Human Cytomegalovirus-Associated Periodontitis in Renal Transplant Patients

H. Nowzari, M.G. Jorgensen, S. Aswad, N. Khan, E. Osorio, A. Safarian, H. Shidban, and S. Munroe

ABSTRACT

Background. Human cytomegalovirus (HCMV) infection is associated with renal transplant failure. Periodontal pockets may be reservoirs for HCMV replication.

Objectives. This study was done to determine active HCMV replication in saliva and gingival crevicular fluid of renal transplant patients affected by periodontitis.

Methods. HCMV pp67-mRNA amplification was analyzed in oral fluids of 38 transplant recipients at 6 months' posttransplantation. Patients received antiviral therapy until 3 months' posttransplantation. The HCMV-positive cell line VR-977 was the positive control, and oral fluids from healthy volunteers served as the negative control. Periodontitis was diagnosed by clinical examination. Serum HCMV IgG and IgM were analyzed to differentiate recent and latent infection.

Results. Prevalence of gingival overgrowth was 68.4%. HCMV gene transcripts were detected in the saliva of 21% and the gingival crevicular fluid of 18% of patients. All patients (100%) with HCMV pp67-mRNA detected in saliva demonstrated clinical manifestations of viral infection, as did 86% of patients with HCMV pp67-mRNA detected in the gingival crevicular fluid. Serum IgM was positive in 7.9% of patients and IgG in 65.8%; however, associations with active mRNA replication were not statistically significant.

Conclusions. Renal transplant patients affected by periodontitis are at risk of viral replication within the periodontal tissues despite antiviral therapy. This study suggests that use of HCMV pp67-mRNA detection in saliva and gingival crevicular fluid provides markers of active viral infection, and evidence for a link between HCMV-associated periodontitis and renal transplant complications.

HUMAN cytomegalovirus (HCMV) is emerging as an important pathogen in immunocompromised individuals and organ transplant patients.[1] Active viral infection is frequently associated with severe clinical consequences including transplant failure. Stress, immunosuppression, or immune dysfunction may trigger viral activation, causing vascular damage to both the transplant and the host. In organ transplant patients, HCMV infection is often complicated by invasive visceral disease.

Jiwa et al[2] detected HCMV viremia in 21% of bone marrow transplant patients and 21% of kidney transplant patients. Wolf and Spector[3] reported that among 11 patients who were virus-positive by plasma PCR, 8 (73%) subsequently developed HCMV disease. In bone marrow transplant recipients, plasma-PCR was associated with a positive predictive value of 60% for disease development and a negative predictive value of 97%. The PCR detection preceded disease development by a median interval of 3 weeks.

Kas-Deelen et al[4] identified cytomegalic endothelial cells in blood samples of 54 kidney transplant recipients. Cytomegalic endothelial cells were detected in patients affected by moderate or high HCMV antigenemia. The occurrence of rejection episodes before HCMV infection was an im-

From the University of Southern California School of Dentistry (H.N., M.G.J., A.S., S.M.) and National Institute of Transplantation (S.A., N.K., E.D., H.S.), Los Angeles, California.

Supported by the National Institute of Transplantation.

Address reprint requests to Michael G. Jorgensen, USC School of Dentistry, 925 West 34th Street, Room 4274, Los Angeles, CA 90089-0641. E-mail: jorgensm@usc.edu

0041-1345/03/$–see front matter
doi:10.1016/j.transproceed.2003.10.026

portant risk factor for the occurrence of cytomegalic endothelial cells in blood during HCMV infection ($P < .001$).

Recent studies by Contreras et al[5–7] have presented strong evidence for the role of herpesviruses in the pathogenesis of human periodontal disease. Hanookai et al[8] studied active periodontitis lesions in 19 trisomy-21 patients detecting HCMV in 5 (26%) patients. In healthy periodontal sites, only one revealed HCMV. Subgingival debridement using a combination of hand and ultrasonic instruments did not reduce the presence of genomic herpesvirus. The investigators suggested that viral infection may reduce periodontal defense mechanisms and promote growth of putative periodontopathic bacteria such as *Tenarella forsythensis* (formerly *Bacteroides forsythus*), *Prevotella intermedia,* and *Capnocytophaga* species. Viral-bacterial co-infections were observed in trisomy-21–associated destructive periodontal disease. HCMV–*Actinobacillus actinomycetemcomitans* co-infection in localized aggressive periodontitis was reported by Nowzari et al in a patient suffering from Fanconi anemia.[9]

Contreras et al[10] examined biopsies of periodontitis lesions from 20 adults and detected HCMV in periodontal tissue biopsies and gingival cell fractions separated by immunomagnetic cell sorting. Periodontitis-derived monocytes and macrophages revealed HCMV in 11 (55%) patients. T lymphocytes harbored HCMV in 4 (20%) patients. The researchers suggested that HCMV mainly infects periodontal monocytes, macrophages, and less frequently T lymphocytes.

In a follow-up study, HCMV was detected in 9 (64%) periodontal pocket samples and 12 (86%) gingival tissue samples obtained from periodontitis patients by Contreras et al, who confirmed the frequent presence of HCMV in periodontitis lesions.[11]

Medications used in transplantation seem to potentiate gingival overgrowth.[12,13] Explanatory hypotheses include drug-induced gingival fibroblast proliferation, impaired collagen degradation, deficiency in collagen phagocytosis by fibroblasts, impaired collagenase activity, intracellular calcium imbalance, and nitric oxide activity and production. Thomason et al[14] and Yamada et al[15] reported altered expression of MMP-1 and TIMP-1, important mediators of periodontal collagen, by cyclosporine (CyA), an antirejection immunosupressive agent in allogenic organ transplantation. Cyclosporine primarily suppresses the cell-mediated immune system and inhibits the production and release of cytokines. The specific lymphocytes that are inhibited are the T-helper cells.

Based on available information, it seems reasonable to hypothesize that periodontal pockets may be important reservoirs for HCMV infection in organ transplant patients affected by periodontitis. The objective of this study was to determine active HCMV replication in saliva and gingival crevicular fluid of renal transplant patients affected by periodontitis and to relate these finding to clinical viral infection.

MATERIALS AND METHODS

The protocol for this investigation was approved by the Internal Review Board of the University of Southern California. Thirty-eight renal transplant recipients (14 women, 24 men, ages: 18 to 65 years) participated after giving written informed consent. Medical history and current medications were determined by chart review and patient interview. All patients had received 200 to 400 mg Zovirax, acyclovir, or ganciclovir per day as prophylactic antiviral therapy for the first 3 months posttransplantation and were currently receiving CyA as a component of a triple immunosuppressive regimen. Following clinical examination, a dichotomous scoring system was used to record gingival overgrowth by calibrated periodontists (100% reproducibility). Demographic data were obtained from medical charts and by interview. Saliva and gingival crevicular fluid samples were collected at least 6 months posttransplantation and processed using automated NucliSens Extractor protocol for nucleic acid isolation. Saliva was collected using sterile cotton swabs; gingival crevicular fluid was obtained by placing sterile endodontic paper points subgingivally in three sites per patient. Amplification and detection of HCMV-pp67-mRNA followed the protocol of kit modules of CMV mRNA assay. The HCMV-positive cell line VR-977 was used as the positive control. Saliva and gingival crevicular fluid from healthy volunteers served as the negative control. Serum samples were analyzed for HCMV immunoglobulin G (IgG) and immunoglobulin M (IgM).

Viral Examination

Extraction of nucleic acid. Saliva was collected on sterile cotton swabs, and gingival crevicular fluid was collected on sterile endodontic paper points. Cotton swabs or paper points were placed in 900 μL of nucleic acid sequence-based amplification (NASBA) lysis buffer (4.7 mol/L guanidium thiocyanate; 46 mmol/L Tris at pH 6.4; 20 mmol/L EDTA; 1.2% Triton X-100) (BioMerieux). Samples were either frozen at −70°C until further use or processed immediately for nucleic acid extraction. Frozen samples were thawed at 37°C. After addition of silica and a standard amount of system control (SC) RNA, which served as positive control for isolation, amplification, and detection of RNA during the NASBA procedure, samples were homogenized by vortexing. Samples were washed once with wash buffer and loaded on an automated nucleic acid extractor. Total nucleic acid was eluted in 50 μL of TE buffer and stored at −70°C.

Amplification. The NASBA amplification reaction was performed with two primers that were designed to amplify part of the mRNA encoding HCMV pp67 (the UL 65 gene product). The NASBA reactions were carried out in a 20-μL reaction mixture containing 40 mmol/L Tris at pH 8.5, 12 mmol/L $MgCl_2$, 70 mmol/L KCl, 15% dimethyl sulfoxide, 5 mmol/L dithiothreitol, each deoxynucleoside triphosphate at a concentration of 1 mmol/L, 0.08U of RNase H, 32U of T7 RNA polymerase, 6.4U of avian myeloblastosis reverse transcriptase, and 5 mL of nucleic acids. Prior to the addition of enzymes, the NASBA reaction mixtures were incubated for 5 minutes at 65°C to destabilize the secondary RNA structures and then cooled for 5 minutes to 41°C to allow primer annealing. Following the addition of enzymes, the reaction mixtures were incubated at 41°C for 90 minutes and then stored at −70°C.

Detection. The amplification products (wild-type and SC RNA) were then placed in detection dilutent and incubated for 30 minutes at 41°C with a biotinylated pp67-specific capture probe bound to 5 μg streptavidin-coated magnetic beads and 3×10^{11} molecules of ruthenium-labeled oligonucleotide detection probe.

Table 1. Associations Between Clinical Viral Infection and Detection of HCMV mRNA in Saliva and Gingival Crevicular Fluid

		Clinical Viral Infection		
		No (%)	Yes (%)	*P* Value
Saliva positive HCMV mRNA	No	23 (77)	7 (23)	.0001*
	Yes	0 (0)	8 (100)	
Gingival crevicular fluid positive HCMV mRNA	No	20 (69)	9 (31)	.01*
	Yes	1 (14)	6 (86)	

*Two-sided *P* value for the Fisher exact test.

These probes were specific for either pp67-mRNA or SC RNA. As a negative control, the detection dilutent was also incubated with the wild-type RNA probe and the oligonucleotide bound to magnetic beads. Following incubation and addition of an assay buffer solution, the tubes were placed in an electrochemiluminescence instrument (NASBA QR System, bioMerieux) for final reading of the results.

RESULTS

The HCMV-active gene transcripts were detected in saliva of 8 (21%) and gingival crevicular fluid of 7 (18%) patients. Prevalence of gingival overgrowth was 68.4% (n = 26). The IgG serum antibodies for HCMV were detected in 25 patients (65.8%); IgM serum antibodies for HCMV were detected in 3 patients (7.9%). There was no statistically significant association between IgM and IgG HCMV mRNA and salivary/crevicular fluid HCMV mRNA.

Clinical viral infections were observed in 15 patients (39.5%), all of whom were diagnosed with gingival overgrowth ($P < .00001$).

The Fishers exact test assessed statistically significant associations between clinical viral infection and detection of HCMV pp67-mRNA in either saliva ($P = .0001$) or in gingival crevicular fluid ($P = 0.01$) (Table 1). Active viral infection and clinical complications did not show a statistically significant association with age (Wilcoxon rank sum test, $P > .05$). There was also no statistically significant association between active viral detection and any of the three following medications: prednisone, Norvasc (amlodipine), and Procardia (nifedipine).

DISCUSSION

Renal transplant patients affected by periodontitis are at risk of harboring active HCMV regardless of prior prophylactic antiviral therapy. Although patients in the present study received systemic antiviral therapy for the first 3 months' posttransplantation, overgrown gingival tissue and periodontal pockets were sites of active HCMV replication.

The danger of CMV infections for organ transplant patients has been extensively reported. Yilmaz et al, in a controlled study using rats, observed a significant increase in acute rejection episodes and complications in RCMV-infected transplant recipients.[16] Rosen and co-workers[17] reported that CMV genotype B was associated with an increased frequency of systemic infection leading to rejection of liver transplants. Jeejeebhoy[18] described an episode of CMV-associated thrombotic microangiopathy 6 weeks' posttransplantation followed by CMV-associated pneumonitis. Subsequent treatment of the CMV infection resolved the thrombotic microangiopathy. Peggs et al[19] proposed extended PCR surveillance and pre-emptive anti-CMV therapy after transplantation to decrease the morbidity associated with prolonged ganciclovir administration.

In the present study, transplant complications requiring urgent hospitalization were observed in 15 patients. All of these patients were diagnosed with gingival overgrowth and periodontitis. HCMV-associated periodontitis was diagnosed in 6 of these patients. Active viral infection was detected in four saliva samples and in three gingival crevicular fluid samples from areas of gingival overgrowth. Overgrown gingival tissue may be an indicator of viral activity and the oral cavity may serve as a reservoir for the virus at times when it is not detected in the serum.

The incidence of CyA-associated gingival overgrowth is reported to vary from 27% to 49%.[20] The higher prevalence of gingival overgrowth diagnosed in the present study (68.4%) may be related to the dichotomous scoring system employed. Patients affected by small or localized zones of gingival overgrowth were scored positive. The present findings are in accordance with previously reported higher prevalence of gingival overgrowth in females (72.2% in females vs 64.7% of males).

Yamasaki and colleagues[21] in 1987 reported increased proportions of microfilament bands, nuclear indentations, and basal lamina–associated stromal junctions in CyA-associated gingival overgrowth. A rise in the cellular production of type I procollagen has been hypothesized.[22,23]

The prevalence of gingival overgrowth will likely increase as organ transplantation is performed more frequently. Additionally, CyA is currently used in the treatment of type I diabetes, systemic lupus erythematosus, psoriasis, rheumatoid arthritis, and multiple sclerosis.[24] Patients exhibiting different degrees of gingival overgrowth showed similar drug levels, leading Varga to conclude[25] that the differences were due to patient variables rather dose to dependence.[25] Ross et al[26] in 1989 reported a significant positive correlation between gingival overgrowth and bacterial plaque and gingivitis. Poor oral hygiene and gingival inflammation were identified as primary risk factors along with salivary and blood concentrations of CyA.[27] While risk factors such as age are disputable, a regimented oral hygiene program and frequent monitoring by oral healthcare professionals can prevent or minimize gingival enlargement.[28–30] In the present study, active viral infection and posttransplant clinical complications did not show a statistically significant association with age.

The present study has described a detection method and identified a potential reservoir for HCMV, which may prove to have long-term implications for organ transplant

recipients. A statistically significant association between viral infection and salivary ($P = .0001$) and gingival crevicular fluid detections of active HCMV pp67-mRNA was shown. ($P = .01$), emphasizing their diagnostic value. Saliva can be used to detect pp67-mRNA as a marker for active HCMV infection in renal transplant patients.

The results of the current study clearly demonstrate an association between periodontal status and potential renal transplant complications. It would seem prudent for transplant candidates to receive a thorough periodontal examination, including analysis of saliva and gingival crevicular fluid for active HCMV infection. Following transplantation, frequent monitoring of a recipient's periodontal health (and promptly rendered appropriate treatment) may reduce the risk of transplant complications due to HCMV infection. Long-term prospective studies are needed to clarify the magnitude of these benefits.

REFERENCES

1. Soderberg-Naucler C, Emery VC: Viral infections and their impact on chronic renal allograft dysfunction. Transplantation 71:SS24, 2001
2. Jiwa NM, Van Gemert GW, Raap AK, et al: Rapid detection of human cytomegalovirus DNA in peripheral blood leukocytes of viremic transplant recipients by the polymerase chain reaction. Transplantation 48:72, 1989
3. Wolf DG, Spector SA: Early diagnosis of human cytomegalovirus disease in transplant recipients by DNA amplification in plasma. Transplantation 56:330, 1993
4. Kas-Deelen AM, de Maar EF, Harmsen MC, et al: Uninfected and cytomegalic endothelial cells in blood during cytomegalovirus infection. effect of acute rejection. J Infect Dis 181:721, 2000
5. Contreras A, Slots J: Mammalian viruses in human periodontitis. Oral Microbiol Immunol 11:381, 1996
6. Contreras A, Zadeh HH, Nowzari H, et al: Herpesvirus infection of inflammatory cells in human periodontitis. Oral Microbiol Immunol 14:206, 1999
7. Contreras A, Umeda M, Chen C, et al: Relationship between herpesviruses and adult periodontitis and periodontopathic bacteria. J Periodontol 70:478, 1999
8. Hanookai D, Nowzari H, Contreras A, et al: Herpesviruses and periodontopathic bacteria in trisomy 21 periodontitis. J Periodontol 71:376, 2000
9. Nowzari H, Jorgensen MG, Ta TT, et al: Aggressive periodontitis associated with Fanconi's anemia. A case report. J Periodontol 72:1601, 2001
10. Contreras A, Slots J: Active cytomegalovirus infection in human periodontitis. Oral Microbiol Immunol 13:225, 1998
11. Contreras A, Nowzari H, Slots J: Herpesvirus in periodontal pocket and gingival tissue specimens. Oral Microbial Immunol 15:15, 2000
12. King GN, Fullinfaw R, Higgins TJ, et al: Gingival hyperplasia in renal allograft recipients receiving cyclosporin-A and calcium antagonists. J Clin Periodontol 20:286, 1993
13. Lowry LY, Welbury RR, Seymour RA, et al: Gingival overgrowth in paediatric cardiac transplant patients. a study of 19 patients aged between 2 and 16 years. Int J Paediatr Dent 5:217, 1995
14. Thomason JM, Sloan P, Seymour RA: Immunolocalization of collagenase (MMP-1) and stromelysin (MMP-3) in the gingival tissues of organ transplant patients medicated with cyclosporin. J Clin Periodontol 25:554, 1998
15. Yamada H, Nishimura F, Naruishi K, et al: Phenytoin and cyclosporin A suppress the expression of MMP-1, TIMP-1, and cathepsin L, but not cathepsin B in cultured gingival fibroblasts. J Periodontol 71:955, 2000
16. Yilmaz S, Koskinen PK, Kallio E, et al: Cytomegalovirus infection-enhanced chronic kidney allograft rejection is linked with intercellular adhesion molecule-1 expression. Kidney Int 50:526, 1996
17. Rosen HR, Corless CL, Rabkin J, et al: Association of cytomegalovirus genotype with graft rejection after liver transplantation. Transplantation 66:1627, 1998
18. Jeejeebhoy FM, Zaltzman JS: Thrombotic microangiopathy in association with cytomegalovirus infection in a renal transplant patient. a new treatment strategy. Transplantation 65:1645, 1998
19. Peggs KS, Preiser W, Kottaridis PD, et al: Extended routine polymerase chain reaction surveillance and pre-emptive antiviral therapy for cytomegalovirus after allogeneic transplantation. Br J Haematol 111:782, 2000
20. Spratt H, Boomer S, Irwin CR, et al: Cyclosporin associated gingival overgrowth in renal transplant recipients. Oral Dis 5:27, 1999
21. Yamasaki A, Rose GG, Pinero GJ, et al: Ultrastructure of fibroblasts in cyclosporin A-induced gingival hyperplasia. J Oral Pathol 16:129, 1987
22. Schincaglia GP, Forniti F, Cavallini R, et al: Cyclosporin-A increase type I procollagen production and mRNA level in human gingival fibroblasts in vitro. J Oral Pathol Med 21:181, 1992
23. Harding MW, Handschumacher RE, Speicher DW: Isolation and amino acid sequence of cyclophilin. J Biol Chem 261:8547, 1986
24. Bach JF: Immunosuppressive therapy of autoimmune diseases. Immunol Today 14:322, 1993
25. Varga E, Lennon MA, Mair LH: Pre-transplant gingival hyperplasia predicts severe cyclosporin-induced gingival overgrowth in renal transplant patients. J Clin Periodontol 25:225, 1998
26. Ross PJ, Nazif MM, Zullo T, et al: Effects of cyclosporin A on gingival status following liver transplantation. ASDC J Dent Child 56:56, 1989
27. Daley TD, Wysocki GP, Day C: Clinical and pharmacological correlations in cyclosporine-induced gingival hyperplasia. Oral Surg Oral Med Oral Pathol 62:417, 1986
28. Hall EE: Prevention and treatment considerations in patients with drug-induced gingival enlargement. Curr Opin Periodontol 4:59, 1997
29. Somacarrera ML, Lucas M, Scully C, et al: Effectiveness of periodontal treatments on cyclosporine-induced gingival overgrowth in transplant patients. Br Dent J 183:89, 1997
30. Ilgenli T, Atilla G, Baylas H: Effectiveness of periodontal therapy in patients with drug-induced gingival overgrowth. Long-term results. J Periodontol 70:967, 1999