Effects of initial periodontal therapy on the incidence of *P. gingivalis* and EBV DNA in chronic periodontitis patients

（慢性歯周炎患者における *P. gingivalis* とエプスタインバーウイルスの
検出率に対する歯周基本治療の効果）

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Preface

This article is based on a main paper, “Quantitative changes of *P. gingivalis* and EBV DNA in saliva before and after initial periodontal therapy in chronic periodontitis patients” in the International Journal of Oral-Medical Sciences, and a reference paper, “Effects of initial periodontal therapy on the prevalence of Epstein-Barr virus DNA and *Porphyromonas gingivalis* in Japanese chronic periodontitis patients” in the International Journal of Oral-Medical Sciences.

Abstract

Background: Chronic periodontitis (CP) is a most prevalent disease consisting of chronic inflammation of the periodontium that is caused by the accumulation of dental plaque. Recently, Epstein-Barr virus (EBV) is thought to be involved in the pathogenesis of periodontitis as well as *Porphyromonas gingivalis* which is the representative periodontopathic bacteria. The purpose of initial periodontal therapy (IPT) is to enhance motivation and remove calculus, periodontopathic bacteria and their byproducts, in order to restore periodontal health. To elucidate the effects of IPT on incidence of *P. gingivalis* and EBV DNA, we used whole saliva and subgingival plaque from the CP patients.

Methods: Twenty CP patients for whole saliva and 17 CP patients for subgingival plaque

samples were recruited and determined periodontal status by probing pocket depth (PD), bleeding on probing (BOP) and clinical attachment level (CAL; only the patients for subgingival plaque samples), and whole saliva or subgingival plaque samples were collected from two periodontal sites with PD of ≤3 mm (healthy sites: HS) or ≥5 mm (periodontitis sites: PS) at first visit and after IPT. Saliva and subgingival plaque samples were subjected to real-time PCR to detect *P. gingivalis* and EBV DNA.

Results: *P. gingivalis* and EBV DNA were detected in 20 (100%) and 14 (70%) saliva samples from the CP patients at first visit. After IPT, number of detections of *P. gingivalis* and EBV DNA were decreased to 17 (85%) and 9 (45%) saliva samples from the patients. Coexistence of *P. gingivalis* and EBV DNA were detected in 14 (70%) saliva samples from the patients at first visit, and significantly decreased to 8 (40%) after IPT. EBV DNA and *P. gingivalis* were detected 9 (52.9%) and 14 (82.3%) sites within the subgingival samples from HS, and 13 (76.5%) and 14 (82.3%) sites within the PS at first visit. After IPT, number of detections of EBV DNA and *P. gingivalis* were decreased to 5 (29.4%) and 13 (76.5%) sites within the subgingival samples from HS, and 9 (52.9%) and 10 (58.8%) sites within the PS. Coexistence of EBV DNA and *P. gingivalis* in the subgingival samples from PS at first visit (12 sites; 70.6%) were significantly decreased after IPT (6 sites; 35.3%). Significant improvements in PD and BOP were observed after

IPT.

Conclusion: These results suggest that the IPT was effective in improvement of clinical parameters such as PD and BOP and reducing the coexistence of *P. gingivalis* and EBV in the saliva and subgingival plaque in PS from CP patients. However, IPT could not eradicate the EBV and *P. gingivalis*. Further research would be necessary for improving the periodontal treatment strategy.

Introduction

Periodontitis, an inflammatory disease, is caused by three risk factors such as bacterial, environmental and host factors. Severe periodontitis provokes destruction of periodontium, gingival swelling, alveolar bone resorption, and eventual tooth loss (1). Bacterial plaque is key etiological factor in the onset and progression of periodontitis (2).

EBV is one of the most prevalent viruses in the world. It is estimated that over 90% of adults are EBV seropositive (3, 4). Primary infections of infants with EBV are usually asymptomatic, but the infection of adolescence and young adult with EBV causes infectious mononucleosis, a self-limiting, lymphoproliferative disease. Spread within families is thought to be a common route of EBV transmission by salivary contact. The virus infects first within oropharyngeal epithelium, and later primarily within B lymphocytes are invaded via CD21 receptors, where it establishes a lifelong latent

infection (5-9). EBV has been linked to the development of several malignant tumors, including Burkitt's lymphoma, Hodgkin's disease certain forms of T-cell lymphoma, lymphoproliferative disease in immunosuppressed individuals, nasopharyngeal carcinoma and a proportion of gastric cancers (10-12). ZEBRA is an early lytic protein of EBV encoded BZLF1 gene. In the latent state, hypoacetylation of histone in the BZLF1 promoter by histone deacetylases is involved in maintaining EBV latency. The reactivation of EBV from latent infection occurs frequently and multiplies with the epithelium cells of the pharyngeal and is exhausted in saliva (7, 13-15).

There were several studies describing relationship between periodontal disease and EBV infections (16-21). Therefore, we have examined the coexistence of *P. gingivalis* and EBV in the subgingival plaque from two periodontal pocket sites with probing pocket depth (PD) of $\leq$3 mm or $\geq$5 mm. *P. gingivalis* and EBV DNA were detected in higher copy numbers in the deep periodontal pockets and found higher incidence of coexistence as compared with shallow periodontal pockets (18, 19). In these studies, we suggested that EBV may serve as pathogenic factors lead to periodontal disease among Japanese patients. *P. gingivalis* could increase the virulence of EBV via reactivation of EBV through butyric acid (7, 18, 19). EBV and human cytomegalovirus are significantly related to chronic periodontitis (CP) (20). Coexistence of *P. gingivalis* and EBV could

promote the progression of CP in pregnant women (21). Therefore, interactions between *P. gingivalis* and EBV might be involved in the onset and progression of CP.

The purpose of this study was to examine the effects of initial periodontal therapy (IPT) on the prevalence of *P. gingivalis* and EBV DNA in the saliva and subgingival plaque.

Methods

Clinical examination and characteristics of participants

Periodontal examination comprising determination of PD, bleeding on probing (BOP) and, clinical attachment level (CAL). At first visit and after IPT, periodontal examinations were performed by a trained periodontist using PCP11 probe (Hu-Friedy, Chicago, IL, USA) according to the method published previously (22, 23). CP patients were defined as the presence of at least two sites with PD $\geq$5 mm and CAL of more than 5 mm. Twenty CP patients for whole saliva and 17 CP patients for subgingival plaque samples were included in this study. All subjects were systemically healthy and had no history of periodontal treatment or any type of antibiotic therapy for at least 3 months prior to the present study. The Institutional Review Board at the Nihon University School of Dentistry at Matsudo approved the study (EC17-16-15-005-2). Written informed consent was obtained from each study subject after all experiments were fully explained. They

received IPT, such as oral hygiene instructions, scaling and root planing (SRP) and mechanical tooth cleaning (within 12 months) at Nihon University Hospital School of Dentistry at Matsudo, Japan.

Sampling

Saliva samples were collected from 20 CP patients at first visit and after initial periodontal therapy. Whole saliva was collected from each subject by chewing on a gum base, containing neither fragrance nor flavored ingredients for 5 min (24).

Seventeen subgingival plaque samples were collected from one periodontally healthy site (HS) with PD ($\leq$3 mm), and one periodontitis site (PS) with PD ($\geq$5 mm) of 17 CP patients at first visit and after initial periodontal therapy. Before sampling, supragingival plaque was removed with Gracey curette. Sterile paper points were inserted to the sample site (three times), retained for 30 sec, pooled in Eppendorf tubes, and then stored at -80 °C (18).

DNA extraction and real-time PCR

DNA samples from the whole saliva and subgingival plaque were prepared using High Pure Viral Nucleic Acid Kit (Roche Applied Science, Mannheim, Germany). Real-time

polymerase chain reaction (PCR) was used to measure the copy numbers of *P. gingivalis* and EBV DNA in the samples, using the specific primer sets described previously (19, 25). The dynamic ranges of the real-time PCR assays were determined through serial dilution of DNA extracts either as AKATA cells or *P. gingivalis* TDC60 of the standards in the range of 10^9~10^1copies/ml (26, 27).

Statistical analysis

Significant differences between baseline values of PD and BOP (for whole saliva), and values after IPT were analyzed using paired *t*-test. The chi squared test for independence, confirmed by Fisher's exact probability test, was used to determine whether individual pathogens and BOP (for subgingival plaque) were changed by IPT.

Results

The age, sex, PD and BOP of the patients for whole saliva samples are summarized in Table 1. Six males and 14 females were included in this study. The mean PD at first visit were 2.95 ± 0.77 mm, and then it was changed to 2.15 ± 0.43 mm after IPT. BOP was detected in 46.4 ± 27.1% at first visit, and then BOP changed in 14.9 ± 16.2% after IPT. PD and BOP at first visit were significant improved after IPT.

The age, sex, PD, CAL and BOP of the patients for subgingival plaque samples are summarized in Table 2. Seven males and 10 females were included in this study. The mean PD of the HS and PS at first visit were 2.94 ± 0.24 mm and 7.35 ± 1.54 mm, and then they were changed to 2.82 ± 0.39 mm (HS) and 5.76 ± 1.68 mm (PS) after IPT. The mean CAL of the HS and PS at first visit were 3.65 ± 1.37 mm and 8.76 ± 1.92 mm, and then they were changed to 3.82 ± 1.51 mm (HS) and 7.47 ± 1.74 mm (PS) after IPT. BOP was detected in 2 (11.8%) HS and 17 (100%) PS at first visit, and then BOP could not detect in HS and detected in 11 (65%) PS after IPT. PD and BOP of the PS at first visit were significant improved after IPT.

Table 3 shows gender, age, clinical data and counts of *P. gingivalis* and EBV DNA (copies/ml) of each subject at first visit and after IPT. *P. gingivalis* and EBV DNA were detected in saliva taken from 20 (100%, range from 5.76 to 2.83×10^{9} copies/ml) and 14 (70%, range from 3.01×10^{2} to 2.78×10^{7} copies/ml) participants at first visit and their incidence decreased to 17 (85%, range from 3.15×10^{1} to 4.67×10^{7} copies/ml) and 9 participants (45%, range from 2.90×10^{1} to 1.24×10^{7} copies/ml) after IPT (Table 3). Coexistence of *P. gingivalis* and EBV DNA at first visit (14; 70%) were significantly decreased after IPT (8; 40%) (Table 4).

Table 5 and 6 show gender, age, clinical data and counts of EBV DNA and *P. gingivalis*

(copies/ml) of each subject in the HS and PS at first visit and after IPT. EBV DNA and *P. gingivalis* were detected 9 sites (52.9%, range from 2.52×10^2 to 1.09×10^4 copies/ml) and 14 sites (82.3%, range from 5.29×10^1 to 4.71×10^8 copies/ml) in the subgingival samples from HS at first visit and changed to 5 sites (29.4%, range from 6.12×10^2 to 8.41×10^3 copies/ml) and 13 sites (76.5%, range from 4.34 to 7.61×10^7 copies/ml) from HS after IPT (Table 5). EBV DNA and *P. gingivalis* were detected 13 sites (76.5%, range from 3.78×10^1 to 2.55×10^4 copies/ml) and 14 sites (82.3%, range from 2.28×10^3 to 6.21×10^9 copies/ml) in the subgingival samples from PS at first visit and changed to 9 sites (52.9%, range from 6.50×10^2 to 8.59×10^3 copies/ml) and 10 sites (58.8%, range from 9.97×10^1 to 2.70×10^9 copies/ml) from PS after IPT.

The prevalence of EBV DNA and *P. gingivalis* in the subgingival samples from HS or PS are listed in Table 7 and 8. Occurrence of EBV DNA and *P. gingivalis* in the HS or PS were decreased after IPT, but not statistically significant. Coexistence of EBV DNA and *P. gingivalis* in the PS at first visit (12; 70.6%) were significantly decreased after IPT (6; 35.3%) (Table 8). However, coexistence of EBV DNA and *P. gingivalis* in the HS did not decreased significantly after IPT (Table 7).

Discussion

In the present study, we showed that higher numbers of *P. gingivalis*, EBV DNA and coexistence of *P. gingivalis* and EBV DNA were detected in the whole saliva of CP patients and they were decreased by IPT. It's notable that PD, BOP and coexistence of *P. gingivalis* and EBV DNA in the saliva at first visit were significantly decreased after IPT. In the second study, we demonstrated that high incidence of EBV DNA, *P. gingivalis* and coexistence of EBV DNA and *P. gingivalis* were detected in the subgingival plaque from HS and PS of CP patients and they were decreased by IPT. Especially, PD, BOP and coexistence of EBV DNA and *P. gingivalis* in the PS at first visit were significantly decreased after IPT. These results suggest that IPT is effective in improvement of PD and BOP and reducing the coexistence of *P. gingivalis* and EBV in the saliva and subgingival plaque. We wished to examine the effect of IPT on the incidence of *P. gingivalis* and EBV DNA in the saliva and subgingival plaque, because several studies suggest that *P. gingivalis* and EBV act synergistically to potentiate progression of periodontitis and tissue destruction of periodontium (18-21, 28, 29).

Periodontopathic bacteria is crucial risk factor for periodontal disease, it might be associated with systemic conditions. Especially, *P. gingivalis* triggers changes to the composition and amount of the oral commensal bacteria inducing inflammation and bone

resorption. Changes in the composition of the gut bacterium have been implicated in several inflammatory diseases. Therefore, targeting of possible keystone bacteria, such as *P. gingivalis* could help treat periodontal disease of polymicrobial etiology (30). EBV were associated with the severity of periodontal disease and with major periodontopathic bacteria (31, 32). These reports suggested that high copy numbers of *P. gingivalis* and EBV DNA may correlate with severity of periodontitis.

We previously reported, *P. gingivalis* were detected in the subgingival plaque from 20 (80%) deep periodontal pockets (PS; PD ≥5 mm) and 9 (36%) shallow periodontal pockets (HS; PD of ≤3 mm), and EBV DNA were detected in 20 (80%) PS and 10 (40%) HS of 25 Japanese CP patients (19). These results showed that detection rate of EBV DNA and *P. gingivalis* in the PS at first visit (Table 8) were similar, whereas detection rate in the HS at first visit (Table 7) were higher than the results of we reported previously (19). These results also showed that detection rate of *P. gingivalis* in the PS (80%) were lower than the detection rate in the saliva (100%), whereas detection rate of EBV DNA in the PS (80%) were similar detection rate in the saliva (70%) at first visit (Table 4). *P. gingivalis* and EBV DNA coexist in the saliva of CP patient's high frequency (70%) at first visit (Table 4). In the subgingival plaque samples, EBV DNA and *P. gingivalis* coexist in the PS at first visit at high frequency (70.6%) (Table 8). These values correlated with the data of previous

study that the detection rate (68%) of coexistence of *P. gingivalis* and EBV DNA in the deep periodontal pockets of the CP patients (19).

We have previously reported immunostaining using B cell marker CD19 showed large number of B cells infiltrated into the inflamed gingival connective tissues, and EBV-encoded small RNA (EBRE) positive B cells were detected in the same location using *in-situ* hybridization (18). Latent EBV might be induced into the lytic replication cycle by several inducers, such as phorbol 12-*O*-tetradecanoylphorbol-13-acetate, calcium ionophores, butyric acid and anti-immunoglobulin (7, 8, 33). BamHI Z EBV replication activator (ZEBRA) is an early lytic protein of EBV encoded by BZLF1 gene which is involved in converting the EBV from the latent to the lytic form. Histone deacetylase (HDAC) induces hypoacetylation of the BZLF1 promoter, and involved in the maintaining of EBV latency. *P. gingivalis* produces butyric acid which is an inhibitor of HDAC, increased histone acetylation and induced transcription of the BZLF1 gene (7, 8). These findings suggest that *P. gingivalis* is a risk factor for EBV reactivation in the periodontal tissues.

Results of this study provides evidence for potential interactions between *P. gingivalis* and EBV in the etiology of periodontitis. Periodontopathic bacteria and EBV co-existence apparently leads to additive effects and exacerbates the progress of periodontitis (33).

EBV-infected periodontium tends to harbor high levels of periodontopathic bacteria. Bacterial and viral co-existences were reported more frequently in deeper PD sites of CP patients (28, 29).

PD, BOP and coexistence of *P. gingivalis* and EBV DNA in the saliva and subgingival plaque at first visit were significantly decreased after IPT. Therefore, the results suggest that IPT is very important to treat periodontal disease and maintain periodontal health.

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Table 1 Characteristics of patients for whole saliva samples

CP patients (n =20)		
	First visit	**After IPT**
Age (years)	56.1 ± 15.4	
Males	6 (30%)	
Females	14 (70%)	
Mean PD (mm)	2.95 ± 0.77	2.15 ± 0.43**
BOP (%)	46.4 ± 27.1	14.9 ± 16.2**

Chronic periodontitis (CP), initial periodontal therapy (IPT),
probing pocket depth (PD), bleeding on probing (BOP),
Statistically significant; $P<0.01$**, mean ± SD

Table 2 Characteristics of participants for subgingival plaque samples

CP patients (n =17)		
	First visit	**After IPT**
Age (years)	44.8 ± 14.9	
Males	7 (41.2%)	
Females	10 (58.8%)	
PD(mm)	2.94 ± 0.24(HS)	2.82 ± 0.39 (HS)
	7.35 ± 1.54(PS)	5.76 ± 1.68 (PS)**
CAL (mm)	3.65 ± 1.37(HS)	3.82 ± 1.51 (HS)
	8.76 ± 1.92(PS)	7.47 ± 1.74 (PS)
BOP	2 (11.8%) (HS)	0 (0%)
	17 (100%) (PS)	11(65%)**

Clinical attachment level (CAL), healthy sites (HS), periodontitis sites (PS)
Statistically significant; $P<0.01$**, mean ± SD

Table 3 Clinical data and counts of EBV DNA and *P. gingivalis* at first visit and after IPT for whole saliva samples

			First visit				After IPT			
Subject No.	**Gender**	**Age**	**Mean PD** (mm)	**BOP (%)**	**(Copies/ml) *P. gingivalis***	**(Copies/ml) EBV**	**Mean PD** (mm)	**BOP (%)**	**(Copies/ml) *P. gingivalis***	**(Copies/ml) EBV**
1	Female	64	2.86	43.5	1.32×10^4	7.59×10^4	1.08	1.4	ND	ND
2	Female	32	2.38	22.8	1.25×10^4	1.24×10^3	1.85	0	1.37×10^2	6.53×10^3
3	Male	58	3.13	29.2	4.09×10^4	6.38×10^5	2.56	16.7	ND	ND
4	Female	69	2.07	53.7	1.83×10^4	3.27×10^4	1.79	21.6	3.15×10^1	2.90×10^1
5	Female	52	2.66	22.4	2.10×10^3	ND	1.73	1.2	1.16×10^6	ND
6	Male	47	3.4	51.2	2.46×10^5	3.64×10^5	2.07	12.3	9.59×10^4	1.86×10^5
7	Female	59	1.83	1.2	8.63×10^6	2.78×10^7	1.8	0	3.59×10^6	1.81×10^6
8	Female	41	3.97	60.7	7.08×10^4	1.30×10^4	3.01	45.2	1.95×10^6	ND
9	Female	72	2.42	5.3	5.86×10^6	3.04×10^5	2.38	5.3	2.26×10^6	3.84×10^5
10	Female	47	2.63	33.3	8.44×10^6	1.12×10^5	1.99	6.7	6.92×10^6	ND
11	Female	57	2.48	38.7	5.76	ND	2.17	19.6	3.50×10^5	4.18×10^2
12	Female	54	2.84	73.1	1.85×10^3	ND	2.25	6.4	4.17×10^6	1.24×10^7
13	Male	65	2.32	40.7	2.51×10^3	7.64×10^3	2.2	24.1	6.28×10^6	ND
14	Female	83	2.63	65.5	2.46×10^4	1.13×10^5	1.99	4.2	ND	1.45×10^4
15	Male	59	4.44	95.2	3.51×10^8	1.05×10^7	2.84	58.8	3.05×10^7	6.04×10^5
16	Female	16	3.83	79.2	8.38×10^4	ND	2.38	38.7	1.85×10^4	ND
17	Female	44	2.36	12.5	1.25×10^5	7.16×10^4	2.01	1.8	2.63×10^4	ND
18	Female	78	2.64	44	1.81×10^4	ND	2.04	6.5	3.01×10^4	ND
19	Male	55	3.75	60.3	1.52×10^5	ND	2.71	14.1	3.62×10^4	ND
20	Male	70	4.45	94.9	2.83×10^9	3.01×10^2	2.23	13.2	4.67×10^7	ND

Not detectable (ND)

Table 4 Occurrence of EBV DNA and *P. gingivalis* in the saliva at first visit and after IPT for whole saliva samples

	Detection frequency		Significance (*P*-value)
Infectious agents	First visit	After IPT	First visit vs After IPT
P. gingivalis	20 (100%)	17 (85%)	0.07
EBV	14 (70%)	9 (45%)	0.1
P. gingivalis + EBV	14 (70%)	8 (40%)	0.05*

Initial periodontal therapy (IPT)

Table 5 Clinical data and counts of EBV DNA and *P. gingivalis* in the HS at first visit and after IPT for subgingival plaque samples

			First visit					After IPT				
Subject No.	**Gender**	**Age**	**(≦3) PD** (mm)	**CAL** (mm)	**BOP**	**(Copies/ml) EBV**	**(Copies/ml) *P. gingivalis***	**(≦3) PD** (mm)	**CAL** (mm)	**BOP**	**(Copies/ml) EBV**	**(Copies/ml) *P. gingivalis***
1	Female	27	3	3	−	ND	9.21×10^3	3	3	−	ND	6.35×10^3
2	Female	39	3	8	+	4.49×10^3	4.71×10^8	3	9	−	ND	7.61×10^7
3	Female	28	2	2	−	ND	5.71×10^7	3	3	−	ND	ND
4	Male	38	3	3	−	ND	ND	2	5	−	ND	ND
5	Female	41	3	3	−	4.48×10^3	1.11×10^4	3	3	−	ND	8.24×10^3
6	Female	70	3	3	−	3.40×10^3	2.40×10^3	3	3	−	ND	ND
7	Female	31	3	4	−	ND	1.34×10^3	3	4	−	1.03×10^3	1.45×10^2
8	Female	42	3	3	−	ND	8.24×10^3	3	3	−	ND	2.75×10^3
9	Male	72	3	5	−	3.18×10^3	4.30×10^4	2	3	−	ND	6.29×10^4
10	Female	59	3	5	−	1.09×10^4	3.61×10^6	2	4	−	ND	9.50×10^3
11	Male	47	3	3	−	8.34×10^3	8.14×10^5	3	4	−	7.94×10^3	3.70×10^4
12	Male	73	3	3	−	ND	5.29×10^1	3	3	−	ND	4.34
13	Female	46	3	4	−	2.52×10^2	8.59×10^2	3	3	−	6.12×10^2	1.65×10^3
14	Male	24	3	3	−	ND	ND	3	3	−	ND	ND
15	Male	43	3	3	−	3.75×10^3	ND	3	3	−	2.10×10^3	1.23×10^3
16	Male	37	3	3	−	4.56×10^3	3.81×10^3	3	4	−	8.41×10^3	4.13×10^1
17	Female	44	3	4	+	ND	3.39×10^5	3	5	−	ND	1.27×10^3

Table 6 Clinical data and counts of EBV DNA and *P. gingivalis* in the PS at first visit and after IPT for subgingival plaque samples

			First visit					After IPT				
Subject No.	**Gender**	**Age**	**(≧5) PD (mm)**	**CAL (mm)**	**BOP**	**(Copies/ml) EBV**	**(Copies/ml) *P. gingivalis***	**(≧5) PD (mm)**	**CAL (mm)**	**BOP**	**(Copies/ml) EBV**	**(Copies/ml) *P. gingivalis***
1	Female	27	6	7	+	2.98×10^3	8.41×10^8	6	7	−	ND	1.91×10^8
2	Female	39	8	12	+	2.38×10^3	3.56×10^8	6	11	+	1.47×10^3	2.70×10^9
3	Female	28	8	9	+	8.36×10^3	3.69×10^8	6	7	+	8.59×10^3	ND
4	Male	38	6	7	+	ND	ND	5	6	+	ND	ND
5	Female	41	8	10	+	ND	2.84×10^9	8	9	+	1.78×10^3	2.87×10^8
6	Female	70	10	10	+	ND	2.28×10^3	9	10	+	ND	ND
7	Female	31	6	8	+	9.04×10^2	1.89×10^4	5	7	+	6.03×10^3	2.20×10^5
8	Female	42	6	7	+	8.36×10^3	1.89×10^5	6	6	−	6.29×10^3	1.53×10^3
9	Male	72	8	11	+	5.65×10^3	6.21×10^9	6	8	−	6.50×10^2	8.23×10^5
10	Female	59	7	9	+	2.55×10^4	4.43×10^9	3	5	−	5.72×10^3	7.66×10^6
11	Male	47	6	7	+	3.78×10^1	1.42×10^6	6	7	+	ND	1.05×10^9
12	Male	73	8	10	+	5.88×10^3	4.13×10^6	3	7	+	1.15×10^3	ND
13	Female	46	6	6	+	1.57×10^2	2.14×10^8	3	5	−	5.29×10^3	ND
14	Male	24	10	11	+	2.37×10^2	2.75×10^7	6	8	+	ND	ND
15	Male	43	6	6	+	ND	ND	6	6	+	ND	ND
16	Male	37	6	8	+	8.89×10^3	ND	6	8	−	ND	9.97×10^1
17	Female	44	10	11	+	2.03×10^3	2.20×10^8	8	10	+	ND	2.24×10^4

Table 7 Occurrence of EBV DNA and *P. gingivalis* in the subgingival samples from HS at first visit and after IPT for subgingival plaque samples

	Detection frequency		Significance (*P*-value)
Infectious agents	First visit	After IPT	First visit vs After IPT
EBV	9 (52.9%)	5 (29.4%)	0.148
P. gingivalis	14 (82.3%)	13 (76.5%)	0.5
EBV + *P. gingivalis*	8 (47.1%)	5 (29.4%)	0.241

Healthy sites (HS)

Table 8 Occurrence of EBV DNA and *P. gingivalis* in the subgingival samples from PS at first visit and after IPT for subgingival plaque samples

	Detection frequency		Significance (*P*-value)
Infectious agents	First visit	After IPT	First visit vs After IPT
EBV	13 (76.5%)	9 (52.9%)	0.141
P. gingivalis	14 (82.3%)	10 (58.8%)	0.129
EBV + *P. gingivalis*	12 (70.6%)	6 (35.3%)	0.042*

Periodontitis sites (PS), Statistically significant; $P<0.05$*