Herpesvirus carriage in saliva and posttreatment apical periodontitis: Searching for association

Bianca P.S. Guilherme, DDS, a Dennis C. Ferreira, MSc, b Isabela N. Rôças, PhD, c José C. Provenzano, MSc, c Kátia R.N. Santos, PhD, d and José F. Siqueira Jr, PhD, c
Rio de Janeiro, RJ, Brazil
ESTÁCIO DE SÁ UNIVERSITY AND FEDERAL UNIVERSITY OF RIO DE JANEIRO

Objective. Herpesvirus infection can cause immunosuppression and then act as a modifier of apical periodontitis, influencing the disease severity and response to treatment. The purpose of this study was to investigate if herpesvirus infection, as inferred by salivary carriage, may influence the endodontic treatment outcome.

Study design. The study population included 72 patients who had root canals treated more than 1 year previously because of necrotic pulps and apical periodontitis. At the follow-up examination, 27 of these patients presented with posttreatment apical periodontitis (failure) and 45 individuals exhibited healed/healing periradicular tissues (success). Saliva was collected from these individuals, DNA was extracted, subjected to multiple displacement amplification, and screened by polymerase chain reaction (PCR) assays for the presence of 6 herpesviruses: herpes simplex virus types 1 and 2 (HSV-1/2), Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), human herpesvirus-6 (HHV-6), and human herpesvirus-8 (HHV-8).

Results. Except for HSV-1/2, all other herpesviruses were detected in saliva from both healed/healing and diseased groups. HHV-8 was the most frequent herpesvirus found in saliva (84% in success, 89% in failure), followed by HCMV (22% in success, 30% in failure), EBV (16% in success, 18.5% in failure) and HHV-6 (7% in success, 15% in failure). No significant association of herpesvirus carriage in saliva with poor treatment outcome was discernible in the population studied (P > .05).

Conclusions. Data from the present study suggest that herpesvirus infection may not influence the outcome of endodontic treatment. Further longitudinal studies are warranted to confirm these findings. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:678-683)

Apical periodontitis is primarily caused by bacteria, but other conditions may conceivably influence its progression, form, severity, and response to treatment.1 These conditions are usually referred to as disease modifiers. The activity of disease modifiers, as for instance altering the host defense to infection, might help explain why some asymptomatic teeth become symptomatic overnight, why some lesions take too long to heal after endodontic treatment, and why some apparently well-treated root canals still result in failure.1,2 There is some evidence showing that diabetes may function as a modifier of apical periodontitis.3,4 Other potential disease modifiers of endodontic interest include polymorphisms in genes related to the immune response5 and smoking.6

The past years have witnessed an increasing interest in the potential role of herpesviruses in the pathogenesis of apical periodontitis. Members of this group of viruses have been detected in symptomatic apical periodontitis lesions,7 endodontic abscesses,8,9 large lesions,10 and lesions from human immunodeficiency virus (HIV)-positive patients.11 It has been hypothesized that herpesviruses may be implicated in the pathogenesis of apical periodontitis as a direct result of virus infection or as a result of virally induced impairment of local host defenses, which might give rise to overgrowth of pathogenic bacteria in the very apical part of the root canal.12 Therefore, considering that herpesvirus infection can cause focal immunosuppression, it is possible to surmise that it may act as a disease modifier and influence apical periodontitis progression, severity, and response to treatment.
The overall prevalence of herpesviruses in the general adult population may reach values as high as 90%.13 Herpesviruses represent the most prevalent group of viruses found in human saliva, and their occurrence is usually caused by shedding of virions from infected oral sites, including salivary glands, oral mucosa, or gingival sulcus.14,15 Interestingly, even systemically healthy adults may continually and asymptomatically shed detectable herpesvirus DNA in saliva.13

The hypothesis for the present study is that herpesvirus infection may negatively affect the response of apical periodontitis to endodontic treatment. The rationale is that herpesvirus-infected defense cells are attracted to the periradicular tissues in response to bacterial infection and, once there, the viruses become activated and induce local immunosuppression. This would favor pathogenicity of bacteria persisting at the apical canal and therefore impair periradicular healing. To evaluate this hypothesis, periradicular sampling would be the best approach for detection of herpesvirus infection, but it would be ethically impossible to collect these samples from healed or healing cases. Thus, saliva may be the best sampling material to survey for the presence of herpesvirus infection. If the hypothesis of this study is confirmed, herpesvirus detection in saliva at the time of treatment might be further investigated for its possible role as predictor of poor prognosis. Highly sensitive and specific molecular microbiology methods have greatly facilitated the detection of virus in clinical material.16 Hence, these methods hold the potential of using herpesvirus salivary detection to indicate a risk for poor endodontic treatment outcome.

The present study was undertaken to search for an association between herpesvirus infection (as inferred by their presence in saliva) and the endodontic treatment outcome. For this, patients subjected to follow-up examination for analysis of the outcome of the endodontic treatment had their saliva collected and screened for the presence of 6 human herpesviruses.

MATERIAL AND METHODS

Individuals and inclusion criteria

The population sample that met the inclusion criteria, described later in this article, involved 72 adult individuals (41 females and 31 males). A questionnaire was given to all individuals participating in the study so as to obtain information regarding their general health and habits. The systemic conditions and acquired habits of interest for this study, as they might act as disease modifiers and then as covariates, included diabetes (7 individuals), hypertension (14 individuals), HIV infection (none reported), and smoking (9 individuals). The protocol for this study was approved by the Ethics Committee of the Estácio de Sá University.

Treatment outcome was determined on the basis of radiographic and clinical evaluations. Immediate postoperative radiographs at the time of treatment available in the Dental School records and follow-up radiographs of treated teeth were taken using film holders and treatment outcome was categorized as follows:

1. **Healed**: Contour and width of the periodontal ligament space (PDL) were normal or PDL contour was widened mainly around excess filling. Appearance of the surrounding bone was normal. These cases were categorized as success (controls).

2. **Healing**: Periradicular radiolucency was clearly decreasing in size. For the purpose of this study, these teeth were also categorized as success (controls), because a significant level of healing was observed. Cases with uncertain healing were excluded.

3. **Diseased (not healed)**: Periradicular radiolucency was unchanged or increased in size. These cases were categorized as failure.

In teeth with more than one root, the least-favorable outcome was registered. Two experienced endodontists independently analyzed the radiographs under magnification and in the only 4 cases where disagreement occurred, a third observer was consulted. Clinical examination was also performed to check for other signs/symptoms of treatment failure, including pain, swelling, and sinus tracts.

Rigid inclusion criteria were used to select patients. To be enrolled, each individual had to exhibit only 1 root canal–treated tooth or more than 1 treated tooth exhibiting the very same periradicular status at the follow-up examination (i.e., healthy/healing or diseased). All individuals presented with pulp necrosis and apical periodontitis at the time of treatment, which in turn should have been completed for at least 1 year previously. Treated teeth had both adequate root canal fillings and adequate coronal restorations. Root canal treatment was ranked as adequate based on the following aspects: all canals were obturated, no voids in the filling mass were present, and the apical terminus of the filling was 0 to 2 mm short of the radiographic apex. Coronal restoration was ranked as adequate when it was a permanent restoration that appeared clinically and radiographically intact, with no detectable signs of overhangs, open margins, or recurrent caries. All endodontic treatments were conducted by either graduate students or specialists.

Following these inclusion criteria, the diseased group (failure) was composed of 27 patients and the healed/healing group (success) was composed of 45 individuals.
Sample taking and processing

Saliva samples were taken from the individuals at the time of follow-up examination. Sampling and DNA extraction procedures were performed as described previously.\(^5\),\(^17\) Samples from 48 patients were available from a previous study and stored frozen.\(^5\) Fresh samples were taken from another 24 patients who had not participated in that previous study. To improve the performance of polymerase chain reaction (PCR) assays for virus detection, DNA extracts from saliva were subjected to multiple displacement amplification (MDA) by using the Illustra GenomiPhi V2 DNA amplification kit (GE Healthcare, Piscataway, NJ) following the manufacturer’s instructions.

PCR assays

Initially, to ascertain the availability of human DNA for analysis, aliquots of 2 µL of the MDA products from salivary DNA were subjected to PCR using primers directed to the T-cell receptor Vα22 gene.\(^18\) These results were confirmed by using PCR directed to the human β-globin gene.\(^19\)

The human viruses targeted in this study were the following: herpes simplex virus types 1 and 2 (HSV-1/2), Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), human herpesvirus-6 (HHV-6), and human herpesvirus-8 (HHV-8). A multiplex nested PCR approach was used to simultaneously detect HSV-1/2, HCMV, and EBV.\(^20\) Nested PCR assays were used for detection of EBV,\(^8\) HCMV,\(^8\) HHV-6,\(^21\) and HHV-8.\(^22\) Aliquots of 2 µL of MDA products were used as templates in each individual PCR reaction for virus detection. All PCR reactions and cycling parameters for virus detection are summarized in a previous study,\(^9\) except for the nested PCR assays targeting HCMV and EBV, which followed the protocols by Chen et al.\(^8\)

All PCR analyses were performed in duplicate. Positive and negative controls were included in all batches of samples analyzed. Positive controls for viruses consisted of DNA extracted from clinical samples (blood or saliva) previously tested positive for each target virus as determined by PCR and sequencing. One negative control consisting of sterile ultrapure water instead of sample was included for every 5 samples in all batches of samples analyzed.

Amplicons were separated by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and viewed under ultraviolet transillumination. A 100–base pair DNA ladder digest (New England Biolabs, Beverly, MA) served as the molecular size standard. Representative products from positive PCR reactions were sequenced to confirm identification.

Statistical analysis

The data concerning the presence of the different herpesviruses in saliva of individuals with healed/healing or diseased teeth were statistically analyzed by means of the Pearson χ² test or the Fisher 2-tailed exact test. The latter was used whenever at least one cell of the 2 × 2 contingency table had a value less than 5. Several other factors (systemic conditions, smoking, race, and gender) were also evaluated for associations with treatment outcome to check if they could act as covariates. The Student t test was used for analyses of the age distribution between the 2 groups. Significance level was always set at \(P\) less than .05. Prevalence of the different herpesviruses in saliva from subpopulations according to systemic conditions, smoking, and gender were also recorded, but statistical tests were not performed for these subdivided data because of the resulting too low sample size.

RESULTS

The characteristics of the study individuals and their relationship with the periradicular status are depicted in Table I. Overall analysis showed that no systemic condition or acquired habit was significantly associated with posttreatment disease (\(P > .05\)). Gender and race had no significant influence on treatment outcome either (\(P > .05\)). Therefore, none of the conditions were found to serve as covariates.

Table II displays the results of the prevalence of the different herpesviruses in saliva of patients with or without radiographic evidence of posttreatment apical periodontitis. Except for HSV-1/2, all other herpesviruses were detected in saliva from both healed/healing and diseased groups. Overall, HHV-8 was the most frequent herpesvirus found in saliva (62/72 individuals, 86%), followed by HCMV (18/72, 25%), EBV (12/72, 17%), and HHV-6 (7/72, 10%). Only 4 patients (all of them healed/healing cases) showed none of the target viruses. No significant associations were detected between any of the target viruses and treatment outcome (\(P > .05\)).

DISCUSSION

One of the models proposed for the role of herpesvirus infection in the pathogenesis of apical periodontitis claims that the virus causes localized immunosuppression, which consequently favors overgrowth of bacteria in the apical root canal.\(^12\) Localized inflammation in the periradicular tissues caused by intraradicular bacterial infection results in attraction of host defense cells infected by herpesviruses. As these cells infiltrate and accumulate in the inflamed tissues, the herpesviruses can be reactivated spontaneously, by concomitant bacterial infection or during periods of reduced host
remaining bacteria are in levels that are compatible with tissue healing. However, the possibility exists that the host response can be influenced by disease modifiers and, as a consequence, cases with low numbers of residual bacteria might not heal or might take longer to remit in patients with some unfavorable disease modifier, in spite of adequate root canal treatment.

Although herpesvirus infection might be included in this category of disease modifier influencing the response of apical periodontitis to treatment, this was not observed in the present study for any of the target herpesviruses. Whereas HSV-1/2 was not found in any of the samples, all of the other 4 herpesviruses were detected in saliva from both success and failure groups. Prevalences for these 4 viruses were slightly higher in failure cases, but not enough to reach statistical significance. Therefore, no association of herpesvirus carriage in saliva with poor treatment outcome was discernible in the population studied.

One important limitation of this study is that although the evaluation of treatment outcome was retrospective, analysis of virus presence was cross sectional, as this information was available only at the time of recall. Because saliva samples were taken at the time of follow-up examination, it is not possible to infer when infected individuals acquired the virus. Also, the virus presence in saliva may have been only transient after a recent transfer from another infected individual, which would then represent a carriage state with no infection. For the hypothesis of this study to be confirmed and virus infection be considered a predictor of poor outcome or delayed healing response, the individual should have been infected before the endodontic treatment or shortly thereafter. However, this study was just a preliminary cross-sectional analysis searching for association. Longitudinal studies are required to confirm or refute the present findings.

### Table I. Characteristics of study subjects and associations with endodontic treatment outcome

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Success (healed/healing)</th>
<th>Failure (diseased)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>28 (67.5)</td>
<td>13 (32.5)</td>
<td>.24*</td>
</tr>
<tr>
<td>Male</td>
<td>17 (56)</td>
<td>14 (44)</td>
<td></td>
</tr>
<tr>
<td>Female + EBV</td>
<td>4 (50)</td>
<td>4 (50)</td>
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<tr>
<td>Male + EBV</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td></td>
</tr>
<tr>
<td>Female + HCMV</td>
<td>7 (54)</td>
<td>6 (46)</td>
<td></td>
</tr>
<tr>
<td>Male + HCMV</td>
<td>3 (60)</td>
<td>2 (40)</td>
<td></td>
</tr>
<tr>
<td>Female + HHV-6</td>
<td>3 (60)</td>
<td>2 (40)</td>
<td></td>
</tr>
<tr>
<td>Male + HHV-6</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td></td>
</tr>
<tr>
<td>Female + HHV-8</td>
<td>21 (68)</td>
<td>10 (32)</td>
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</tr>
<tr>
<td>Male + HHV-8</td>
<td>17 (55)</td>
<td>14 (45)</td>
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<tr>
<td>Race</td>
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<tr>
<td>White</td>
<td>38 (67)</td>
<td>19 (33)</td>
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</tr>
<tr>
<td>African-Brazilian</td>
<td>7 (47)</td>
<td>8 (53)</td>
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<td>Systemic condition</td>
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<tr>
<td>Diabetic</td>
<td>3 (43)</td>
<td>4 (57)</td>
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</tr>
<tr>
<td>Nondiabetic</td>
<td>42 (65)</td>
<td>23 (35)</td>
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<tr>
<td>Diabetic + EBV</td>
<td>2 (100)</td>
<td>0 (0)</td>
<td></td>
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<tr>
<td>Diabetic + HCMV</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + HHV-6</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + HHV-8</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td></td>
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<tr>
<td>Hypertensive</td>
<td>11 (79)</td>
<td>3 (21)</td>
<td>.22†</td>
</tr>
<tr>
<td>Nonhypertensive</td>
<td>34 (59)</td>
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<td></td>
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<td>Hypertensive + HCMV</td>
<td>1 (50)</td>
<td>1 (50)</td>
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</tr>
<tr>
<td>Hypertensive + HHV-6</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>Hypertensive + HHV-8</td>
<td>10 (83)</td>
<td>2 (17)</td>
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<td>Habits</td>
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<tr>
<td>Smoker</td>
<td>3 (33)</td>
<td>6 (67)</td>
<td>0.07†</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>42 (67)</td>
<td>21 (33)</td>
<td></td>
</tr>
<tr>
<td>Smoker + EBV</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td></td>
</tr>
<tr>
<td>Smoker + HCMV</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Smoker + HHV-6</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Smoker + HHV-8</td>
<td>3 (37.5)</td>
<td>5 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>42.5 (±18.1)‡</td>
<td>46.6 (±15.1)‡</td>
<td>0.33§</td>
</tr>
</tbody>
</table>

EBV, Epstein-Barr virus; HCMV, human cytomegalovirus; HHV-6, human herpesvirus-6; HHV-8, human herpesvirus-8.

*Pearson χ².
†Fisher exact test.
§Student t test.
‡Mean (± standard deviation).

### Table II. Prevalence of different herpesviruses in saliva and association with endodontic treatment outcome

<table>
<thead>
<tr>
<th>Target virus</th>
<th>Success (healed/healing)</th>
<th>Failure (diseased)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHV-8</td>
<td>38 (84%)</td>
<td>24 (89%)</td>
<td>.73*</td>
</tr>
<tr>
<td>HCMV</td>
<td>10 (22%)</td>
<td>8 (30%)</td>
<td>.48†</td>
</tr>
<tr>
<td>EBV</td>
<td>7 (16%)</td>
<td>5 (18.5%)</td>
<td>.74†</td>
</tr>
<tr>
<td>HHV-6</td>
<td>3 (7%)</td>
<td>4 (15%)</td>
<td>.41*</td>
</tr>
<tr>
<td>HSV-1/2</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>—</td>
</tr>
</tbody>
</table>

EBV, Epstein-Barr virus; HCMV, human cytomegalovirus; HHV-6, human herpesvirus-6; HHV-8, human herpesvirus-8; HSV-1/2, herpes simplex virus types 1 and 2.

*Fisher exact test.
†Pearson χ².
Samples were collected from saliva for 2 reasons. First, although failure cases might have been treated by surgery and virus detection ascertained directly using the lesion as a source of DNA, this would not be possible and ethically viable for healed/healing cases. Second, had any association with poor outcome been disclosed for the test viruses, development of a test to predict the treatment outcome would be easier because saliva is promptly available and no invasive technique is required for sampling.

In general, the most prevalent herpesvirus was HHV-8 (86%), followed by HCMV (25%), EBV (17%), and HHV-6B (10%). The prevalence values for most viruses (except for HHV-8) were relatively lower when compared with many other studies. However, HHV-8 was prevalent in saliva, and this result is in disagreement with some studies in healthy general adult populations from the United States and northern Europe. Our previous investigation of endodontic abscesses in Brazilian patients also demonstrated a high prevalence of HHV-8. This suggests a geographic influence on the occurrence of HHV-8. In fact, all these discrepancies in prevalence, for more or less depending on the target herpesvirus, may be a reflection of the geographic differences among the populations studied, different oral conditions of the sampled population, and the different methods used for sample taking/processing and virus identification.

Other potential disease modifiers, such as diabetes, hypertension, and smoking, were also tested for their association with treatment outcome so as not to act as confounding variables. None of them was significantly associated with endodontic treatment outcome. As for diabetes, the present findings are in disagreement with the literature. Hypertension as well has not been shown to be a factor influencing apical periodontitis, whereas data related to smoking are still conflicting. However, these analyses were not the main scope of this study and are substantially underpowered, as the very low numbers of individuals with diabetes (n = 7), hypertension (n = 14), or smoking habit (n = 9) do not allow for a robust statistical analysis to be performed.

In conclusion, the current study of a relatively small sample size demonstrated that herpesvirus infection as inferred from salivary carriage was not associated with poor outcome of endodontic treatment in Brazilians. Further longitudinal studies involving a larger sample size and from different geographic populations are needed to help clarify this issue.

REFERENCES


Reprint requests:
José F. Siqueira Jr, DDS, MSc, PhD
Faculty of Dentistry
Estácio de Sá University
Av. Alfredo Baltazar da Silveira, 580/cobertura
Recreio, Rio de Janeiro, RJ
Brazil 22790-710
jf_siqueira@yahoo.com; siqueira@estacio.br