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

Keywords: Epstein-Barr virus, initial periodontal therapy, Porphyromonas gingivalis, periodontitis, prevalence

Posted Date: July 15th, 2019

DOI: https://doi.org/10.21203/rs.2.11335/v1

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 measured periodontal clinical parameters such as probing pocket depth (PD) and bleeding on probing (BOP), 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%) in the subgingival samples from HS, and 13 (76.5%) and 14 (82.3%) in 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%) in the HS, and 9 (52.9%) and 10 (58.8%) in 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; 70.6%) were significantly decreased after IPT (6; 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 common viruses, infecting more than 90% of the adult population worldwide [3, 4]. EBV infection is usually asymptomatic during childhood, but the infection leads to infectious mononucleosis when it is delayed until adolescence. It is transmitted from host to host by salivary contact, and the virus passes through the oropharyngeal epithelium to B lymphocytes, where it establishes a lifelong latent infection [5-7]. EBV is associated with Burkitt's lymphoma, Hodgkin's lymphoma, natural killer/T-cell lymphoma, post transplantation lymphoproliferative disorder and nasopharyngeal cancer [8-10]. EBV is adsorbed to CD21 receptors which are expressed on B lymphocyte [11, 12]. The reactivation of EBV from latent infection occurs frequently and multiplies with the epithelium cells of the pharyngeal and is exhausted in saliva [13-15].

Association between EBV infection and periodontitis has been reported [16, 17]. Therefore, we have studied the relationship between EBV and *Porphyromonas gingivalis* which is representative periodontal disease pathogen in chronic periodontitis (CP). EBV DNA and *P. gingivalis* were detected in deeper periodontal pockets of Japanese CP patients [18, 19]. Our findings suggest that EBV DNA may serve as a pathogenic factor leading to CP. We also provide evidence for potential interactions between EBV and *P. gingivalis* in the etiopathogenesis of CP [18]. A systematic review indicated that herpesviruses including EBV are significantly associated with CP [20]. Lu et al. [21] reported an EBV and *P. gingivalis* coinfection may promote the development of CP among pregnant women.

Peri-implantitis (PI) is an inflammatory reaction associated with functional deterioration of supporting bones around dental implants [22]. PI is the most frequent long-term complications of dental implants. It provokes bone destruction with suppuration, and encountered swelling and bleeding on probing (BOP) around implants [22-25]. We have previously reported higher levels of EBV and *P. gingivalis* were detected in peri-implant sulcus of PI patient [26].

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

Methods

Subjects and subject distribution

CP patients were defined as the presence of at least two sites with probing pocket depth (PD) ≥5 mm and attachment loss 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. Periodontal status was assessed by PD and BOP using PCP11 probe (Hu-Friedy, Chicago, IL, USA). 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 following primer sets: EBV forward, 5'-CCTGGTCATCCTTTGCCA-3'; EBV reverse, 5'-TGCTTCGTTATAGCCGTAGT-3'; *P. gingivalis* forward, 5'-AGGCAGCTTGCCATACTGCG-3'; *P. gingivalis* reverse, 5'-ACTGTTAGCAACTACCGATGT-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward, 5'-GCACCGTCAAGGCTGAGAAC-3'; and GAPDH reverse, 5'-ATGGTGGTGAAGACGCCAGT-3' [19, 26]. SYBR Premix Ex Taq in a TP800 thermal cycler dice real-time system (Takara-bio, Tokyo, Japan) was used for real-time PCR reaction in a total volume of 25 μl (12.5 μl 2× SYBR Premix Ex Taq, 0.2 μl forward and reverse primers, and 12.1 μl as the DNA

sample (300 ng per 1 well) and the PCR products comprised of 95 bp for EBV, 404 bp for *P. gingivalis*, and 142 bp for GAPDH. The thermal cycling conditions were 10 s at 95 °C, 45 cycles of 5 s at 95 °C and 30 s at 60 °C. 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 109～101copies/ml [27, 28].

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 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 were 2.88 ± 0.32 mm and 7.35 ± 1.49 mm (mean ± SD). BOP was detected in 2 HS and 17 PS. PD and BOP of the PS at first visit were significant improved after IPT. Table 2 shows clinical data and counts of EBV DNA and *P. gingivalis* (copies/ml) in the HS at first visit and after IPT. EBV DNA and *P. gingivalis* were detected 9 (52.9%, range from 2.52×102 to 1.09×105 copies/ml) and 14 (82.3%, range from 5.29×101 to 4.71×108 copies/ml) in the subgingival samples from HS at first visit and changed to 5 (29.4%, range from 6.12×102 to 7.94×104 copies/ml) and 13 (76.5%, range from 4.34 to 7.61×108 copies/ml) from HS after IPT. Table 3 describes clinical data and counts of EBV DNA and *P. gingivalis* in the PS. EBV DNA and *P. gingivalis* were detected 13 (76.5%, range from 3.78×101 to 2.55×104 copies/ml) and 14 (82.3%, range from 2.28×103 to 6.21×109 copies/ml) in the subgingival samples from PS at first visit and changed to 9 (52.9%, range from 6.50×102 to 8.59×103 copies/ml) and 10 (58.8%, range from 9.97×101 to 2.70×109 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.

Although bacteria play an essential role in the etiology of periodontal disease, it has become increasingly clear that herpes viruses, especially EBV, are involved in the etiology of several types of periodontal disease because bacterial activity alone does not adequately explain several clinical characteristics of periodontal disease, such as rapid bone resorption with minimal amount of plaque, site specific development, and presence of quiescence and active phase [18, 29, 30]. 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, 31, 32]. 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 [33, 34]. These reports suggested that high copy numbers of EBV DNA and *P. gingivalis* may reflect the severity of inflammation. In the previous report, range of counts in PCR-positive sites of periodontitis patients and periodontally normal subjects for EBV DNA (detected in 60% periodontitis lesions and 13% normal periodontal sites) were 2.1 x 103 ~ 8.3 x 108 and 2.4 x 103 ~ 3.2 x 104 copies/ml, and for *P. gingivalis* (detected in 87% periodontitis lesions and 13% normal periodontal sites) were 5 x 103 ~ 1 x 1010 and 2.1 x 104 ~ 3.1 x 106 copies/ml [34]. The results showed that positive rate and copy numbers of EBV DNA and *P. gingivalis* in the periodontitis lesions were similar 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-β [7, 8, 35]. 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 [35, 36]. 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 [31, 34]. 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 establish the new treatment could be developed as a strategy to keep latency of or reducing the EBV.

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

Not applicable.

Funding

The study was supported by Grant-in-Aid for Scientific Research (C; No. 17K11994, C; No.16K11526, Young (B); No.17K17361) from the Ministry of Education, Science, Sports and Culture of Japan, and Nihon University Multidisciplinary Research Grant for 2017.

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.

Ethical 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)	2.88 ± 0.33 (HS)	2.82 ± 0.39 (HS)
	7.35 ± 1.54 (PS)	5.59 ± 1.80 (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),

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

	First visit				**After IPT**			
Subject No.	**(≦3) PD** (mm)	**BOP**	**(Copies/ml) EBV**	**(Copies/ml)** ***P. gingivalis***	**(≦3) PD** (mm)	**BOP**	**(Copies/ml) EBV**	**(Copies/ml)** ***P. gingivalis***
1	3	−	ND	9.21×10^3	3	−	ND	6.35×10^3
2	3	+	4.49×10^3	4.71×10^8	3	−	ND	7.61×10^8
3	2	−	ND	5.71×10^7	3	−	ND	ND
4	3	−	ND	ND	2	−	ND	ND
5	3	−	4.48×10^3	1.11×10^5	3	−	ND	8.24×10^3
6	3	−	3.40×10^3	2.40×10^3	3	−	ND	ND
7	3	−	ND	1.34×10^3	3	−	1.03×10^3	1.45×10^2
8	3	−	ND	8.24×10^3	3	−	ND	2.75×10^3
9	3	−	3.18×10^3	4.30×10^4	2	−	ND	6.29×10^4
10	3	−	1.09×10^5	3.61×10^6	2	−	ND	9.50×10^3
11	3	−	8.34×10^4	8.14×10^5	3	−	7.94×10^4	3.70×10^4
12	3	−	ND	5.29×10^1	3	−	ND	4.34
13	3	−	2.52×10^2	8.59×10^2	3	−	6.12×10^2	1.65×10^3
14	2	−	ND	ND	3	−	ND	ND
15	3	−	3.75×10^4	ND	3	−	2.10×10^3	1.23×10^3
16	3	−	4.56×10^4	3.81×10^3	3	−	8.41×10^3	4.13×10^1
17	3	+	ND	3.39×10^5	3	−	ND	1.27×10^3

not detectable (ND), initial periodontal therapy (IPT)

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

	First visit				After IPT			
Subject No.	(≦3) PD (mm)	BOP	(Copies/ml) EBV	(Copies/ml) *P. gingivalis*	(≦5) PD (mm)	BOP	(Copies/ml) EBV	(Copies/ml) *P. gingivalis*
1	6	+	2.98×10^3	8.41×10^8	6	−	ND	1.91×10^8
2	8	+	2.38×10^3	3.56×10^8	6	+	1.47×10^3	2.70×10^9
3	8	+	8.36×10^3	3.69×10^8	6	+	8.59×10^3	ND
4	6	+	ND	ND	5	+	ND	ND
5	8	+	ND	2.84×10^9	8	+	1.78×10^3	2.87×10^8
6	10	+	ND	2.28×10^3	9	+	ND	ND
7	6	+	9.04×10^2	1.89×10^4	5	+	6.03×10^3	2.20×10^5
8	6	+	8.36×10^3	1.89×10^5	6	−	6.29×10^3	1.53×10^6
9	8	+	5.65×10^3	6.21×10^9	3	−	6.50×10^2	8.23×10^5
10	7	+	2.55×10^4	4.43×10^9	3	−	5.72×10^3	7.66×10^6
11	6	+	3.78×10^1	1.42×10^6	6	+	ND	1.05×10^9
12	8	+	5.88×10^3	4.13×10^6	3	+	1.15×10^3	ND
13	6	+	1.57×10^2	2.14×10^8	3	−	5.29×10^3	ND
14	10	+	2.37×10^2	2.75×10^7	6	+	ND	ND
15	6	+	ND	ND	6	+	ND	ND
16	6	+	8.89×10^3	ND	6	−	ND	9.97×10^1
17	10	+	2.03×10^3	2.20×10^8	8	+	ND	2.24×10^4

not detectable (ND) , initial periodontal therapy (IPT)

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

	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), initial periodontal therapy (IPT)