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Periodontitis lesions are the main source of salivary cytomegalovirus

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Background: Herpesviruses play causal or cooperative roles in childhood infections, tumorigenesis, ulcerogenesis, and periodontitis. Saliva is a common vehicle of herpesvirus horizontal transmission, but the source of salivary herpesviruses remains obscure. To evaluate the significance of periodontal disease in shedding of oral herpesviruses, this study determined the genome-copy counts of human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV) in whole saliva of subjects with periodontitis, gingivitis, or no natural teeth.
Methods: Whole saliva was collected from 14 periodontitis patients, 15 gingivitis patients and 13 complete denture wearers. The study subjects were systemically healthy and had not received periodontal treatment in the past 3 months. Real-time TaqMan polymerase chain reaction was used to determine the salivary load of HCMV and EBV.
Results: Salivary HCMV was detected in seven (50%) periodontitis patients, but not in any gingivitis or edentulous subjects ($P < 0.001$). Salivary EBV was detected in 11 (79%) periodontitis patients, in five (33%) gingivitis patients, and in seven (54%) edentulous subjects ($P = 0.076$). Salivary samples showed copy counts of HCMV in the range of 3.3×10^3–4.2×10^4/ml and of EBV in the range of 3.6×10^2–1.6×10^9/ml.
Conclusions: HCMV and EBV are commonly present in the saliva of periodontitis patients. Periodontitis lesions of systemically healthy subjects seem to constitute the main origin of salivary HCMV, but do not comprise the sole source of salivary EBV.

Key words: cytomegalovirus; Epstein–Barr virus; periodontal disease; saliva

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Herpesvirus infections can remain asymptomatic, but still give rise to viral shedding, or can cause illness, ranging from classic infectious diseases to benign and malignant tumors (21). Human cytomegalovirus (HCMV) can cause serious infections in immunologically immature (e.g. congenital infection) and in immune-compromised hosts (e.g. acquired immune deficiency syndrome patients and organ transplant recipients). HCMV diseases in immunocompetent individuals may be more common than believed previously (15). HCMV and Epstein–Barr virus (EBV) are significantly associated with human periodontitis (20, 22).

Saliva can contain high genome-copy counts of herpesviruses, and is a vehicle for herpesviral transmission among close individuals. Medically healthy persons have revealed salivary EBV copy counts in the range of 6×10^6–2.2×10^6 per 0.5 μg DNA (24). Human herpesvirus 6 (HHV-6) and HHV-7 can occur in saliva with prevalences exceeding 90% and in concentrations of several million genome-copies (11). HHV-6 was detected in the whole saliva of 68% of healthy volunteers from Brazil (14). HHV-8 reached salivary loads of 2.6×10^6–4.1×10^6 genome-copies/ml in a renal allograft recipient (1). Individuals infected with human immunodeficiency virus (HIV) harbor higher salivary HCMV counts than non-HIV-infected persons (9, 10), and HIV-infected individuals receiving anti-retroviral therapy have higher salivary prevalence and copy counts of herpesviruses than normal subjects (12). HIV-infected individuals have been found to have salivary median genome-copy counts of 3.3×10^3/ml for HCMV and 5.3×10^5/ml for EBV (6).

Conventional periodontal treatment has reduced salivary HCMV copy counts by 65-fold and salivary EBV genome-copy counts by 13-fold (17); salivary EBV counts in two Kostmann syndrome children by more than 100-fold (26); and the average EBV counts per ml saliva from 946,000 to 9010, and six of 11 (54%) study patients showing salivary EBV pretreatment did not reveal the virus posttreatment (7). The available data point to diseased periodontal sites as an important source for oral shedding of herpesviruses.

To identify the source of oral herpesviruses, this study focused on periodontal lesions as the origin of salivary HCMV and EBV. The salivary genome-copy counts of HCMV and EBV were quantified in subjects with periodontitis, gingivitis, or no natural teeth using real-time polymerase chain reaction (PCR).

Materials and methods

Subjects

Forty-two systemically healthy individuals were enrolled in the study, including 14 subjects with chronic periodontitis (six men, eight women; ages 38–53 years), 15 with gingivitis (eight men, seven women; ages 30–41 years), and 13 with no teeth and complete dentures for at least 2 years (eight men, five women; ages 56–70 years). The periodontitis subjects had ≥ 20 teeth and at least four teeth with periodontal pocket depth > 5 mm and/or four teeth with periodontal attachment loss > 5 mm. Patients with gingivitis revealed no teeth with periodontal pocket depth > 4 mm and presented gingival bleeding on probing in ≥ 15 teeth. Patients were excluded if they were pregnant, lactating, had received periodontal or antibiotic therapy within the previous 3 months, or exhibited a systemic condition that could influence the course of periodontal disease. Written informed consent was obtained from each study subject after all procedures had been fully explained. The Institutional Internal Review and Ethics Board at the Gülhane Military Medical Academy Sciences of Dentistry approved the study.

Periodontal examination consisted of measurements of periodontal probing depth and clinical attachment level obtained at four sites per tooth, the dental plaque index, the gingival index, and per cent periodontal sites that bled on probing (18). Periodontal examination and virological sampling were performed by the same investigator.

Unstimulated whole saliva was collected in plastic tubes before periodontal probing and virological sampling.

Virological analysis

A 5′-nuclease (TaqMan) real-time PCR assay was used to determine the genomic copy counts of HCMV and EBV (8). DNA was extracted from the unstimulated saliva sample using an alkali phenol–chloroform–isoamyl alcohol procedure (16) and then dissolved in 100 μl distilled water. Previously described PCR primers and probes were employed in the study (18).

Statistical analysis

Statistical evaluation was carried out using the SPSS 15.0 statistical package (SPSS Inc, Chicago, IL). Descriptive data were reported as mean ± standard deviation, range and median. Virus genomic copy counts were normalized by conversion to natural logarithmic values. The Kruskall–Wallis test was used to compare virus occurrence across subject groups. The Mann–Whitney *U*-test with the Bonferroni correction was applied for the post-hoc comparison analysis among the groups of viruses. *P*-values < 0.05 were considered statistically significant.

Results

Whole-mouth examination of patients with periodontitis and gingivitis, respectively, showed pocket depth means of 3.3 ± 0.2 and 2.2 ± 0.1 mm, plaque index means of 1.6 ± 0.2, and 0.9 ± 0.2, gingival index means of 1.8 ± 0.1 and 1.1 ± 0.2, and percentage bleeding-on-probing means of 70 ± 6 and 17 ± 10.

Table 1 shows the logarithmically normalized copy counts of the study viruses in saliva samples from periodontitis, gingivitis, and toothless patients. HCMV was detected in 50% of the periodontitis patients but was not identified in gingivitis or in edentulous patients ($P \leq 0.001$). EBV was detected in 79% of periodontitis, in 33% of gingivitis, and in 54% of edentulous patients ($P = 0.076$). Virus-positive saliva samples showed genome-copy counts of HCMV in the range of 3.3×10^3–4.2×10^4/ml and of EBV in the range of 3.6×10^2–1.6×10^9/ml.

Patients with teeth exhibited an increased risk for harboring HCMV [odds ratio (OR) = 1.318; 95% of OR, 1.074–1.619]. The risk ratio for EBV was not affected by the presence of teeth.

Discussion

The present study identified salivary HCMV in 50% and salivary EBV in 79% of periodontitis patients. In a parallel study, the same research group detected HCMV in 53% and EBV in 60% of periodontitis lesions (18). The similar percentages of HCMV-positive salivary and periodontitis samples are consistent with a periodontitis origin of salivary HCMV. The higher EBV-positivity in samples from saliva than from periodontitis may suggest a shedding of EBV from oral sites other than the inflamed periodontium.

The presence of HCMV in the saliva of 50% of periodontitis patients, but the absence of the virus from saliva of gingivitis and edentulous subjects, strengthens the notion of periodontitis sites being the primary source of salivary HCMV, and also underscores the linkage between

Table 1. Human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV) genome-copy counts in saliva of dental patients[1]

Clinical diagnosis	% HCMV-positive subjects	Mean ± SD of HCMV in HCMV-positive subjects	Range of HCMV in HCMV-positive subjects	Median of HCMV in HCMV-positive subjects	% EBV-positive subjects	Mean ± SD of EBV in EBV-positive subjects	Range of EBV in EBV-positive subjects	Median of EBV in EBV-positive subjects
Periodontitis ($n = 14$)	50	9.28 ± 1.10	8.10–10.65	8.79	79	11.69 ± 3.41	5.89–18.15	12.26
Gingivitis ($n = 15$)	0	0	0	N/A	33	10.40 ± 3.45	5.74–13.67	11.04
Toothless ($n = 13$)	0	0	0	N/A	54	12.75 ± 4.41	8.19–21.19	12.64

N/A, not applicable.
[1]HCMV and EBV genome-copy counts are expressed natural logarithmically; *P*-values among patient groups are < 0.001 for HCMV and 0.076 for EBV.

HCMV and periodontitis (22). Periodontal HCMV resides in T cells and macrophages (3), and increasing disease severity is associated with higher inflammatory cell and HCMV genome-copy counts (22). Gingivitis sites that are not associated with alveolar bone loss demonstrate low levels of HCMV and other herpesviruses (22), which in part may account for the non-progressiveness of the disease. Tissue/site tropism of HCMV may explain the propensity of the virus to colonize and produce disease in discrete periodontal sites.

EBV inhabited 60% of deep periodontal pockets and reached levels of 2.1×10^3–8.3×10^8/ml in advanced lesions (18). As the periodontal pocket content is emptied into saliva every 90 s (5), it is reasonable to expect egress of EBV from periodontal sites. Conventional periodontal treatment suppressed salivary EBV to undetectable levels in six of 11 patients (7), probably as the result of a decline in periodontal clinical inflammation and EBV-infected inflammatory cells. The five patients, who revealed EBV post-treatment, may have received insufficient periodontal therapy, or EBV-infected, non-periodontal sites may have sustained the shedding into saliva. The detection of salivary EBV in 54% of the edentulous study subjects points to a non-periodontal source of the virus. EBV can infect tonsil epithelium (13) and normal oral epithelium (25), and may egress from these sites.

The finding of periodontitis lesions being the major source of salivary herpesviruses provides the rationale for new approaches to herpesvirus disease prevention. Sunde et al. (23) treated a patient, who exhibited refractory periodontitis and high EBV periodontal pocket counts, with valacyclovir-HCl, 500 mg twice a day for 10 days. The treatment suppressed periodontal EBV to undetectable levels for at least 1 year and resulted in a 'dramatic' clinical improvement of the periodontal disease (23). Anti-herpesvirus chemotherapy can decrease the salivary viral load as well. A short course of valacyclovir, 2 g twice on the day of treatment and 1 g twice the following day, resulted in a significant decrease in the salivary occurrence of EBV compared with controls (11). Valacyclovir, 500 mg orally twice daily for 1 month, given to elite male distance runners, reduced the salivary load of EBV by 82% compared with placebo (4). Valacyclovir therapy, 3 g per day for 14 days, caused more than a 100-fold reduction of EBV genome-copies in oral wash fluid of patients with acute infectious mononucleosis (2). Treatment of periodontitis with both conventional antibacterial debridement and an antiviral drug such as valacyclovir may reduce the risk of herpesvirus shedding and infection of close individuals.

The present findings have potentially important public-health implications. The potential of periodontal therapy to reduce or eliminate gingival inflammation and decrease herpesvirus salivary levels may reduce the risk of horizontal transmission of herpesviruses and spread of herpesvirus-related diseases. Gingival inflammation can be controlled in most individuals by simple and low-cost antimicrobial treatments that have virtually no adverse effects and lend themselves to self-administration (19).

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