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Periodontitis lesions are a source of salivary cytomegalovirus and Epstein–Barr virus

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Aim: Several herpesvirus species can be detected in periodontal pockets and saliva. This study compared human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV) DNA copy counts in periodontitis sites and in whole saliva, and evaluated the potential of periodontal therapy to reduce the salivary level of the two viruses.

Material and methods: A total of 20 systemically healthy periodontitis patients, 21–56 years of age, participated in the study. All 20 patients were examined at baseline, and seven patients also at 3 months after periodontal therapy. Treatment included oral hygiene instruction, scaling and root planing, and surgery. Clinical parameters were evaluated using established methods. In each patient, virological samples were collected from one periodontal pocket of 6–10 mm probing depth, from the adjacent inflamed periodontal pocket wall, and from unstimulated whole saliva. Relationships between subgingival, gingival tissue and salivary herpesvirus counts were evaluated using Spearman's and Kendall's rank correlation coefficient tests. The 5′-nuclease (TaqMan®) real-time polymerase chain reaction (PCR) assay was employed to quantify genomic copies of periodontal HCMV and EBV.

Results: At baseline, the 20 periodontitis patients showed significant positive correlations between gingival tissue and salivary counts of HCMV DNA ($p = 0.003$) and EBV DNA ($p = 0.045$). Periodontal pocket depth was positively correlated with salivary EBV DNA counts ($p = 0.002$). Periodontal therapy reduced average full-mouth periodontal pocket depth from 4.6 mm to 1.4 mm, plaque index from 2.1 to 0.9, and gingival index from 2.1 to 0.4. Following treatment, HCMV DNA counts decreased 37.5 fold in subgingival sites and 64.6 fold in saliva, and EBV DNA counts decreased 5.7 fold in subgingival sites and 12.9 fold in saliva.

Conclusions: The present study provides compelling evidence of a periodontitis source for salivary HCMV and EBV. The potential of periodontal therapy to decrease herpesvirus salivary counts may help diminish herpesvirus transmission from person to person and herpesvirus-related diseases in exposed individuals. Further research is warranted to determine the relationship between periodontal herpesvirus counts and the risk of viral transmission to close acquaintances.

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Human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV), two members of the Herpesviridae family, can cause serious infectious diseases in immunologically immature and immunocompromised individuals, and may contribute to oncogenesis (1). In immunocompetent individuals, an HCMV infection rarely results in clinical disease, except in newborns, where HCMV represents the major infectious cause of pregnancy complications and

birth defects (2). HCMV comprises the most common life-threatening infection in HIV-infected patients and, due to reactivation from immunosuppressive chemotherapy, is the most frequent infectious reason for rejection of solid organ transplants and bone marrow and stem cell grafts (3). HCMV may also participate in the development of primary atherosclerosis, postangioplasty restenosis, and post-transplantation arteriosclerosis (4). EBV is the main causative agent of infectious mononucleosis in adolescents and young adults (5). EBV may also mediate oncogenic growth, as evidenced by its close relationship with certain tumors arising in lymphoid tissue, such as Burkitt's lymphoma, post-transplant B-cell lymphoma and Hodgkin's disease, and in epithelial tissue, such as undifferentiated nasopharyngeal carcinoma and various types of gastric carcinoma (6).

Herpesviruses are transmitted from person to person during the period of primary infection or episodes of reactivation. Individuals frequently acquire herpesviruses at an early age, sometimes *in utero* (7). Over the lifetime of the infected host, bursts of herpesvirus reactivation may occur with no evidence of clinical disease, but with low-level infection having the potential to spread to close acquaintances. HCMV seroconversion takes place in all age groups and, in Germany, with relatively high frequency in 30–35-year-old individuals (8).

Salivary gland tissue and ductal epithelial cells have long been assumed to be the main reservoirs for salivary herpesviruses (9). Saliva of both immunocompetent and immunocompromised subjects may periodically contain infectious herpesvirus species, and serve as a vehicle for viral transmission from person to person (10, 11). Acquisition of EBV often occurs through salivary exchange in the oropharynx (12), and mononucleosis is sometimes referred to as the 'kissing disease' (13). Breast feeding (14) and sexual contact (15) constitute other risk events for herpesvirus transmission.

Studies have identified HCMV and EBV in advanced types of marginal and periapical periodontitis, acute necrotizing ulcerative gingivitis, and periodontal abscesses (1). Particularly high herpesvirus DNA loads are detected in aggressive periodontitis lesions (16). Data also indicate that periodontal therapy can result in a marked decrease in subgingival herpesvirus counts, often to undetectable levels even by sensitive polymerase chain reaction (PCR) techniques (17–20). A reduction in EBV subgingival counts seems to parallel a decrease in salivary EBV counts (20). Those findings raise the intriguing possibility that, by reducing subgingival and salivary herpesvirus counts, periodontal treatment may diminish the risk of infecting close acquaintances. To begin testing the hypothesis that the inflamed periodontium is a source of salivary herpesviruses, we studied the correlation between HCMV and EBV periodontal and salivary counts, and determined the effect of periodontal therapy on periodontal and salivary herpesvirus counts. A 5′-nuclease (TaqMan®, Roche Diagnostics, Basel, Switzerland) real-time PCR assay was used to identify and quantify HCMV and EBV DNAs.

Material and methods

The present study included 20 periodontitis patients, 21 to 56 years of age. The clinical characteristics of the patients have been described previously (16). All study patients were systemically healthy. 9 individuals below 35 years of age were assigned the diagnosis of aggressive periodontitis, and 11 individuals 36-56 years of age the diagnosis of chronic [adult] periodontitis. Periodontal therapy consisted of oral hygiene instruction, full-mouth scaling and root planing, and modified Widman flap surgery of all deep periodontal sites. 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.

Virological samples were obtained following presurgical depuration, but prior to definitive periodontal treatment. Immediately prior to periodontal surgery, unstimulated whole saliva was collected in a glass cylinder, followed by a periodontal pocket sample from the single deepest probing site in the dentition (6–10 mm probing depths). After removing supragingival plaque with sterile cotton pellets, a sterile periodontal curette was gently inserted to the bottom of the test periodontal pocket, and subgingival material was removed by a single stroke. Subsequently, a gingival tissue specimen was harvested in conjunction with surgery (21). After raising buccal and palatal gingival flaps, a total of 36–50 mg (mean, 40 mg) of pocket epithelium, underlying connective tissue, and granulation tissue was removed by a periodontal curette from the periodontal lesion. At 3 months posttreatment, 2 aggressive and 5 chronic periodontitis patients were available for virological sampling of the test subgingival sites and saliva, as described above.

A 5′-nuclease (TaqMan®) real-time PCR assay was used to identify and quantify genomic copies of HCMV and EBV. DNA extraction, PCR primers, TaqMan probes and TaqMan real-time assay conditions are described elsewhere (16). The relationship between subgingival, gingival tissue and salivary herpesvirus counts was evaluated using the non-parametric Spearman's and Kendall's tau rank correlation coefficient (SPSS 10.0 statistical package; SPSS Inc, Chicago, IL, USA). p-values equal to or less than 0.05 were considered statistically significant.

Results

At baseline, most of the 20 study patients revealed HCMV DNA and EBV DNA in periodontal samples and in saliva (Table 1). Significant positive correlations were identified between gingival tissue and salivary levels of HCMV ($\zeta = 0.63, p = 0.003$) and EBV ($\zeta = 0.45, \quad p = 0.045$). Periodontal pocket depth at sample sites correlated with EBV DNA counts in saliva ($\zeta = 0.65, p = 0.002$). In the subgroup of nine aggressive periodontitis patients, HCMV DNA counts in gingival tissue were positively correlated with HCMV

Table 1. Human cytomegalovirus [HCMV] and Epstein–Barr virus [EBV]DNA copy counts in subgingival sites, gingival tissue and saliva from 20 untreated periodontitis patients (baseline data)

Patient number	Patient age in years	HCMV DNA, subgingival counts/ml	HCMV DNA, gingival tissue counts	HCMV DNA, salivary counts/ml	EBV DNA, subgingival counts/ml	EBV DNA, gingival tissue counts	EBV DNA, salivary counts/ml
1	34	34,000	750,000	2,000,000	2,400	17,000	430,000
2	27	2,800,000	110,000	110,000	10,000	7,700	540,000
3	34	0	0	0	2,800	0	0
4	32	0	0	33,000	0	16,000	55,000,000
5	32	3,400	0	23,000	3,700	40,000	43,000
6	24	5,200	540,000	40,000	10,000	260,000	0
7	34	280	0	2,600	6,500	0	43,000
8	21	13,000	0	0	9,100	27,000	77,000
9	34	690	0	160,000	12,000	5,000	240,000
10	49	3,200	7,300	520,000	5,100	0	0
11	36	0	0	100,000	2,900	0	85,000
12	51	0	0	25,000	0	6,400	0
13	40	3,400	0	0	730	0	54,000
14	36	0	0	730	0	3,400	24,000
15	36	0	0	41,000	0	28,000	36,000
16	45	0	0	0	0	0	0
17	56	2,600	0	0	0	0	0
18	56	0	0	0	3,200	13,000	20,000,000
19*	40	24,000	0	110,000	20,000	820,000,000	470,000,000
20	47	770	0	0	0	0	0

*The patient showed a rapid breakdown of periodontal attachment and alveolar bone and had not received presurgical periodontal depuration prior to virological sampling.

DNA counts in saliva ($\zeta = 0.83$, $p = 0.006$). The 11 chronic periodontitis patients showed positive correlation between periodontal pocket depth at sample sites and EBV DNA counts in saliva ($\zeta = 0.65$, $p = 0.03$). No statistical correlations were found between HCMV or EBV DNA counts in periodontal pockets and saliva.

Periodontal therapy caused a reduction in full-mouth average periodontal pocket depth from 4.6 mm to 1.4 mm, in plaque index from 2.1 to 0.9, and in gingival index from 2.1 to 0.4. Following treatment, HCMV DNA counts decreased 37.5 fold in subgingival sites and 64.6 fold in saliva, and EBV DNA counts decreased 5.7 fold in subgingival sites and 12.9 fold in saliva (Table 2).

Discussion

The present study identified significant correlations between gingival tissue and salivary DNA copy counts of HCMV and EBV in untreated periodontitis patients. Also, to statistically predict their salivary presence, HCMV and EBV only required sampling from a single periodontitis lesion, whereas periodontopathic bacteria seem to necessitate sampling of several subgingival sites (22). It may be that sampling of additional periodontal sites in each untreated patient also would produce significant correlations between subgingival and salivary HCMV and EBV DNA counts. At any rate, the lack of a statistical correlation between subgingival and salivary herpesvirus copy counts is

Table 2. Human cytomegalovirus [HCMV] and Epstein–Barr virus [EBV]DNA copy counts in subgingival sites and saliva from 7 periodontitis patients at baseline and at 3 months following periodontal therapy

Patient number	HCMV DNA, subgingival counts/ml – Pretreatment	HCMV DNA, subgingival counts/ml – Posttreatment	HCMV DNA, salivary counts/ml - Pretreatment	HCMV DNA, salivary counts/ml - Posttreatment	EBV DNA, subgingival counts/ml – Pretreatment	EBV DNA, subgingival counts/ml – Posttreatment	EBV DNA, salivary counts/ml - Pretreatment	EBV DNA, salivary counts/ml - Posttreatment
2	2,800,000*	210	110,000	1,200	10,000	720	540,000	66,000
8	13,000	0	0	0	9,100	450	77,000	1,700,000**
10	3,200	570	520,000	3,500	5,100	0	0	0
11	0	550	100,000	0	2,900	3,900	85,000	7,600
17	2,600	0	0	6,600	0	1,600	0	0
18	0	0	0	1,700	3,200	2,100	20,000,000	94,000
19	24,000	0	110,000	0	20,000	0	470,000,000*	0

*'Outlier' number was not included in the calculation of the pretreatment average count of viral DNA.
**EBV DNA was not detected at 4 months posttreatment.

consistent with little or no salivary contamination of periodontal sites during the virological sampling. The available data point to a close relationship between HCMV and EBV in inflamed periodontal tissue and in saliva, and provide evidence at least for a partial periodontal origin of salivary herpesviruses.

The present finding of a reduction of salivary EBV counts after periodontal therapy has been described previously (20). However, at 3 months post-treatment, the seven study patients demonstrated some degree of gingival inflammation, as measured by the gingival index. The employment of more diligent oral hygiene efforts (23) may yield an even greater reduction in salivary herpesvirus counts than reported here. Moreover, the oropharyngeal epithelium of asymptomatic virus carriers can sustain an EBV infection and may play a role in the egress of EBV into saliva (24), which may partly explain the relatively high post-treatment counts of salivary EBV in some study patients. It needs to be determined if periodontitis patients with high post-treatment counts of oral herpesviruses are at greater risk of disease recurrence than treated patients with little or no detectable herpesvirus presence.

The gingival tissue–salivary herpesvirus linkage gives a reason for reconsidering the long-standing belief that salivary glands comprise the main nidus of salivary herpesviruses (9, 25). Undoubtedly, mumps (26, 27) and other acute viral infections of the salivary glands (28–30) give rise to the excretion of specific pathogenic viruses into saliva. However, normal salivary gland tissue may only contain sparse amounts of HCMV (31, 32) and EBV (33–35). In the present study, abundant herpesviruses were detected in saliva of periodontitis patients showing no evidence of salivary gland disease. Similar to herpesviruses, TT viral DNA counts in gingival tissue seem to be related to the presence of periodontitis and to the salivary level of the virus (35). Salivary hepatitis C virus may emanate from the periodontium as well (36). It may be that periodontal sites with destructive disease comprise a main source for various viruses in saliva of normal individuals.

Since saliva constitutes an important vehicle for herpesvirus transmission from person to person, the potential of periodontal therapy to suppress salivary herpesvirus counts may have important public health implications. Reducing or eliminating the viral load in saliva may significantly diminish the risk for or, at least, delay viral transmission among individuals in close personal contact, and help reduce the incidence of periodontitis and non-oral viral diseases in exposed subjects. However, since periodontal therapy may merely postpone herpesvirus transmission to an older age where acute viral infections may cause accentuated disease (37–40), the life-long health benefit of suppressing salivary herpesviruses through periodontal therapy needs to be determined. Nonetheless, as recently reported (8), HCMV seroconversion may take place in virtually all age groups without extraordinary complications, at least in immunocompetent healthy individuals.

In conclusion, the present findings are consistent with the notion that periodontitis lesions are sites for HCMV and EBV oral persistence and constitute an important source of infective herpesviruses in saliva. Since herpesvirus species frequently spread between individuals through salivary contact, our findings provide the rationale for prompting larger studies to determine whether antimicrobial periodontal therapy and follow-up care, with subsequent reduction in salivary herpesvirus load, may decrease the rate of herpesvirus transmission and related oral and non-oral diseases among close family members and acquaintances.

References

1. Slots J. Herpesviruses in periodontal diseases. *Periodontol 2000* (in press).
2. Gaytant MA, Steegers EA, Semmekrot BA, Merkus HM, Galama JM. Congenital cytomegalovirus infection. review of the epidemiology and outcome. *Obstet Gynecol Surv* 2002;**57:**245–256.
3. Froberg MK. Review: CMV escapes! *Ann Clin Lab Sci* 2004;**34:**123–130.
4. O'Connor S, Taylor C, Campbell LA, Epstein S, Libby P. Potential infectious etiologies of atherosclerosis: a multifactorial perspective. *Emerg Infect Dis* 2001;**7:**780–788.
5. Papesch M, Watkins R. Epstein–Barr virus infectious mononucleosis. *Clin Otolaryngol* 2001;**26:**3–8.
6. Pagano JS. Epstein–Barr virus: the first human tumor virus and its role in cancer. *Proc Assoc Am Physicians* 1999;**111:**573–580.
7. Gabrielli L, Lazzarotto T, Foschini MP *et al*. Horizontal in utero acquisition of cytomegalovirus infection in a twin pregnancy. *J Clin Microbiol* 2003;**41:**1329–1331.
8. Hecker M, Qiu D, Marquardt K, Bein G, Hackstein H. Continuous cytomegalovirus seroconversion in a large group of healthy blood donors. *Vox Sang* 2004; **86:**41–44.
9. Morgan DG, Niederman JC, Miller G, Smith HW, Dowaliby JM. Site of Epstein–Barr virus replication in the oropharynx. *Lancet* 1979;**2:**1154–1157.
10. Gautheret-Dejean A, Aubin JT, Poirel L *et al*. Detection of human Betaherpesvirinae in saliva and urine from immunocompromised and immunocompetent subjects. *J Clin Microbiol* 1997;**35:**1600–1603.
11. Ikuta K, Satoh Y, Hoshikawa Y, Sairenji T. Detection of Epstein–Barr virus in salivas and throat washings in healthy adults and children. *Microbes Infect* 2000;**2:**115–120.
12. Rickinson E, Kieff E. Epstein–Barr virus. In: Knipe DM, Howley PM, eds. *Fields virology*, 4th edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2001: 2575–2627.
13. Carbary LJ. Infectious mononucleosis: the kissing disease. *Nurs Care* 1975;**8:**22–24.
14. Stagno S, Reynolds DW, Pass RF, Alford CA. Breast milk and the risk of cytomegalovirus infection. *N Engl J Med* 1980; **302:**1073–1076.
15. Fowler KB, Pass RF. Sexually transmitted diseases in mothers of neonates with congenital cytomegalovirus infection. *J Infect Dis* 1991;**164:**259–264.
16. Kubar A, Saygun I, Özdemir A, Yapar M, Slots J. Real-time PCR quantification of human cytomegalovirus and Epstein–Barr virus in periodontal pockets and the adjacent gingiva of periodontitis lesions. *J Periodont Res* 2005;**40:**97–104.
17. Pacheco JJ, Coelho C, Salazar F, Contreras A, Slots J, Velazco CH. Treatment of Papillon–Lefevre syndrome periodontitis. *J Clin Periodontol* 2002;**29:**370–374.
18. Yapar M, Saygun I, Özdemir A, Kubar A, Sahin S. Prevalence of human herpesviruses in patients with aggressive periodontitis. *J Periodontol* 2003;**74:**1634–1640.

19. Saygun I, Yapar M, Özdemir A, Kubar A, Slots J. Human cytomegalovirus and Epstein–Barr virus type 1 in periodontal abscesses. *Oral Microbiol Immunol* 2004;**19:**83–87.
20. Idesawa M, Sugano N, Ikeda K *et al.* Detection of Epstein–Barr virus in saliva by real-time PCR. *Oral Microbiol Immunol* 2004;**19:**230–232.
21. Ramfjord SP, Nissle RR. The modified Widman flap. *J Periodontol* 1974;**45:**601–607.
22. Umeda M, Contreras A, Chen C, Bakker I, Slots J. The utility of whole saliva to detect the oral presence of periodontopathic bacteria. *J Periodontol* 1998; **69:**828–833.
23. Slots J, Jorgensen MG. Effective, safe, practical and affordable periodontal antimicrobial therapy: where are we going, and are we there yet? *Periodontol 2000* 2002;**28:**298–312.
24. Pegtel DM, Middeldorp J, Thorley-Lawson DA. Epstein–Barr virus infection in ex vivo tonsil epithelial cell cultures of asymptomatic carriers. *J Virol* 2004; **78:**12613–12624.
25. Wilmes E, Wolf H. [Epstein–Barr virus infections. New pathogenic and clinical aspects]. *Laryngorhinootologie* 1989;**68:** 36–43 (German).
26. Afzal MA, Buchanan J, Dias JA *et al.* RT–PCR based diagnosis and molecular characterisation of mumps viruses derived from clinical specimens collected during the 1996 mumps outbreak in Portugal. *J Med Virol* 1997;**52:**349–353.
27. Lee JY, Na BK, Kim JH *et al.* Regional outbreak of mumps due to genotype H in Korea in 1999. *J Med Virol* 2004;**73:**85–90.
28. Nigatu W, Nokes DJ, Enquselassie F *et al.* Detection of measles specific IgG in oral fluid using an FITC/anti-FITC IgG capture enzyme linked immunosorbent assay (GACELISA). *J Virol Meth* 1999; **83:**135–144.
29. Chernetsova OV, Lopatkina TN, Popova IV, Vorob'ev AA, Shipulina O, Iu Safonova TN. [The rate of HCV-RNA detection in the serum, saliva, and tissue of the minor salivary glands in chronic hepatitis C with Sjogren's syndrome]. *Klin Med (Mosk)* 2003;**81:**37–40 (Russian).
30. Torres JR, Liprandi F, Goncalvez AP. Acute parotitis due to dengue virus. *Clin Infect Dis* 2000;**31:**E28–E29.
31. Mariette X, Cazals-Hatem D, Agbalika F *et al.* Absence of cytomegalovirus and Epstein–Barr virus expression in labial salivary glands of patients with chronic graft-versus-host disease. *Bone Marrow Transplant* 1996;**17:**607–610.
32. Karja V, Syrjänen K, Syrjänen S. No Epstein Barr and cytomegalovirus DNA found in salivary gland tumours. *ORL J Otorhinolaryngol Relat Spec* 1997;**59:**97–99.
33. Merne ME, Syrjänen SM. Detection of Epstein–Barr virus in salivary gland specimens from Sjogren's syndrome patients. *Laryngoscope* 1996;**106:**1534–1539.
34. Liavaag PG, Cheung RK, Kerrebijn JD, Freeman JL, Irish JC, Dosch HM. The physiologic reservoir of Epstein–Barr virus does not map to upper aerodigestive tissues. *Laryngoscope* 1998;**108:**42–46.
35. Rotundo R, Maggi F, Nieri M, Muzzi L, Bendinelli M, Pini Prato GP. TT virus infection of periodontal tissues: a controlled clinical and laboratory pilot study. *J Periodontol* 2004;**75:**1216–1220.
36. Maticic M, Poljak M, Kramar B *et al.* Detection of hepatitis C virus RNA from gingival crevicular fluid and its relation to virus presence in saliva. *J Periodontol* 2001;**72:**11–16.
37. Lin YJ, Huang LM, Lee CY *et al.* A seroepidemiological study of varicella-zoster virus in Taipei City. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi* 1996;**37:**11–15.
38. Darby SC, Ewart DW, Giangrande PL, Spooner RJ, Rizza CR. Importance of age at infection with HIV-1 for survival and development of AIDS in UK haemophilia population. UK Haemophilia Centre Directors' Organisation. *Lancet* 1996; **347:**1573–1579.
39. Stark GL, Wood KM, Jack F *et al.* Hodgkin's disease in the elderly: a population-based study. *Br J Haematol* 2002;**119:**432–440.
40. Ouyang Q, Wagner WM, Walter S *et al.* An age-related increase in the number of CD8+ T cells carrying receptors for an immunodominant Epstein–Barr virus (EBV) epitope is counteracted by a decreased frequency of their antigen-specific responsiveness. *Mech Ageing Dev* 2003;**124:**477–485.