaga* species-positive and -negative participants. (A) *C. ochracea* (B) *C. sputigena*. *C.*, *Capnocytophaga*. *P<0.05.

significant difference in plaque control record scores was found between *C. ochracea*-positive and -negative participants. *C. sputigena*-positive participants recorded higher plaque control record scores (21.0%) than *C. sputigena*-negative participants (15.0%; Fig. 1B).

The associations between *Capnocytophaga species* and periodontal condition are summarized in Table II. *C. ochracea*-positive participants recorded a higher rate of ≥4 mm periodontal pockets with BOP *than C. ochracea*-negative participants, but this difference was not significant. There was no significant association between the presence of *C. ochracea* and ≥6 mm periodontal pockets with BOP. *C. ochracea*-positive participants recorded higher PISA scores than *C. ochracea*-negative participants, but this difference was not significant. *C. sputigena*-positive participants recorded a higher rate of ≥4 mm periodontal pockets with BOP than

Table II. Association between *Capnocytophaga* species and periodontal condition.

Periodontal condition	*C. ochracea*			*C. sputigena*		
	Negative (n=44)	Positive (n=92)	P-value	Negative (n=23)	Positive (n=113)	P-value
≥4 mm periodontal pocket with BOP (n=65)	17 (38.6%)	48 (52.2%)	0.15[a]	7 (30.4%)	58 (51.3%)	0.11[a]
≥6 mm periodontal pocket with BOP (n=29)	9 (20.5%)	20 (21.7%)	>0.99[a]	3 (13%)	26 (23%)	0.41[a]
Median PISA, mm^2 (IQR)	38.9 (74.8)	71.2 (150.9)	0.06[b]	31.7 (126.2)	60.0 (137.6)	0.13[b]
Median PESA, mm^2 (IQR)	1,005.2 (478.0)	1,036.9 (397.0)	0.14[b]	913.9 (478.2)	1,038.0 (430.6)	0.15[b]

IQR, interquartile range; PISA, periodontal inflamed surface area; BOP, bleeding on probing; PESA, periodontal epithelial surface area; *C., Capnocytophaga*. [a]Fisher's exact; [b]Mann-Whitney U test.

Table III. Association between the prevalence of *C. ochracea*/*C. sputigena* and clinical characteristics.

Characteristic	*C. ochracea*/*C. sputigena*		
	Negative (n=53)	Positive (n=83)	P-value
Median age, years (IQR)	74.0 (14.0)	72.0 (14.0)	0.25[a]
Age, years (%)			
30-39 (n=2)	1 (1.9)	1 (1.2)	0.32[b]
40-49 (n=7)	1 (1.9)	6 (7.2)	
50-59 (n=12)	2 (3.8)	10 (12.0)	
60-69 (n=33)	13 (24.5)	20 (24.1)	
70-79 (n=50)	22 (41.5)	28 (33.7)	
80-89 (n=30)	14 (26.4)	16 (19.3)	
90-99 (n=2)	0 (0.0)	2 (2.4)	
Sex (%)			
Male (n=43)	12 (22.6)	31 (37.3)	0.09[c]
Female (n=93)	41 (77.4)	52 (62.7)	
Hypertension (n=30)	12 (22.6%)	18 (21.7%)	>0.99[c]
Diabetes (n=19)	9 (17.0%)	10 (12.0%)	0.45[c]
Dyslipidemia (n=25)	10 (18.9%)	15 (18.1%)	>0.99[c]
Median number of teeth (IQR)	25.0 (8.5)	25.0 (8.0)	0.55[a]
Denture use (n=54)	24 (45.3%)	30 (36.1%)	0.37[c]

IQR, interquartile range; *C., Capnocytophaga*. [a]Mann-Whitney U; [b]χ^2; [c]Fisher's exact test.

C. sputigena-negative participants, but this difference was not significant. There was no significant association between presence of *C. sputigena* and ≥6 mm periodontal pockets with BOP. *C. sputigena*-positive participants recorded higher PISA scores than *C. sputigena*-negative participants, but this difference was not significant.

Association of dual Capnocytophaga species infection with oral hygiene and periodontal condition. The associations between the presence of dual *Capnocytophaga species* and clinical characteristics are summarized in Table III. No significant association was found between the prevalence of *C. ochracea*/*C. sputigena* and clinical factors. *C. ochracea*/*C. sputigena* dual-positive participants exhibited significantly higher plaque control record scores (21%) than non-dual-positive participants (14%; Fig. 2). Additionally, *C. ochracea*/*C. sputigena* dual-positive participants exhibited significantly higher PISA scores than non-dual-positive participants (Table IV).

Association between prevalence of dual Capnocytophaga species and oral health status after propensity score matching. Propensity score matching was applied to the *C. ochracea*/*C. sputigena* dual-positive and the non-dual-positive group, resulting in 51 matched participant pairs (Table SI).

Table IV. Association between prevalence of *C. ochracea*/*C. sputigena* and periodontal condition.

	C. ochracea/*C. sputigena*		
Periodontal condition	Negative (n=53)	Positive (n=83)	P-value
≥4 mm periodontal pocket with BOP (n=65)	20 (37.7%)	45 (54.2%)	0.08[a]
≥6 mm periodontal pocket with BOP (n=29)	9 (17.0%)	20 (24.1%)	0.39[a]
Median PISA, mm^2 (IQR)	46.2 (79.9)	82.3 (151.1)	0.04[b]
Median PESA, mm^2 (IQR)	1,006.6 (483.1)	1,038.0 (394.2)	0.09[b]

IQR, interquartile range; PISA, periodontal inflamed surface area; BOP, bleeding on probing; PESA, periodontal epithelial surface area; *C.*, *Capnocytophaga*. [a]Fisher's exact test. [b]Mann-Whitney U test.

Table V. Association between the prevalence of *C. ochracea*/*C. sputigena* and periodontal condition after propensity score matching.

	C. ochracea/*C. sputigena*		
Periodontal condition	Negative (n=51)	Positive (n=51)	P-value
≥4 mm periodontal pocket with BOP (n=50)	20 (39.2%)	30 (58.8%)	0.07[a]
≥6 mm periodontal pocket with BOP (n=21)	9 (17.6%)	12 (23.5%)	0.63[a]
Median PISA, mm^2 (IQR)	46.2 (76.1)	82.9 (155.0)	0.12[b]
Median PESA, mm^2 (IQR)	1,025.3 (478.2)	1,004.5 (514.1)	0.16[b]

[a]Fisher's exact; [b]Wilcoxon signed-rank test. IQR, interquartile range; PISA, periodontal inflamed surface area; BOP, bleeding on probing; PESA, periodontal epithelial surface area; *C.*, *Capnocytophaga*.

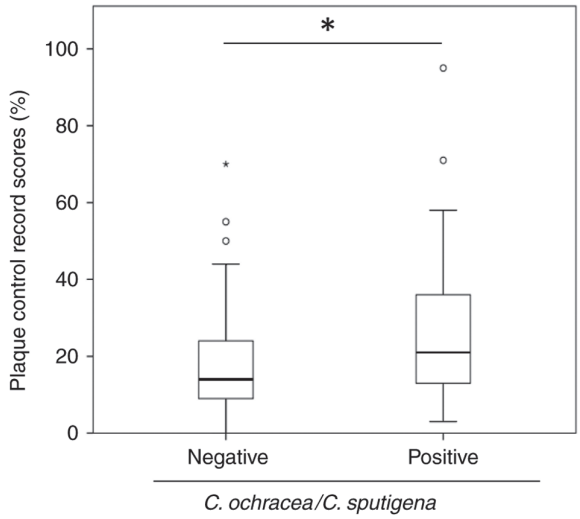

Figure 2. Plaque control record score in *C. ochracea*/*C. sputigena* dual-positive or -negative participants. *C.*, *Capnocytophaga*. *P<0.05.

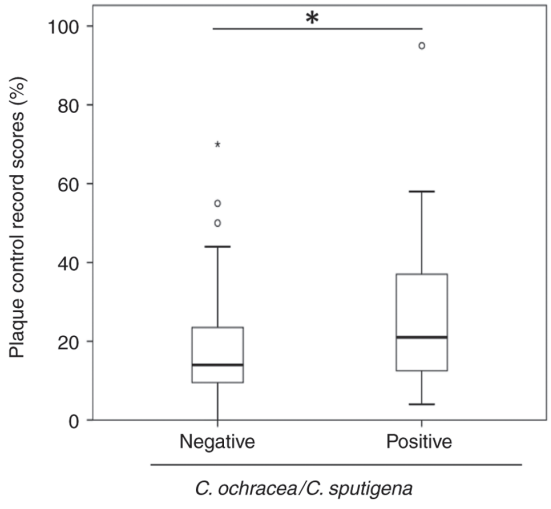

Figure 3. Plaque control record score in *C. ochracea*/*C. sputigena* dual-positive or -negative participants after propensity score matching. *C.*, *Capnocytophaga*. *P<0.05.

There was no significant difference in PISA or PESA scores between the *C. ochracea*/*C. sputigena* dual-positive group and the non-dual-positive group (Table V). *C. ochracea*/*C. sputigena* dual-positive participants exhibited significantly higher plaque control record scores (21%) than non-dual-positive participants (14%; Fig. 3). The statistical power was 93% based on 51 matched participants, suggesting that the statistical power was sufficient.

Discussion

The oral hygiene of *Capnocytophaga* species-positive participants was poor compared with that of *Capnocytophaga* species-negative participants in the present study. Another study reported an association between the abundance of *Capnocytophaga* species and dental plaque accumulation

and halitosis (18,19). The concentration of volatile sulfur compounds in the oral cavity is associated with the abundance of *C. gingivalis* in patients with periodontitis who receive periodontal therapy (20). *C. ochracea*/*C. sputigena* were associated with dental plaque accumulation in middle-aged and older people before and after propensity score matching in the present study. *C. ochracea* plays a key role in the initial phase of dental plaque formation and may be involved in co-aggregation with other bacteria to establish mature dental biofilm (21). These findings indicated that *Capnocytophaga* species infection may be associated with poor oral hygiene. By contrast, the amount of dental plaque accumulation and calculus is not associated with the prevalence of oral *Capnocytophaga* in patients aged 7-15 years (22). The prevalence rate of oral *Capnocytophaga* species is 46% in children (aged 2-6 years) with healthy gingiva, indicating that oral *Capnocytophaga* infection may be established in young children and is commonly detected in children without gingivitis (9). Further research targeting both young and older people is required to clarify the association between the prevalence of oral *Capnocytophaga* and oral hygiene status.

The relative abundance of oral *Capnocytophaga* species is greater in periodontally healthy individuals than in individuals with periodontitis or gingivitis (23). Additionally, the abundance of *Capnocytophaga* species is lower in patients with severe periodontitis than in those with non-severe periodontitis (23). The prevalence of oral *Capnocytophaga* species is 87% in healthy individuals and 73% in patients with periodontitis (24). Therefore, patients with periodontitis may be less likely to have oral *Capnocytophaga* infection than periodontally healthy individuals. Here, *Capnocytophaga* species were found in 96% of participants receiving supportive periodontal therapy (professional tooth cleaning, scaling and toothbrushing instruction). However, the present study did not compare oral *Capnocytophaga* prevalence between patients with and without periodontitis.

No association was found between dual infection with *C. ochracea*/*C. sputigena* and PISA scores in middle-aged and older people following propensity score matching, suggesting that *C. ochracea*/*C. sputigena* was not associated with active periodontitis. *Capnocytophaga* species are periodontal pathogens that may be associated with stable periodontitis with mild periodontal inflammation, by contrast with *P. gingivalis*, *T. forsythia* and *T. denticola,* which are major periodontal pathogens (6). To the best of our knowledge, there is no standard for employing the PISA value to assess the severity of periodontitis. Leira *et al* (25) equated active periodontitis with a PISA score >130. The median PISA score of participants in the present study was 55.2 and the maximum score was 958. These findings suggested that many participants in this study had mild periodontitis. Therefore, it was hypothesized that patients with mild periodontitis are more likely to have oral *Capnocytophaga* infection than those with moderate or severe periodontitis. Additionally, no significant association was found between the presence of *Capnocytophaga* species and ≥6 mm deep periodontal pockets with BOP (an indicator of severe periodontitis). The prevalence of oral *Capnocytophaga* species may not be associated with severity of periodontitis. Among *Capnocytophaga* species, *C. gingivalis* is associated with periodontal inflammation (26). However, the association between oral *C. gingivalis* and severity of periodontitis remains unknown. In our previous study, dual infection with *T. forsythia* and *T. denticola* was associated with severity of periodontal inflammation in older Japanese people (8). Therefore, it is hypothesized that *T. forsythia* and *T. denticola* are more importantly associated with the progression of periodontitis than *Capnocytophaga* species. To the best of our knowledge however, no previous studies have investigated the association between *Capnocytophaga* species and *T. forsythia* /*T. denticola*.

Oral microbiome diversity is associated with the development and progression of periodontitis (27,28). It is hypothesized that oral microorganisms are cooperatively and synergistically involved in periodontitis, rather than single bacteria alone (27,28). *Fusobacterium nucleatum* coaggregates with *C. ochracea; F. nucleatum*-induced dental plaque biofilm formation is enhanced by the soluble component of *C. ochracea* (29). Such interactions between *Capnocytophaga* species and other periodontal disease-associated bacteria may contribute to induction of periodontal inflammation. Therefore, it is essential to maintain good oral health with daily toothbrushing, together with regular professional dental care, including dental scaling.

Cleaning the tongue surface as well as brushing the teeth is key for reducing the growth of oral bacteria. The application of topical tetracycline ointment on the tongue surface has been shown to decrease bacterial presence (30), suggesting that topical antibiotic administration is effective in inhibiting bacterial growth on the tongue surface. Notably, previous studies have investigated the effectiveness of adjunct periodontal therapy using ozonized gels, photobiomodulation and probiotics in patients with periodontitis (31-33). However, the effects of such therapies on the bacterial population on the tongue dorsum remain unknown. Additional research is required to elucidate the effects of antibiotic application and adjunct periodontal therapies on microbiota of the tongue surface.

In addition to periodontopathic bacteria, periodontal herpes virus also serves a major role in the pathology of periodontal disease (34). The cytopathic impact of the herpes virus on immune cells may lead to host immune dysfunction (34). Additionally, herpes virus infection in host cells may induce production of inflammatory cytokines and chemokines via activation of signaling pathways such as p38 MAP kinase, JNK and NF-κB (35). Virus-enhanced local inflammatory response may be involved in the progression of periodontitis. Among herpes viruses, the Epstein-Barr virus (EBV) is associated with inflammation (36). Our previous study reported that oral EBV has a prevalence rate of 20% and is associated with PISA and plaque control record scores (37). Additionally, oral human herpesvirus-7 is commonly found in individuals with deep periodontal pockets (38). However, the association between *Capnocytophaga species* and periodontal herpes virus remains unclear.

There were several limitations in the present study. First, the association between the prevalence of single *Capnocytophaga* species and oral health status after adjusting for the effect of clinical confounding factors was not investigated. Second, the prevalence of oral *Capnocytophaga* species positivity in young people without periodontitis remains unclear. Third, the association between *Capnocytophaga* species other than *C. ochracea* and *C. sputigena* and periodontal inflammation remains unclear. Fourth, the presence of *Capnocytophaga*

species in inflammatory periodontal pockets was not elucidated. It is important to demonstrate the presence of *Capnocytophaga* species in the gingival sulcus to clarify the association between *Capnocytophaga* species and periodontal inflammation. Fifth, a causal association between the prevalence of oral *Capnocytophaga* species and dental caries or periodontitis remains uncertain because the present study was a cross-sectional study. Finally, the generalizability of the results is limited because the present study was performed at a single hospital in Japan.

In conclusion, oral *Capnocytophaga* species were prevalent in middle-aged and older Japanese people with periodontitis. Additionally, dual oral *Capnocytophaga* species infection (*C. ochracea*/*C. sputigena)* may be associated with poor oral hygiene and mild periodontal inflammation.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

HS conceived the study, performed experiments, analyzed data and wrote and reviewed the manuscript. NH, YK and YN performed experiments. TT analyzed and interpreted data and supervised the study. KO analyzed and interpreted data and reviewed the manuscript. HS and KO confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The Ethical Committee of Hiroshima University approved the study (approval no. E-1115), and all participants signed informed consent agreement prior to participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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