Original Article The prevalence of oral herpes simplex virus type 1 and its association with oral hygiene and periodontal condition

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Abstract: The objective of this study was to clarify the association between the prevalence of oral herpes simplex virus type 1 (HSV-1) and oral hygiene and periodontal condition. We targeted 168 participants (median age 69 years) who visited Hiroshima University Hospital. Real time polymerase chain reaction (PCR) analysis was employed to detect HSV-1 DNA in the samples collected from the tongue dorsum. We calculated plaque control record scores by evaluating the extent of dental plaque accumulation to evaluate the oral hygiene condition. Additionally, periodontal inflamed surface area (PISA) scores, which indicate periodontitis severity, were calculated by measuring pocket depth and bleeding on probing. HSV-1 was determined as positive in 25 of 168 participants (14.9%). Participants in their 50s showed a higher prevalence rate of HSV-1 (26.3%) than the other age groups. There was no significant association between the prevalence of oral HSV-1 and clinical factors (i.e., age, sex, medical history, number of remaining teeth, denture use). HSV-1 DNA positive participants recorded higher median plaque control record scores than HSV-1 DNA negative participants. Additionally, HSV-1 DNA positive participants showed higher median PISA scores compared to HSV-1 DNA negative participants. However, there was no significant difference in plaque control record or PISA scores between HSV-1 positive and negative participants. Our results suggest that oral HSV-1 prevalence may be associated with poor oral hygiene and periodontitis in middle-aged and older people.

Keywords: HSV-1, oral hygiene, periodontitis, PISA, real-time PCR

Introduction

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are alpha herpes viruses with a doublestranded DNA genome [1]. Most HSV-1 and HSV-2 infections are latent and asymptomatic, but often cause oral and genital lesions [2, 3]. HSV has a mechanism to escape from the host innate immune system by impairing T-cell immune function [4]. Periodontitis is a chronic oral inflammatory disease that causes the destruction of the periodontal ligament and alveolar bone, and is characterized by dysbiosis of periodontal disease-related bacteria [5]. Interestingly, HSV-1 DNA has been detected in the periodontal tissue of individuals with chronic periodontitis, suggesting that HSV-1 contributes to the progression of periodontal disease [6]. Additionally, serum HSV-1 antibody-positive individuals exhibited a higher risk of periodontitis than serum HSV-1 antibody-negative individuals [7]. However, the association between the prevalence of oral HSV-1 and periodontitis has not been fully investigated in middle-aged and older Japanese people.

Oral hygiene practice (i.e., regular toothbrushing with a correct brushing technique, appropriate toothbrush, and adequate brushing time) is required to maintain good oral hygiene [8, 9]. It is speculated that inadequate oral hygiene practice causes inflammation in periodontal tissues. Therefore, poor oral hygiene may be associated with the prevalence of oral herpes virus as well as periodontopathic bacteria. However, there is limited understanding regarding the association of HSV-1 with oral hygiene in Japanese people. Therefore, the objective of this study was to elucidate the association of oral HSV-1 infection with oral hygiene condition and periodontitis in middle-aged and older Japanese people.

Materials and methods

Participants

One hundred sixty-eight patients (median age 69 years) who visited the Hiroshima University Hospital from 2019 to 2021 were targeted in this cross-sectional study. The Ethical Committee of Hiroshima University approved this study (Approval no. E-1115) and all participants signed an informed consent form before participating. In accordance with our previous study [10], dental plaque was stained using plaque disclosing solution and then plaque control record scores were calculated by assessing plaque staining at six points (mesiobuccal, mesiolingual, midbuccal, midlingual, distobuccal, and distolingual) for each individual tooth. Probing pocket depth and bleeding on probing (BOP) were examined at six points for each individual tooth. Periodontal inflamed surface area (PISA) scores were employed to assess the surface area of the bleeding periodontal pocket epithelium. PISA and periodontal epithelial surface area (PESA) scores were calculated in accordance with the methods reported by Nesse et al. [11]. Age, sex, systemic disease, denture use, and the number of remaining teeth were also examined.

DNA extraction from swab samples

Oral samples were collected non-invasively by swabbing the tongue dorsum softly using an Orcellex[®] Brush (Rovers Medical Devices, Oss, The Netherlands). Collected samples were immediately dissolved in lysis buffer (Invitrogen, Carlsbad, CA, USA) and stored at -20°C until further processing. DNA extraction was conducted using a PureLink[™] Microbiome DNA Purification Kit (Invitrogen) in accordance with the manufacturer's instructions.

Real time polymerase chain reaction (PCR) analysis

Real time PCR was performed using the Thermal Cycler Dice[®] Real Time System III (Takara, Osaka, Japan) to detect HSV-1 DNA. A standard curve for HSV-1 was generated using a dilution series of the HSV-1 DNA positive samples. The PCR amplification protocol was as follows: 95°C for 10 min, followed by 50 cycles of 95°C for 20 sec and 60°C for 1 min and 40°C for 30 sec. The sequence of primers and probes for HSV-1 used in this study was previously reported by another group as follows: Forward primer; 5'-CTGTTCTCGTTCCTC-ACTGCCT-3', Reverse primer; 5'-CAAAAACGAT-AAGGTGTGGATGAC-3', Probe; 5'-FAM-CCCTGG-ACACCCTCTTCGTCGTCAG-TAMRA-3' [12].

Human endogenous retrovirus group 3 member 1 (ERV3-1) was employed as an internal control gene. Real-time PCR analysis was performed to detect ERV3-1 using a Thermal Cycler Dice® Real Time System III (Takara) and THUNDERBIRD SYBR qPCR Mix (Toyobo Life Science, Osaka, Japan) in accordance with our previous study [13]. The amplification protocol was conducted as follows: one cycle of 95°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec. The ERV3-1 primer sequences were as follows: Forward primer; 5'-CATGGGAAGCAAGGGAACT-AATG-3', Reverse primer; 5'-CCCAGCGAGCAAT-ACAGAATTT-3'. ERV3-1 expression was confirmed in all analyzed samples.

Statistical analysis

SPSS software, version 24.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The χ^2 test or Fisher's exact test was used to analyze the association between HSV-1 and clinical factors. The Mann-Whitney U test was used to evaluate significant differences in age and the remaining teeth between HSV-1 negative and positive participants. Logistic regression analysis was conducted to examine the relationship between HSV-1 and clinical data. Clinical measurements with a P value of less than 0.20 through univariate analysis were used as explanatory variables. We conducted post hoc power analysis using G*Power (version 3.1.9.4, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Statistical power of the χ^2 test or Mann-Whitney U test was calculated from the total sample size, with an effect size of 0.5, and significance level of 0.05. Statistical power of 80% was considered acceptable [14]. Post hoc power analysis of the χ^2 test revealed that the statistical power was 99%. Additionally, post hoc power analysis of the Mann-Whitney U test revealed that the statistical power was 61%, indicating that it was below the required threshold. P values < 0.05 were considered significant.

Clinical factor (n)	HSV-1		
	Negative (143)	Positive (25)	P value
Age, median (IQR)	70 (12.3)	69 (12.0)	0.93ª
Age group, n (%)			
30s (1)	1 (0.7%)	0 (0%)	0.59 ^b
40s (8)	8 (5.6%)	0 (0%)	
50s (19)	14 (9.8%)	5 (20%)	
60s (60)	51 (35.7%)	9 (36%)	
70s (55)	46 (32.2%)	9 (36%)	
80s (24)	22 (15.4%)	2 (8%)	
90s (1)	1 (0.7%)	0 (0%)	
Gender, n (%)			
Man (11)	51 (35.7%)	10 (40%)	0.66°
Women (34)	92 (64.3%)	15 (60%)	
Hypertension (34), n (%)	28 (19.6%)	6 (24%)	0.60°
Diabetes (19), n (%)	16 (11.2%)	3 (12%)	>0.99°
Dyslipidemia (29), n (%)	23 (16.1%)	6 (24.0%)	0.39°
Cardiovascular disease (13), n (%)	9 (6.3%)	4 (16%)	0.11°
The number of remaining teeth, median (IQR)	25 (7.3)	25 (3.5)	0.27ª
Denture user (50), n (%)	44 (31%)	6 (24%)	0.64°

IQR: Interquartile range. ^aMann-Whitney U test. ^bχ² test. ^cFisher's exact test. *P*-values <0.05 were considered significant.

Results

Association between HSV-1 and clinical characteristics

The association between HSV-1 DNA positivity and clinical characteristics in participants is summarized in Table 1. HSV-1 was determined positive in 25 of 168 participants (14.9%). HSV-1 DNA was not detected in participants in their 30s and 40s. Participants in their 50s showed a higher prevalence rate of HSV-1 (26.3%) than other age groups. HSV-1 DNA positive participants showed higher prevalence rates of dyslipidemia or cardiovascular disease (24% and 16%) compared to HSV-1 DNA negative participants (16.1% and 6.3%). There was no significant association between the prevalence of oral HSV-1 and clinical factors (i.e., age, sex, medical history, number of remaining teeth, denture use).

Association between HSV-1 and periodontal condition

HSV-1 DNA positive participants recorded higher median plaque control record scores (24.0) than HSV-1 DNA negative participants (21.0) (**Figure 1A**). However, there was no significant difference in plaque control record scores between HSV-1 DNA negative and positive participants. HSV-1 DNA positive participants showed a higher rate of ≥ 6 mm deep periodontal pockets (36%) compared to HSV-1 DNA negative participants (28.7%), but no significant difference was found (Table 2). HSV-1 DNA positive participants recorded higher median PISA (178.8) and PESA scores (1210.2) compared to HSV-1 DNA negative participants (77.5 and 1022.7) (Figure 1B and 1C). However, no significant difference in PISA and PESA scores was found between HSV-1 DNA negative and positive participants. These results suggest that HSV-1 DNA positive participants were more likely to have poor oral hygiene and periodontitis than HSV-1 DNA negative participants.

Logistic regression analysis with HSV-1 as the objective variable

Logistic regression analysis with variables with a *P* value of less than 0.20 in univariate analysis (i.e., cardiovascular disease and PESA score) as explanatory variables and HSV-1 as the objective variable was performed. The results of logistic regression analysis are summarized in **Table 3**. There was no significant association between HSV-1 and cardiovascular disease and PESA score.



Figure 1. Plaque control record, PISA, and PESA scores in HSV-positive and HSV-negative participants. A. Plaque control record scores; B. PISA scores; C. PESA scores. There was no significant difference in plaque control record, PISA, or PESA scores (P=0.52, P=0.27, P=0.18) between HSV-1 DNA negative and positive participants.

Discussion

Several pathogenic bacteria are believed to facilitate the development of periodontitis [15]. Subgingival microbial complexes composed of Porphyromonas gingivalis (P. gingivalis), Treponema denticola (T. denticola), and Prevotella intermedia (P. intermedia) (so-called red complex bacteria) are highly pathogenic for periodontal disease [16]. P. gingivalis-produced lipopolysaccharide is one of the major pathogenic factors of periodontitis and enhances the production of inflammatory cytokines (i.e., IL-1 β , IL-6, and TNF- α) in immune cells [17]. Double infection of *T. denticola* and *P. intermedia* is associated with more severe periodontal inflammation in older adults [18].

Patients with poor periodontal condition recorded a higher rate of oral Epstein-Barr virus (EBV) prevalence (25.7%) than those with good periodontal condition (0.0%) [19]. Oral EBV prevalence has been associated with poor oral hygiene and increased severity of periodontal inflammation [20]. Furthermore, individuals with deep periodontal pockets with bleeding showed a higher prevalence of oral human cytomegalovirus (HCMV) (9.7%) than those without deep periodontal pockets with bleeding (0.0%) [21]. Participants with oral human herpes virus-7 (HHV-7) positivity exhibited a higher positive rate of ≥ 6 -mm-deep periodontal pockets with bleeding (25.0%) than those without oral HHV-7 (7.9%) [22]. It has been reported that HSV-1 was frequently found in deep periodontal pockets of patients with periodontitis [23]. In this study, nearly 20% of participants with ≥6-mm-deep periodontal pockets were positive for oral HSV-1. Additionally, oral HSV-1 positive participants recorded higher PISA values than HSV-1 negative participants, indicating that HSV-1 is associated with the severity of periodontitis. These results suggest that human herpes viruses such as HSV-1, EBV, HCMV, and HHV-7, as well as periodontopathic bacteria, are important factors in the progression of periodontitis.

A previous study suggested that there is a crucial relationship between oral herpes viruses and periodontopathic bacteria [24]. We previously found that co-infection of oral CMV and *P. gingivalis* was associated with active periodontitis [21]. Interestingly, co-infection of oral EBV and *P. gingivalis* is associated with diabetes in middle-aged and older people [20]. These results suggest that human herpes viruses and periodontopathic bacteria may cooperatively be involved in progression of periodontitis.

Daily tooth brushing is necessary to maintain good oral hygiene. Bristle stiffness and deflection of manual toothbrushes gradually decline as splaying of the bristles increases with extended use [25, 26]. Therefore, regular replacement of the toothbrush (i.e., at least

Devie devete Leeve ditiere	HSV-1	Dualua	
Periodontal condition	Negative (143)	Positive (25)	P-value
Probing pocket depth, n (%)			
<4 mm (52)	43 (30.1%)	9 (36%)	0.45ª
≥4 mm and <6 mm (66)	59 (41.3%)	7 (28%)	
≥6 mm (50)	41 (28.7%)	9 (36%)	
\geq 4 mm periodontal pocket with BOP (70), n (%)	59 (41.3%)	11 (44%)	0.83 ^b
\geq 6 mm periodontal pocket with BOP (32), n (%)	27 (18.9%)	5 (20%)	>0.99 ^b

 Table 2. Association between HSV-1 DNA and periodontal condition

 $^{a}\chi^{2}$ test. b Fisher's exact test. *P* values <0.05 were regarded as significant.

Table 3. Logistic regression analysis with HSV-1 as objective varia

Clinical variables	Odds ratio	95% confidence interval	P-value
PESA	1.06	0.99-1.00	0.99
Cardiovascular disease	3.17	0.88-11.5	0.08

old. Sample size calculation before starting study will be needed for future study. Finally, a causal relationship between oral HSV-1 and periodontitis could not be

every 2 months) and correct toothbrushing technique are necessary to maintain good oral health and reduce gingival inflammation. In this study, the level of dental plaque accumulation was greater in oral HSV-1 positive participants than in oral HSV-1 negative participants. It is likely that oral HSV-1 positive participants exhibit poor oral hygiene. Oral HSV-1 infection may be associated with poor oral hygiene practice in middle-aged and older people. Inflammatory periodontal tissue may act as a reservoir for HSV-1. Our results highlight the importance of maintenance of good oral hygiene to reduce the prevalence of oral HSV-1 infection. However, the impact of oral hygiene practice on oral HSV-1 infection remains unknown. Therefore, a prospective cohort study will be required to clarify the association between oral HSV-1 infection and oral health condition.

There were some limitations to this study. First, this study enrolled middle-aged and older participants with periodontitis; however, oral HSV-1 prevalence in young people remains unknown. Second, the relationship between HSV-1 and periodontopathic bacteria remains unclear. Third, the extent of the presence of HSV-1 in inflammatory periodontal pockets remains unknown. Fourth, we were unable to perform serological test using saliva and blood specimens to determine the presence of HSV-1 antibodies. Therefore, the positive rate of HSV-1 antibodies remains unknown. Furthermore, the statistical power of Mann-Whitney U test was below the required threshdetermined because this study was cross-sectional in design.

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Disclosure of conflict of interest

None.

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