論　文　内　容　要　旨

Association of oral Epstein-Barr virus with oral health status in Japanese adults

（日本人成人における口腔の Epstein-Barr ウイルスと口腔の健康状態との関係）

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Objective: Epstein-Barr virus (EBV), also known as human herpesvirus 4, is a member of the herpesviridae family. It remains unknown whether EBV is associated with poor oral health (i.e., periodontitis, dental plaque accumulation and an increased oral bacterial count) among middle-aged and older Japanese people. The objective of this study was to clarify the relationship between oral EBV and oral health status in Japanese adults.

Methods: A total of 150 patients who visited the Department of Oral Health of Hiroshima University Hospital were enrolled in this study. We excluded subjects with oral cancer or potentially malignant oral disorders (n = 1), cancer patients receiving surgical treatment, chemotherapy or radiotherapy (n = 20), those with severe immunodeficiency (n = 2) and those with auto-immune diseases receiving steroid therapy (n = 3). Finally, we analyzed 124 patients (46 males and 78 females; mean age, 69.2 years; age range, 35–90 years). We also targeted 43 dependent older people (10 men, 33 women; mean age, 87.9 years) who had a certified need for long-term support or nursing care at an adult day care center in Hiroshima. The design of this cross-sectional study was approved by the Ethical Committee of Hiroshima University. All participants signed an informed consent agreement. Oral rinse samples were obtained by asking the subjects to rinse their mouths with 10 ml of saline for 15 sec. Samples were collected from the tongue surface using the Orcellex® Brush (Rovers Medical Devices, Oss, the Netherlands) in dependent older people. DNA was extracted using a PureLink™ Microbiome DNA Purification kit (Thermo Fisher Scientific). Real time PCR analysis was performed to determine EBV DNA copy. PCR amplification of the bacterial 16S rRNA gene was performed to detect periodontal disease-related bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Fusobacterium nucleatum*. The χ^2 test or Fisher's exact test were used to evaluate significant differences between positive rates of EBV DNA and clinical factors. The Mann-Whitney U test was used to compare differences in clinical parameters between two groups. The Kruskal-Wallis test was used to compare differences in clinical parameters among three groups. Propensity scores were calculated by logistic regression analysis of 11 clinical factors (age, sex, remaining teeth, denture use, smoking, hypertension, diabetes, hyperlipidemia, stroke, heart disease, and bone and joint disease). Statistical analysis was performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results: EBV DNA positivity was investigated in 124 oral rinse samples using real-time PCR. EBV DNA was determined as positive in 16 of 124 participants (12.9%). No significant difference was found between EBV DNA and clinical factors (i.e., sex, age, remaining teeth, denture use, smoking or medical history). Ten of the 38 participants with periodontal pockets ≥ 6 mm were EBV DNA positive (26.3%). There was a significant association between EBV DNA positivity and probing depth ($P = 0.01$). Additionally, a significant association was found between bleeding on probing (BOP) and EBV DNA positivity

(P = 0.03). To examine the relationship between virus DNA amplification and periodontal tissue condition (i.e., periodontal pocket depth or BOP), we examined the EBV DNA copy number in 16 EBV positive cases. A significant association was not found between EBV DNA copy number and periodontal pocket depth or BOP. Additionally, there was no significant increase in the EBV DNA copy number in people with ≥ 6 mm periodontal pockets and BOP as compared to those without ≥ 6 mm periodontal pockets and BOP. Next, propensity score matching was performed between participants without ≥ 4 mm periodontal pockets and BOP (i.e., participants with good periodontal health) and those with ≥ 4 mm periodontal pockets, BOP or both (i.e., participants with poor periodontal health) to investigate the relationship between EBV and periodontal health status. We identified 35 matched pairs among the participants. Participants with poor periodontal health exhibited a higher EBV DNA positivity rate (25.7%) than those with good periodontal health (0.0%). A significant association was found between EBV DNA positivity and periodontal health status (P = 0.001). Next, sterilized paper points were used to obtain gingival crevicular fluid. Total 65 samples were collected by inserting paper points into the periodontal pocket. Of 65 cases, 6 cases showed EBV DNA positive, suggesting the presence of EBV in periodontal pocket. Patients with ≥ 4 mm periodontal pockets with BOP recorded a higher EBV DNA positivity rate (15.8%) than those without ≥ 4 mm periodontal pockets with BOP (6.5%). However, no significant difference was found between EBV DNA positivity and BOP or periodontal pockets with BOP.

EBV DNA was detected in 3 of 43 dependent older people (7.0%). EBV DNA was not significantly associated with clinical factors. People with poor oral hygiene showed increased EBV DNA-positive rates (33.3%) compared with those with good or fair oral hygiene statuses (9.1% and 3.4%, respectively), but the association was not significant. EBV DNA was not significantly associated with oral health status (e.g., remaining teeth, denture use, oral wetness degree and oral bacterial numbers). All EBV DNA-positive participants were positive for *F. nucleatum*. However, EBV DNA prevalence was not significantly associated with periodontal disease-related bacteria.

Conclusions: Oral EBV infection is thought to be associated with periodontitis in middle-aged and older Japanese people. EBV prevalence may be associated with enhanced pathogenicity of periodontal disease-related bacteria. Thus, regular oral health care (i.e., subgingival plaque removal using a toothbrush and subgingival scaling) is necessary not only to prevent periodontal disease, but also to prevent EBV infection in periodontal tissue. Oral EBV infection may be associated with oral hygiene in dependent older people. Additional studies are needed to clarify the relationship between EBV and oral health status in larger numbers of dependent older people.