jdhs
Journal of Dental Hygiene Science

J Dent Hyg Sci Vol. 24, No. 2, 2024, pp.97-106
https://doi.org/10.17135/jdhs.2024.24.2.97

RESEARCH ARTICLE

Multiplex Real-Time Polymerase Chain Reaction Analysis of Pathogens in Peri-Implantitis and Periodontitis: A Randomized Trial

Eun-Deok Jo†

Department of Dental Hygiene, College of Health Science, Gachon University, Incheon 21936, Korea

Background: Periodontitis and peri-implantitis are diseases caused by pathogenic microorganisms that cause tissue damage and alveolar bone destruction resulting in the loss of teeth and implants. Due to the biological differences in the tissues surrounding the implants, peri-implantitis progresses more rapidly and intensely than periodontitis, underscoring the importance of understanding the characteristics and interactions of pathogenic bacteria. This study aimed to quantitatively analyze the pathogenic microorganisms associated with periodontitis and peri-implantitis in Korean patients and evaluate the correlation between these bacteria.

Methods: A total of 98 (52 males and 46 females) were randomly selected and classified into three groups (healthy group [HG]=25; periodontitis group [PG]=31; and peri-implantitis group [PIG]=42). The relative expression levels of 11 pathogenic microorganisms collected from the gingival sulcus fluid were determined using multiplex real-time polymerase chain reaction.

Results: *Eikenella corrodens, Fusobacterium nucleatum*, and *Prevotella nigrescens* were highly prevalent in the HG, PG, and PIG patients. The results of the relative quantitative analysis of microorganisms showed that all bacteria belonging to the green, orange, and red complexes were significantly more abundant in the PG and PIG than in the HG ($p<0.05$). *Porphyromonas gingivalis* in the red complex showed a positive correlation with all microorganisms in the orange complex ($p<0.05$). *Campylobacter rectus* in the orange complex showed a significant positive correlation with all microorganisms in the red complex, and with *F. nucleatum, P. nigrescens, Prevotella intermedia*, and *Eubacterium nodatum* ($p<0.05$).

Conclusion: *P. gingivalis, C. rectus*, and *F. nucleatum* exhibit strong interactions. Removing these bacteria can block complex formation and enhance the prevention and treatment of periodontitis and peri-implantitis.

Key Words: Pathogenic microorganisms, Peri-implantitis, Periodontitis, Real-time polymerase chain reaction

Introduction

1. Background

Periodontitis and peri-implantitis are diseases in which teeth and implants are lost due to tissue damage and alveolar bone destruction caused by inflammation[1]. Pathogenic microorganisms are the leading causes[2,3], forming bacterial films on both the root and implant surfaces[4]. Moreover, the crevices of all the surfaces of healthy or inflamed implants contain a greater variety of pathogenic microorganisms compared to the subgingival biofilms of natural teeth[5]. Peri-implantitis progresses faster and more intensely than general periodontitis because implants have different biological structures and less vascular distribution in the surrounding tissues[1]. Because peri-implantitis, such as periodontitis, begins with pathogenic microorganisms, attention should be paid to the characteristics and interrelationships of bacteria growing in colonies[6,7].

The pathogenic microorganisms commonly found in periodontitis and peri-implantitis include *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Streptococcus intermedius*, *Fusobacterium nucleatum*, *Eikenella corr-*

Received: June 4, 2024, **Revised:** June 11, 2024, **Accepted:** June 17, 2024

eISSN 2233-7679

†Correspondence to: Eun-Deok Jo, *https://orcid.org/0000-0002-6098-684X*
Department of Dental Hygiene, College of Health Science, Gachon University, 191 Hambangmoe-ro, Yeonsu-gu, Incheon 21936, Korea
Tel: +82-32-820-4370, Fax: +82-32-820-4370, E-mail: jeundeok@gmail.com

odens, and *Porphyromonas gingivalis*[8-11]. Both diseases are associated with specific bacterial species or complexes[12]. Socransky et al.[13] identified the relationship between species, location, and environmental variables based on staining response, colony type, clinical parameters, and pigments. Based on this, the change patterns were predicted and classified into five types of complexes. Pathogenic bacteria that significantly affect periodontal inflammation are classified into red and orange complexes[13]. The red complex, which is related to pocket depth and bleeding, is mainly found in the late stages of periodontitis, whereas the orange complex is found in the middle stages. Bacteria in these two complexes promote interactions between strains, which may affect the occurrence and progression of periodontal inflammation[14]. Bacteria formed in the early stages of periodontitis are classified as green complexes, while those found in healthy periodontal tissue are classified as yellow and purple complexes[13]. *A. actinomycetemcomitans* is the causative agent of rapid periodontal disease and is aggressive in localized areas. This is not classified as a cluster because the strain's "serum b" is different, but is mainly dealt with[15].

It is essential to understand the characteristics, composition, and interactions of pathogenic microorganisms and develop strategies to prevent and treat diseases according to risk factors. Sculean et al.[16] suggested various treatment methods for patients with periodontitis and peri-implantitis through bacterial analysis, and confirmed that the treatment effect was improved by applying them. Therefore, analysis of pathogenic microorganisms is essential. Existing culture-based methods have limitations such as difficulties in sample collection, low sensitivity and specificity, and cross-reactivity problems[5]. In addition, polymerase chain reaction (PCR), which applies molecular biology technology, can confirm the appearance rate of microorganisms; however, quantitative analysis is not possible[17]. However, by using multiple real-time PCR, the causative bacteria of periodontal and implant infections can be detected and identified rapidly. Therefore, it has been widely used in dentistry because it can identify and quantify periodontal pathogens in various clinical samples such as subgingival plaque, saliva, and gingival crevicular fluid[7,18,19].

2. Objectives

This study aimed to compare the appearance rate and quantity of 11 types of periodontitis-related pathogenic microorganisms in periodontitis and peri-implantitis in Korean patients using multiplex real-time PCR, and to investigate the correlation between the detected bacteria. Thus, it is possible to predict the treatment effect through bacterial reduction in periodontal and implant health management, and to establish an appropriate treatment plan.

Materials and Methods

1. Participant selection

This clinical study was approved by the Gachon Bioethics Committee. The study adhered to the ethical principles outlined in the Helsinki Declaration and followed the CONSORT guidelines[20]. The study was was registered in the Clinical Research Information Service (CRIS No. KCT0008890). G-power (version 3.1.9.7) was used to determine the sample size for this study, with the effect size set to 0.5, significance level α to 0.05, and power to 0.95, and the calculated sample size was 80 people. Among the patients who visited the dental hospital, 130 who showed an interest in the study were selected. All participants agreed to participate in the study after receiving detailed information about the trial through oral and written study information sheets. In addition, 32 patients were excluded: patients with systemic diseases such as diabetes and hepatitis; patients with oral diseases excluding periodontitis; orthodontists; implant wearers used for overdentures; pregnant women; and antibiotic users. Finally, 98 patients (52 males, 46 females) participated in the study and signed a consent form.

2. Experimental procedure

Probing pocket depth (PPD) and bleeding on probing (BOP) tests were performed in all patients to evaluate the periodontal and peri-implant conditions. One dental hygienist examined four sites per tooth: #16, #11, #26, #31, #36, and #46 (mesial buccal, distal buccal, buccal, lingual, and palatal sides), using a WHO Probe (type C; DenMat, Lompoc., CA, USA). Patients were classified into healthy group (HG, n=25), periodontitis group (PG, n=31), and

peri-implantitis group (PIG, n=42). HG indicates no BOP and PPD not exceeding 3 mm. PG indicates presence of BOP in the periodontal area around natural teeth PPD greater than or equal to 4 mm. PIG indicates presence of BOP around the implant that has been placed for more than 12 months as well as PPD exceeding 4 mm[21].

Four samples were collected from the gingival crevicular fluid of each tooth and implant. A sterilized paper point (#35, 04 taper; DiaDent, Cheongju, Korea) was inserted into the deepest periodontal pocket and maintained for 30～40 seconds. The paper points that absorbed gingival sulcus fluid was transferred to a 50 mL conical tube (sterilized; SPL Life Sciences, Pocheon, Korea), stored at 4°C or colder, and then submitted to the OO Bio Corporation for pathogen sampling and analysis.

3. Pathogen sampling and analysis

A total of 11 significantly pathogenic microorganisms[13] were selected and quantitatively analyzed. As shown in Table 1, the pathogenic microorganisms were classified into clusters based on colony morphology, staining reactions, pigment production, and mutual correlation[13], and the causative bacteria leading to acute periodontal disease[15] were included. The genomic DNA (gDNA) of the pathogenic microorganisms was extracted using a column-type Exgene Cell SV DNA Isolation Kit (Exgene Cell SV; GeneAll Biotech, Seoul, Korea) according to the manufacturer's instructionsDue to potential interference between forward and reverse primers designed for specific microbial DNA sequences, we grouped three to four strains into three separate panels (Table 1). These primers were used to analyze gDNA and clone standards isolated from the samples. Subsequently, the prepared forward and reverse primers and probes were mixed, and the analysis was conducted using a CFX96-IVD real-time PCR detection system (Bio-Rad Inc., Hercules, CA, USA). The input values before the analysis were the gDNA of standard strains sold by the Korean Collection for Type Cultures (KCTC, Korea), Korean Culture Center of Microorganisms (KCCM, Korea), and Korean Collection for Oral Microbiology (KCOM, Korea). The conditions were set once at 95°C for 5 minutes, and 41 times at 95°C for 30 seconds, 60°C for 40 seconds, and 72°C for 30 seconds. The strain was quantitatively analyzed based on the total gDNA amount of each sample. The analysis results were converted into an index to easily compare the differences by simply expressing the numerical value calculated as the average value obtained twice per sample.

4. Statistical analysis

Data were statistically analyzed using SPSS for Windows (version 29.00; IBM Corp., Armonk, NY, USA). First, the characteristics of the patients and sampling sites were analyzed using one-way ANOVA and Bonferroni post-hoc tests. The prevalence and number of pathogens in each group were evaluated using Kruskal-Wallis and

Table 1. Used Strains in This Study

	Scientific name	Target strain	Popular
A pannel	*Aggregatibacter actinomycetemcomitans*	KCCM 12227	ATCC 29522
	Porphyromonas gingivalis	KCTC 5332	ATCC 33277
	Tannernella forsythia	KCTC 5666	ATCC 43037
	Treponema denticola	KCTC 15104	ATCC 35405
B pannel	*Fusobacterium nucleatum*	KCTC 2640	ATCC 25586
	Prevotella nigrescens	KCTC 15081	ATCC 33563
	Prevotella intermedia	KCTC 9692	ATCC 25611
	Total bacteria 16s rDNA		
C pannel	*Eubacterium nodatum*	KCTC 15015	ATCC 33099
	Parvimonas micra	KCOM 1535	ATCC 33270
	Campylobacter rectus	KCTC 5636	ATCC 33238
	Eikenella corrodens	KCTC 15198	ATCC 23834

KCCM: Korean Culture Center of Microorganisms, ATCC: American Type Culture Collection, KCTC: Korean Collection for Type Cultures, KCOM: Korean Collection for Oral Microbiology.

Bonferroni post-hoc tests. Correlations between the 11 pathogenic microorganisms were analyzed using Pearson's correlation coefficient. The statistical significance was set at $p < 0.05$.

Results

1. Characteristics of patients and sampling sites

Table 2 presents the mean age and sex of patients in each group. HG was 39.72 years (males: 28.0%; females: 72.0%), PG was 54.81 years (males: 61.3%; females: 38.7%); and PIG, 62.31 years (males: 61.9%; females: 38.1%). Table 2 summarizes the characteristics of the sampling sites. The PPD and BOP of PG (6.09 mm, 27.58%, $p < 0.001$) and PIG (6.05 mm, 31.36%, $p < 0.001$) showed a significant difference from the HG (3.19 mm, 2.96%), but there was no difference between PG (6.09 mm, 27.58%) and PIG (6.05 mm, 31.36%) (Table 2).

2. The relative expression rate of bacterial pathogens in three groups by real-time PCR

P. gingivalis, *Tannernella forsythia*, and *Treponema denticola* in the red complex appeared in the order of PIG (90.5%, 59.5%, and 61.9%, respectively), PG (87.1%, 35.5%, and 48.4%, respectively), and HG (20.0%, 16.0%, and 16.0%, respectively) (p=0.001). *P. intermedia* and *Parvimonas micra* in the orange complex appeared at similarly high levels in PIG (76.2% and 97.6%, respectively) and PG (71.0% and 100%, respectively) and at low levels in HG (8.0% and 76.0%, respectively) (p=0.001). *Eubac-*

Table 2. Characteristics of Patients and Sampling Sites

	HG (n=25)	PG (n=31)	PIG (n=42)	p-value
Age (y)	39.72±10.32	54.81±10.85	62.31±8.06	
Males:females (%)	07 (28.0):18 (72.0)	19 (61.3):12 (38.7)	26 (61.9):16 (38.1)	
PPD (mm)	3.19±0.41	6.09±1.44	6.05±1.16	
p-value		<0.001[a]	<0.001[b]	0.893[c]
BOP (%)	2.96±3.87	27.58±10.87	31.36±11.84	
p-value		<0.001[a]	<0.001[b]	0.351[c]

Values are presented as mean±standard deviation or ratio.
HG: healthy group, PG: periodontitis group, PIG: peri-implantitis group, PPD: probing pocket depth, BOP: bleeding of probing.
[a]Significant difference between the HG & PG.
[b]Significant difference between the HG & PIG.
[c]Significant difference between the PG & PIG.
The p-values are the result of one-way ANOVA and the Bonferroni post-hoc analysis.

Table 3. The Relative Expression Rate of Bacterial Pathogens in Three Groups by Real-time PCR

		HG (n=25)	PG (n=31)	PIG (n=42)	p-value
Red	*Porphyromonas gingivalis*	5 (20.0)	27 (87.1)	38 (90.5)	<0.001
	Tannernella forsythia	4 (16.0)	11 (35.5)	25 (59.5)	0.001
	Treponema denticola	4 (16.0)	15 (48.4)	26 (61.9)	0.001
Orange	*Fusobacterium nucleatum*	23 (92.0)	30 (96.8)	42 (100)	0.184
	Prevotella nigrescens	18 (72.0)	28 (90.3)	37 (88.1)	0.120
	Prevotella intermedia	2 (8.0)	22 (71.0)	32 (76.2)	<0.001
	Parvimonas micra	19 (76.0)	31 (100)	41 (97.6)	0.001
	Eubacterium nodatum	2 (8.0)	20 (64.5)	29 (69.0)	<0.001
	Campylobacter rectus	7 (28.0)	24 (77.4)	36 (85.7)	<0.001
Green	*Eikenella corrodens*	25 (100)	30 (96.8)	41 (97.6)	0.683
	Aggregatibacter actinomycetemcomitans	0 (0.0)	1 (3.2)	4 (9.5)	0.195

Values are presented as n (%).
HG: healthy group, PG: periodontitis group, PIG: peri-implantitis group.
The p-value is the result of the chi-square test.

terium nodatum and *Campylobacter rectus* appeared in the following order: PIG (69.0% and 85.7%, respectively), PG (64.5% and 77.4%, respectively), and HG (8.0% and 28.0%, respectively) ($p<0.001$) (Table 3).

3. Amount of bacterial pathogens in three groups by real-time PCR

All pathogens in the red and orange complexes showed differences in the abundance between HG and PG ($p<0.05$), and HG and PIG ($p<0.01$). The number of microorganisms in the PG and PIG was higher than that in the HG. However, there was no significant difference between PG and PIG. Similarly, *E. corrodens* in the green complex showed significant differences between HG and PG ($p<0.05$), and HG and PIG ($p<0.05$). *A. actinomycetemcomitans* did not differ between the groups (Table 4).

4. Pearson's correlation between bacteria in sample sites

Among the red complexes, *P. gingivalis* and *T. forsythia* showed significant positive correlations ($r=0.344$, $p<0.001$). *P. gingivalis* in the red complex correlated with *F. nucleatum* ($r=0.278$, $p<0.01$), *Prevotella nigrescens* ($r=0.234$, $p<0.05$), *P. intermedia* ($r=0.290$, $p<0.01$), *P. micra* ($r=0.221$, $p<0.05$), *E. nodatum* ($r=0.448$, $p<0.001$) and *C. rectus* ($r=0.338$, $p<0.001$) in the orange complex. In addition, *T. forsythia* correlated with *F. nucleatum* ($r=0.261$, $p<0.01$), *P. nigrescens* ($r=0.216$, $p<0.05$), *E. nodatum* ($r=0.481$, $p<0.001$), and *C. rectus* ($r=0.290$, $p<0.01$). Among the orange complexes, *P. nigrescens* ($r=0.253$, $p<0.05$), *P. intermedia* ($r=0.409$, $p<0.001$), *P. micra* ($r=0.610$, $p<0.001$), *E. nodatum* ($r=0.435$, $p<0.001$), and *C. rectus* ($r=0.260$, $p<0.01$), and *P. nigrescens* correlated with *P. micra* ($r=0.259$, $p<0.01$) and *C. rectus* ($r=0.323$, $p=0.001$). *P. intermedia* correlated with *P. micra* ($r=0.395$, $p<0.001$), *E. nodatum* ($r=0.381$, $p<0.001$), and *C. rectus* ($r=0.376$, $p<0.001$). *P. micra* showed a significant positive correlation with *E. nodatum* ($r=0.353$, $p<0.001$), and *E. nodatum* with *C. rectus* ($r=0.235$, $p<0.05$). No correlation was observed between the green complexes and *A. actinomycetemcomitans* (Table 5).

Discussion

1. Interpretation

Both periodontitis and peri-implantitis are caused by interactions between bacteria in the oral cavity and have bacteriological similarities. However, as suggested in a systematic review by Rajasekar and Varghese[22], studies

Table 4. Amount of Bacterial Pathogens in Three Groups by Real-Time PCR

		HG (n=25)	PG (n=31)	p (HG-PG)	PIG (n=42)	p (HG-PIG)	p (PG-PIG)	p
Red	*Porphyromonas gingivalis*	0.42±2.08	18.24±28.95	<0.001	30.92±33.60	<0.001	0.389	<0.001
	Tannernella forsythia	0.21±0.87	7.47±16.55	0.021	8.33±12.94	0.001	0.241	<0.001
	Treponema denticola	0.00±0.01	0.37±0.62	0.018	2.29±8.32	<0.001	0.539	<0.001
Orange	*Fusobacterium nucleatum*	50.31±68.35	163.08±120.71	<0.001	135.82±95.74	<0.001	>0.999	<0.001
	Prevotella nigrescens	4.01±9.60	13.28±17.71	0.007	15.13±21.87	0.002	>0.999	0.001
	Prevotella intermedia	0.59±2.13	11.64±22.99	<0.001	18.18±28.11	<0.001	0.922	<0.001
	Parvimonas micra	0.27±0.76	3.45±4.00	<0.001	5.48±5.87	<0.001	0.745	<0.001
	Eubacterium nodatum	0.13±0.45	4.45±8.67	<0.001	5.12±7.23	<0.001	>0.999	<0.001
	Campylobacter rectus	0.21±0.68	3.22±4.34	<0.001	2.01±2.41	<0.001	>0.999	<0.001
Green	*Eikenella corrodens*	3.56±3.89	9.97±11.51	0.030	5.45±8.26	0.032	>0.999	0.013
	Aggregatibacter actinomycetemcomitans	0.00±0.00	0.00±0.01	0.256	0.02±0.11	0.061	0.639	0.189
Total		188.01±180.93	460.72±291.58	<0.001	446.06±200.62	<0.001	>0.999	<0.001

Values are presented as mean±standard deviation.
All bacterial counts are expressed in units of 10^6 cells/mL.
HG: healthy group, PG: periodontitis group, PIG: peri-implantitis group.
The p-values result from Kruskal-Wallis test and the Bonferroni post-hoc test by group.

Table 5. Pearson's Correlation between Bacteria in Sample Sites

		Red			Orange						Green	
		P. g	T. f	T. d	F. n	P. n	P. i	P. m	E. n	C. r	E. c	A. a
Red	P. g	1										
	T. f	0.344 (<0.001)	1									
	T. d	0.131 (0.197)	0.017 (0.871)	1								
Orange	F. n	0.278 (0.005)	0.261 (0.009)	0.013 (0.898)	1							
	P. n	0.234 (0.021)	0.216 (0.032)	0.115 (0.261)	0.253 (0.012)	1						
	P. i	0.290 (0.003)	0.088 (0.391)	0.039 (0.706)	0.409 (<0.001)	0.104 (0.308)	1					
	P. m	0.221 (0.028)	0.130 (0.202)	0.035 (0.735)	0.610 (<0.001)	0.259 (0.009)	0.395 (<0.001)	1				
	E. n	0.448 (<0.001)	0.481 (<0.001)	0.068 (0.504)	0.435 (<0.001)	0.088 (0.389)	0.381 (<0.001)	0.353 (<0.001)	1			
	C. r	0.338 (<0.001)	0.290 (0.003)	0.223 (0.027)	0.260 (0.009)	0.323 (0.001)	0.376 (<0.001)	−0.001 (0.992)	0.235 (0.019)	1		
Green	E. c	0.067 (0.509)	−0.047 (0.645)	0.012 (0.908)	0.088 (0.391)	0.186 (0.067)	−0.037 (0.719)	0.082 (0.423)	−0.012 (0.904)	0.034 (0.741)	1	
	A. a	−0.008 (0.941)	0.029 (0.779)	−0.022 (0.831)	0.054 (0.595)	−0.047 (0.643)	−0.059 (0.563)	0.035 (0.731)	0.093 (0.363)	0.073 (0.477)	−0.089 (0.384)	1

HG: healthy group, PG: periodontitis group, PIG: peri-implantitis group.
P.g: *Porphyromonas gingivalis*, T.f: *Tannernella forsythia*, T.d: *Treponema denticola*, F.n: *Fusobacterium nucleatum*, P.n: *Prevotella nigrescens*, P.i: *Prevotella intermedia*, P.m: *Parvimonas micra*, E.n: *Eubacterium nodatum*, C.r: *Campylobacterrectus*, E.c: *Eikenella corrodens*, A.a: *Aggregatibacter actinomycetemcomitans*.
The p-value is the result of Pearson's correlation.

on the differences in pathogenic bacteria present in periodontitis and peri-implantitis compared to those in healthy periodontal conditions are insufficient. Therefore, in this study, patients with healthy periodontal conditions, periodontitis, and peri-implantitis were classified into three groups. The appearance rate and quantity of 11 microorganisms highly related to periodontitis were identified, and the correlation between each bacterium was analyzed.

2. Key results and comparison

As a result of the analysis of the 11 microorganisms, *E. corrodens* of the green complex and *F. nucleatum* and *P. nigrescens* of the orange complex showed high appearance rates in all groups of Korean patients, without any difference between groups. Previous studies have identified *F. nucleatum*[23,24] and *P. nigrescens*[25,26] as microorganisms commonly found in healthy oral cavities. In addition, *A. actinomycetemcomitans*[27], mainly observed because of its strong effect on local periodontitis, is rarely detected in Korean patients. The same results were confirmed in this study.

Microorganisms belonging to orange and red complexes are characterized by virulence factors that promote or sustain inflammatory processes[28,29]. In particular, the highly pathogenic red complex is closely associated with the clinical features of periodontal diseases, especially PPD and BOP. Orange complexes are important because they may attach and bind to various oral bacteria and connect symbiotic colonies of periodontal pathogens[13]. In this study, all microorganisms from the red complex as well as *P. intermedia*, *P. micra*, *E. nodatum*, and *C. rectus* from the orange complex showed a significantly higher incidence of periodontitis and PIG than in the HG ($p \leq 0.001$). Therefore, the pathogenic microorganisms most likely to cause periodontitis and peri-implantitis belong to the red

complex, specifically *P. gingivalis*, *T. forsythia*, and *T. denticola*; some microorganisms of the orange complex were also detected at high frequencies. However, regarding the differences in microbial composition between periodontitis and peri-implantitis, Koyanagi et al.[7] found that peri-implantitis has more diverse microbial composition than periodontitis. Salvi et al.[1], Canullo et al.[30], and Wang et al.[31] reported that the red complex microbial composition showed similar microbial profiles with no differences between periodontitis and peri-implantitis. In addition, Tamura et al.[32] reported that the microbial composition in peri-implantitis differs from that in healthy periodontal conditions. In this study, there was no difference in the microbial composition between the PG and PIG, and the only difference was between the HG and the other groups. Because these results vary depending on the patients, methods, and microbial analysis techniques, follow-up studies reflecting the development of analytical techniques should be conducted.

Relative quantitative analysis of microorganisms showed that the numbers of all microorganisms belonging to the green complex ($p < 0.05$), orange complex ($p < 0.001$), and red complex ($p < 0.001$) were significantly higher in the PG and PIG than in the HG. Ebadian et al.[33] reported that *P. gingivalis* was quantitatively high in both periodontitis and peri-implantitis patients, whereas *T. forsythia*, *P. intermedia*, and *C. rectus* were quantitatively high in patients with periodontitis. In addition, Dabdoub et al.[34] reported that the amount of *T. denticola* in the red complex was higher in periodontitis than in peri-implantitis. However, except for *A. actinomycetemcomitans* in this study, all periodontitis-causing bacteria belonging to the green, orange, and red complexes were abundant in the PG and PIG. Therefore, the microorganisms distributed in periodontitis and peri-implantitis are expected to affect the growth and proliferation of each other.

Gambin et al.[14] reported that the microorganisms in red and orange complexes affected each other. In this study, the correlation between *P. gingivalis* and *T. forsythia* in the red complex was strong, and showed a very high correlation with *E. nodatum* in the orange complex. In particular, *P. gingivalis* was positively correlated with all microorganisms in the orange complex. Based on this, it can be predicted that *P. gingivalis* links the red and orange complexes together. *P. gingivalis* may affect each other's growth while interacting with the microorganisms in the orange complex. Similarly, *C. rectus* in the orange complex significantly correlated with all microorganisms in the red complex, including *P. gingivalis*, *T. forsythia*, and *T. denticola*. It was highly correlated with all the microorganisms in the same complex, except for *P. micra*. Additionally, *F. nucleatum* in the orange complex was highly correlated with all microorganisms within the complex, confirming that it plays a key role in microbial growth in the orange complex. Thus, the red and orange complex microorganisms in the oral cavity may interact with each other to promote proliferation and induce a complex inflammatory response that may aggravate periodontitis or peri-implantitis. In this study, the most influential microorganisms between the complexes were *P. gingivalis* and *C. rectus*, whereas *F. nucleatum* had a strong influence on the orange complex. Therefore, the preferential removal of specific microorganisms that are highly related to other microorganisms, such as *P. gingivalis*, *C. rectus*, and *F. nucleatum*, may prevent colony formation or block the connections between colonies, thereby preventing periodontal disease or delaying its exacerbation.

3. Suggestion

The most important aspect in treating periodontal disease and peri-implantitis known so far is antibacterial treatment in the pathological periodontal pocket and cleaning of the contaminated implant surface[35-37]. The results of this study emphasize the importance of suppressing the number of red and orange complex microorganisms and controlling their proliferation for the treatment and management of periodontitis and peri-implantitis. Since an increase in pathogens may be detected by identifying the composition of oral bacteria in both natural teeth and implant patients, it is possible to identify patients at risk of periodontitis and peri-implantitis in advance and find ways to treat them early. In addition, such bacteriological analysis and understanding may help dentists and dental hygienists provide oral healthcare methods to prevent periodontitis or peri-implantitis that are suitable for each patient.

4. Limitations

This study has limitations in terms of generalization due to the small number of samples, use of various implant products, and the limited number of microorganisms evaluated. To compensate for these limitations, more patients and follow-up studies are required to analyze the correlation between pathogenic bacteria and disease severity. In addition, because various factors may influence the correlation between microbial strains, it is necessary to further clarify the complexity of the interactions between strains through additional research and clinical trials.

5. Conclusion

This study showed that the appearance rate and quantity of 11 microorganisms that cause periodontitis were similar in PG and PIG, and that there were significant differences between the HG and the two other groups. *P. gingivalis* in the red complex and *C. rectus* in the orange complex exhibited the most inter-complex interactions, whereas *F. nucleatum* exhibited significant interactions within the orange complex. These findings suggest that certain microorganisms are associated with periodontitis or peri-implantitis, and may influence disease development and progression through interactions. Eliminating microorganisms that play a significant role in periodontitis and peri-implantitis may help prevent these conditions and contribute to the development of treatment strategies and individualized treatment plans.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by the Institutional Review Board committee of Gachon Bioethics Committee (IRB No. 1044396-202305-HR-082-01). The study was carried out in compliance with the Helsinki Declaration and following the CONSORT guidelines and is registered in the Clinical Research Information Service (CRIS No. KCT0008890). All the patients provided informed consent after completing a form on the survey.

ORCID

Eun-Deok Jo, *https://orcid.org/0000-0002-6098-684X*

Funding

None.

Acknowledgements

None.

Data availability

Raw data is provided at the request of the corresponding author for reasonable reason.

References

1. Salvi GE, Aglietta M, Eick S, Sculean A, Lang NP, Ramseier CA: Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. Clin Oral Implants Res 23: 182-190, 2012. https://doi.org/10.1111/j.1600-0501.2011.02220.x
2. Hajishengallis G, Darveau RP, Curtis MA: The keystone-pathogen hypothesis. Nat Rev Microbiol 10: 717-725, 2012. https://doi.org/10.1038/nrmicro2873
3. Colombo AP, Bennet S, Cotton SL, et al.: Impact of periodontal therapy on the subgingival microbiota of severe periodontitis: comparison between good responders and individuals with refractory periodontitis using the human oral microbe identification microarray. J Periodontol 83: 1279-1287, 2012. https://doi.org/10.1902/jop.2012.110566
4. Al-Ahmad A, Wiedmann-Al-Ahmad M, Faust J, et al.: Biofilm formation and composition on different implant materials in vivo. J Biomed Mater Res B Appl Biomater 95: 101-109, 2010. https://doi.org/10.1002/jbm.b.31688
5. Kumar PS, Mason MR, Brooker MR, O'Brien K: Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants. J Clin Periodontol 39: 425-433, 2012. https://doi.org/10.1111/j.1600-051x.2012.01856.x
6. Heitz-Mayfield LJA, Lang NP: Comparative biology of

chronic and aggressive periodontitis vs. peri-implantitis. Periodontol 2000 53: 167-181, 2010.
https://doi.org/10.1111/j.1600-0757.2010.00348.x

7. Koyanagi T, Sakamoto M, Takeuchi Y, Maruyama N, Ohkuma M, Izumi Y: Comprehensive microbiological findings in peri-implantitis and periodontitis. J Clin Periodontol 40: 218-226, 2013.
https://doi.org/10.1111/jcpe.12047
8. Torrungruang K, Jitpakdeebordin S, Charatkulangkun O, Gleebbua Y: *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Treponema denticola* / *Prevotella intermedia* co-infection are associated with severe periodontitis in a Thai population. PLoS One 10: e0136646, 2015.
https://doi.org/10.1371/journal.pone.0136646
9. Iușan SAL, Lucaciu OP, Petrescu NB, et al.: The main bacterial communities identified in the sites affected by peri-implantitis: a systematic review. Microorganisms 10: 1232, 2022.
https://doi.org/10.3390/microorganisms10061232
10. Hultin M, Gustafsson A, Hallström H, Johansson LA, Ekfeldt A, Klinge B: Microbiological findings and host response in patients with peri-implantitis. Clin Oral Implants Res 13: 349-358, 2002.
https://doi.org/10.1034/j.1600-0501.2002.130402.x
11. Persson GR, Renvert S: Cluster of bacteria associated with peri-implantitis. Clin Implant Dent Relat Res 16: 783-793, 2014.
https://doi.org/10.1111/cid.12052
12. Teles R, Teles F, Frias-Lopez J, Paster B, Haffajee A: Lessons learned and unlearned in periodontal microbiology. Periodontol 2000 62: 95-162, 2013.
https://doi.org/10.1111/prd.12010
13. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr: Microbial complexes in subgingival plaque. J Clin Periodontol 25: 134-144, 1998.
https://doi.org/10.1111/j.1600-051x.1998.tb02419.x
14. Gambin DJ, Vitali FC, De Carli JP, et al.: Prevalence of red and orange microbial complexes in endodontic-periodontal lesions: a systematic review and meta-analysis. Clin Oral Investig 25: 6533-6546, 2021.
https://doi.org/10.1007/s00784-021-04164-4
15. Socransky SS, Haffajee AD: The bacterial etiology of destructive periodontal disease: current concepts. J Periodontol 63: 322-331, 1992.
https://doi.org/10.1902/jop.1992.63.4s.322
16. Sculean A, Deppe H, Miron R, Schwarz F, Romanos G, Cosgarea R: Effectiveness of photodynamic therapy in the treatment of periodontal and peri-implant diseases. Oral Biofilms 29: 133-143, 2021.
https://doi.org/10.1159/000510189
17. Gersdorf H, Meissner A, Pelz K, Krekeler G, Göbel UB: Identification of Bacteroides forsythus in subgingival plaque from patients with advanced periodontitis. J Clin Microbiol 31: 941-946, 1993.
https://doi.org/10.1128/jcm.31.4.941-946.1993
18. Luo L, Xie P, Gong P, Tang XH, Ding Y, Deng LX: Expression of HMGB1 and HMGN2 in gingival tissues, GCF and PICF of periodontitis patients and peri-implantitis. Arch Oral Biol 56: 1106-1111, 2011.
https://doi.org/10.1016/j.archoralbio.2011.03.020
19. Kim HS, Lim SA: Comparison between bacterial culture method and multiplex PCR for identification of Fusobacterium nucleatum and Actinobacillus actinomycetemcomitans from the dental plaques. J Dent Hyg Sci 9: 249-255, 2009.
20. World Medical Association: World Medical Association declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 310: 2191-2194, 2013.
https://doi.org/10.1001/jama.2013.281053
21. Lang NP, Berglundh T; Working Group 4 of Seventh European Workshop on Periodontology: Periimplant diseases: where are we now?--Consensus of the seventh European workshop on periodontology. J Clin Periodontol 38: 178-181, 2011.
https://doi.org/10.1111/j.1600-051x.2010.01674.x
22. Rajasekar A, Varghese SS: Microbiological profile in periodontitis and peri-implantitis: a systematic review. J Long Term Eff Med Implants 32: 83-94, 2022.
https://doi.org/10.1615/jlongtermeffmedimplants.2022043121
23. Field CA, Gidley MD, Preshaw PM, Jakubovics N: Investigation and quantification of key periodontal pathogens in patients with type 2 diabetes. J Periodontal Res 47: 470-478, 2012.
https://doi.org/10.1111/j.1600-0765.2011.01455.x
24. Griffen AL, Beall CJ, Campbell JH, et al.: Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. ISME J 6: 1176-1185, 2012.
https://doi.org/10.1038/ismej.2011.191

25. Maeda N, Okamoto M, Kondo K, et al.: Incidence of *Prevotella intermedia* and Prevotella nigrescens in periodontal health and disease. Microbiol Immunol 42: 583-589, 1998. https://doi.org/10.1111/j.1348-0421.1998.tb02328.x
26. Teanpaisan R, Douglas CW, Walsh TF: Characterisation of black-pigmented anaerobes isolated from diseased and healthy periodontal sites. J Periodontal Res 30: 245-251, 1995. https://doi.org/10.1111/j.1600-0765.1995.tb02129.x
27. Kim MJ, Han GS: Quantitative detection of peri-implantitis bacteria using real-time PCR. J Korean Soc Dent Hyg 21: 555-565, 2021. https://doi.org/10.13065/jksdh.20210053
28. Pinto G, Silva MD, Peddey M, Sillankorva S, Azeredo J: The role of bacteriophages in periodontal health and disease. Future Microbiol 11: 1359-1369, 2016. https://doi.org/10.2217/fmb-2016-0081
29. Harvey JD: Periodontal microbiology. Dent Clin North Am 61: 253-269, 2017. https://doi.org/10.1016/j.cden.2016.11.005
30. Canullo L, Peñarrocha-Oltra D, Covani U, Botticelli D, Serino G, Penarrocha M: Clinical and microbiological findings in patients with peri-implantitis: a cross-sectional study. Clin Oral Implants Res 27: 376-382, 2016. https://doi.org/10.1111/clr.12557
31. Wang HL, Garaicoa-Pazmino C, Collins A, Ong HS, Chudri R, Giannobile WV: Protein biomarkers and microbial profiles in peri-implantitis. Clin Oral Implants Res 27: 1129-1136, 2016. https://doi.org/10.1111/clr.12708
32. Tamura N, Ochi M, Miyakawa H, Nakazawa F: Analysis of bacterial flora associated with peri-implantitis using obligate anaerobic culture technique and 16S rDNA gene sequence. Int J Oral Maxillofac Implants 28: 1521-1529, 2013. https://doi.org/10.11607/jomi.2570
33. Ebadian AR, Kadkhodazadeh M, Zarnegarnia P, Dahlén G: Bacterial analysis of peri-implantitis and chronic periodontitis in Iranian subjects. Acta Med Iran 50: 486-492, 2012.
34. Dabdoub SM, Tsigarida AA, Kumar PS: Patient-specific analysis of periodontal and peri-implant microbiomes. J Dent Res 92(12 Suppl): 168S-175S, 2013. https://doi.org/10.1177/0022034513504950
35. Paul Rosen DC, David Cochran, Stuart Froum, Bradley McAllister, Stefan Renvert, Hom Lay Wang: Peri-implant mucositis and peri-implantitis: a current understanding of their diagnoses and clinical implications. J Periodontol 84: 436-443, 2013. https://doi.org/10.1902/jop.2013.134001
36. Park KH, Han GS: The effects of professional tooth cleaning and plaque control instruction on reduction of peri-implantitis. J Dent Hyg Sci 12: 163-170, 2012.
37. Kim YS, Oh MJ: The effect of following oral health care on implant patients. J Dent Hyg Sci 9: 491-496, 2009.