

# Effects of initial periodontal therapy on the prevalence of Epstein-Barr virus DNA and Porphyromonas gingivalis in Japanese chronic periodontitis patients

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## Research article

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## Abstract

**Background** Initial periodontal therapy (IPT) is cornerstone of periodontal therapy and the first step to control of periodontal risk factors. Scaling and root planing are used to treat root surface irregularities and remove virulent factors caused by periodontal pathogens. This procedure also incorporated into periodontal surgery. To elucidate the effects of IPT on prevalence of Epstein-Barr virus (EBV) DNA and *Porphyromonas gingivalis*, we used subgingival plaque samples from chronic periodontitis (CP) patients.

**Methods** Seventeen CP patients were recruited and determined measured periodontal status clinical parameters such as by probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP) and an X-ray examination, and 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. Plaque samples were subjected to a real-time PCR to detect EBV DNA and *P. gingivalis*.

**Results** 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. Significant improvements in PD and BOP were observed after IPT in 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%).

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

## Background

Patient education, training in oral hygiene, control of risk factors, removal of supra and subgingival bacterial plaque, scaling and root planing (SRP) are contents of implementation of initial periodontal therapy (IPT) [1, 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].

Association between EBV infection and periodontal disease has been reported [16, 17]. Therefore, we have examined the coexistence of EBV and *Porphyromonas gingivalis* which is a representative periodontal pathogen in the subgingival plaque from two periodontal sites with probing depth (PD) of <3 mm or >5 mm. EBV DNA and *P. gingivalis* were detected in higher copy numbers in deep periodontal pockets and showed a higher incidence of the coexistence as compared to shallow periodontal pockets [18, 19]. We showed that EBV may serve as a pathogenic factor leading to CP among Japanese patients. Periodontopathic anaerobic bacteria such as *P. gingivalis* could increase the virulence of EBV via reactivation of EBV through butyric acid [18, 19]. The findings of the meta-analysis suggest that EBV and human cytomegalovirus are significantly associated with CP [20]. EBV and *P. gingivalis* coinfection may promote the development of CP in pregnant women [21]. Therefore, it might be potential interactions between EBV and *P. gingivalis* in the etiopathogenesis of CP.

The purpose of this study was to examine the effects of IPT on the prevalence of Epstein-Barr virus (EBV) DNA and *P. gingivalis* in the subgingival plaque.

## Methods

### Clinical examination and characteristics of participants

Periodontal examination comprising determination of PD, clinical attachment level (CAL), bleeding on probing (BOP) and an X-ray (intraoral radiograph) examination. At each point in time, periodontal examinations were performed by a trained periodontist (YO) using PCP11 probe (Hu-Friedy, Chicago, IL, USA) according to the method has been published previously [23]. CP patients were defined as the presence of at least two sites with PD >5 mm and CAL of more than 5 mm. Seventeen CP patients (mean age, 44.8 ± 14.9 years) 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, SRP and mechanical tooth cleaning (within 12 months) at Nihon University Hospital School of Dentistry at Matsudo, Japan.

### Sampling

Seventeen subgingival plaque samples were collected from one periodontally healthy site (HS) of PD (<3 mm), and one periodontitis site (PS) of PD (>5 mm) among 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 subgingival plaque were prepared using High Pure Viral Nucleic Acid Kit (Roche Applied Science, Mannheim, Germany). Quantitative real-time polymerase chain reaction (PCR) was used to measure the copy numbers of EBV DNA and *P. gingivalis* in the samples, using the specific primer sets described previously. [19, 24]. 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  $10^9 \sim 10^1$  copies/ml [25, 26].

## Statistical analysis

The chi squared test for independence, confirmed by Fisher's exact probability test, was used to determine whether individual pathogens and BOP were changed by IPT. Significant differences between baseline values of PD and values after IPT were analyzed using one-way ANOVA.

## Results

The age, sex, PD, CAL and BOP of the patients are summarized in Table 1. Seven males and 10 females were included in this study. The mean PD of the HS and PS at first visit were  $2.94 \pm 0.24$  mm and  $7.35 \pm 1.54$  mm, and then they were changed to  $2.82 \pm 0.39$  mm (HS) and  $5.76 \pm 1.68$  mm (PS) after IPT. The mean CAL of the HS and PS at first visit were  $3.65 \pm 1.37$  mm and  $8.76 \pm 1.92$  mm, and then they were changed to  $3.82 \pm 1.51$  mm (HS) and  $7.47 \pm 1.74$  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 2 and 3 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  $2.52 \times 10^2$  to  $1.09 \times 10^4$  copies/ml) and 14 sites (82.3%, range from  $5.29 \times 10^1$  to  $4.71 \times 10^8$  copies/ml) in the subgingival samples from HS at first visit and changed to 5 sites (29.4%, range from  $6.12 \times 10^2$  to  $8.41 \times 10^3$  copies/ml) and 13 sites (76.5%, range from  $4.34$  to  $7.61 \times 10^7$  copies/ml) from HS after IPT (Table 2). EBV DNA and *P. gingivalis* were detected 13 sites (76.5%, range from  $3.78 \times 10^1$  to  $2.55 \times 10^4$  copies/ml) and 14 sites (82.3%, range from  $2.28 \times 10^3$  to  $6.21 \times 10^9$  copies/ml) in the subgingival samples from PS at first visit and changed to 9 sites (52.9%, range from  $6.50 \times 10^2$  to  $8.59 \times 10^3$  copies/ml) and 10 sites (58.8%, range from  $9.97 \times 10^1$  to  $2.70 \times 10^9$  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 4 and 5. 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 5). However, coexistence of EBV DNA and *P. gingivalis* in the HS did not decreased significantly after IPT (Table 4).

## Discussion

In this study, we demonstrated that higher levels of EBV DNA, *P. gingivalis* and coexistence of EBV DNA and *P. gingivalis* were detected in the HS and PS of Japanese 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 significant decreased after IPT. The results suggest that IPT is effective in improvement of PD and BOP and reducing the coexistence of EBV and *P. gingivalis* in the subgingival plaque.

Periodontopathic bacteria is major risk factor for periodontal disease, however does not satisfied only by bacterial activity to explain several clinical characteristics of periodontitis, such as rapid bone resorption with minimal amount of plaque, site specific development, and presence of quiescence and active phase. Therefore, EBV could be involved in the etiology of several types of periodontitis [18, 27–29]. In this study, we examined whether higher prevalence of EBV DNA and *P. gingivalis* are associated with PS in Japanese CP patients and IPT could decrease the number of EBV and *P. gingivalis* in the subgingival plaque, because several studies suggest that EBV and *P. gingivalis* act synergistically to potentiate periodontal disease progression and tissue destruction [18, 19, 21, 29, 30]. In this study, EBV DNA and *P. gingivalis* coexist in the PS of CP patients at high frequency (70.6%). This value correlated with previous studies that showed higher levels (68%) of EBV DNA and *P. gingivalis* coexist in the deep PD (> 5 mm) in the CP patients [19]. EBV were associated with major periodontopathic bacteria and with the severity of periodontal disease [31, 32]. These reports suggested that high copy numbers of EBV DNA and *P. gingivalis* may correlate with periodontal disease state. In our previous report, detection rate and counts in PCR-positive deep PD >5 mm (PS) or shallow PD of <3 mm (HS) sites for EBV DNA (detected in 60 80% PS and 40% HC sites) were  $3.74 \times 10^3 \sim 3.70 \times 10^7$  and  $4.37 \times 10^4 \sim 9.13 \times 10^6$  copies/ml, and for *P. gingivalis* (detected in 80% PS and 36% HC sites) were  $2.45 \times 10^7 \sim 1.15 \times 10^9$  and  $4.16 \times 10^6 \sim 2.13 \times 10^9$  copies/ml [19]. The results showed that detection rate of EBV DNA and *P. gingivalis* in the PS were similar in this study, whereas detection rate in the HS were higher in this study (Table 2–5).

We have previously reported that immunostaining using B cell marker CD19 showed large number of B cells infiltrated into the inflamed gingival connective tissues [18]. And the results of *in-situ* hybridization using serial section by EBV-encoded small RNA (EBRE) showed a large number of B cells in the same location were EBER-positive [18].

Latent EBV could be induced into the lytic replication cycle by treatment with several inducers, such as anti-immunoglobulin, butyric acid, calcium ionophore, phorbol 12-myristate 13-acetate and transforming growth factor- $\beta$  [7, 8, 33]. The EBV BZLF1 gene product ZEBRA is a regulator of the transition from latent form to the lytic replication cycle. Histone deacetylase (HDAC) induces hypoacetylation of histone in the BZLF1 promoter, and it is involved in the maintaining of EBV latency. Culture supernatant of *P. gingivalis* contains butyric acid which is an inhibitor of HDAC, increased histone acetylation and transcriptional activity of the BZLF1 gene [7, 8]. These findings suggest that periodontitis is risk factor for EBV reactivation in infected individuals.

Results of this study provides credence for potential interactions between EBV and *P. gingivalis* in the etiopathogenesis of periodontitis. EBV and periodontopathic bacteria 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. Viral and bacterial co-existences were reported more frequently in deeper PD sites of CP patients [29, 30]. We have previously reported that coexistence of EBV DNA and *P. gingivalis* was significantly higher in CP patients with deeper PD sites (> 5 mm; 40%) than in those with shallow PD sites (< 3 mm; 14%) or healthy controls (13%) [18]. In addition, coexistence of EBV DNA and *P. gingivalis* was significantly higher in the deeper PD sites (> 5 mm) of CP patients (68%) than in the PD sites of the healthy controls (15%) and shallow PD sites (< 3 mm) of CP patients (12%) [19]. The data suggest that coexistence of EBV and *P. gingivalis* might increase the risk for developing periodontitis.

IPT was effective in reducing the coexistence of EBV and *P. gingivalis* in the subgingival plaque. Further studies are necessary to develop the new IPT procedure for keeping latency of EBV or reducing the coexistence of EBV and *P. gingivalis*.

## Conclusions

IPT was effective in improvement of inflammatory conditions of periodontitis and reducing the coexistence of EBV and *P. gingivalis* in the subgingival plaque. However, IPT could not eradicate the EBV and *P. gingivalis*. Further research would be necessary for improving the periodontal treatment strategy.

## Abbreviations

BOP: Bleeding on probing; CP: Chronic periodontitis; EBV: Epstein-Barr virus; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HDAC: Histone deacetylase; IPT: Initial periodontal therapy; PCR: Polymerase chain reaction; PD: Probing pocket depth.

## Declarations

## Acknowledgements

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## Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

## Authors' contributions

AK, KI and YO were responsible for design and conception of the study. AK, YI and YO performed follow-up care of patients and sample collection. All authors were involved in the analysis and interpretation of data. AK and YO was responsible for drafting the manuscript. All authors revised it critically and approved the final manuscript.

## Ethics approval and consent to participate

The study had been approved by the Institutional Review Board at the Nihon University School of Dentistry at Matsudo (EC17-16-15-005-2), in accordance with the Declaration of Helsinki. Verbal and written informed consent was received from each subject were then clinically examined.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## Tables

**Table 1** Characteristics of participants

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%)**

Chronic periodontitis (CP), initial periodontal therapy (IPT),

probing pocket depth (PD), bleeding on probing (BOP),

clinical attachment level (CAL), healthy sites (HS), periodontitis sites (PS)

Statistically significant;  $P < 0.01^{**}$

**Table 2** Clinical data and counts of EBV DNA and *P. gingivalis* in the HS at first visit and after IPT

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

not detectable (ND), initial periodontal therapy (IPT), Clinical attachment level (CAL)

**Table 3** Clinical data and counts of EBV DNA and *P. gingivalis* in the PS at first visit and after IPT

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

not detectable (ND), initial periodontal therapy (IPT), Clinical attachment level (CAL)

**Table 4** Occurrence of EBV DNA and *P. gingivalis* in the subgingival samples from HS at

first visit and after IPT

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

Healthy sites (HS), initial periodontal therapy (IPT)

**Table 5** Occurrence of EBV DNA and *P. gingivalis* in the subgingival samples from PS at first visit and after IPT

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

Periodontitis sites (PS), initial periodontal therapy (IPT), Statistically significant;  $P < 0.05^*$