Human Cytomegalovirus and Epstein-Barr Virus Genotypes in Apical Periodontitis Lesions

Aleksandar Jakovljevic, DDS, [*] *Miroslav Andric, DDS, MSc, PhD,* [*] *Aleksandra Knezevic, MD, MSc, PhD,* [†] *Ivan Soldatovic, MD, MSc,* [‡] *Nadja Nikolic, MSc,* [§] *Danijela Karalic, MD,* [†] *and Jelena Milasin, MSc, PhD* [§]

Abstract

Introduction: Different genotypes of human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) possess specific pathogenic abilities because of various interactions with the host's immune system and differences in cell tropism. The aim of this study was to determine the distribution of HCMV and EBV genotypes in apical periodontitis lesions in relation to their clinical and histopathologic features. **Methods:** One hundred samples of apical periodontitis lesions and 25 control samples (healthy pulp tissue) were collected. The presence of HCMV glycoprotein B (gB) and EBV nuclear antigen-2 genotypes was analyzed by nested polymerase chain reaction and restriction fragment length polymorphisms analysis. **Results:** EBV and HCMV were detected in apical periodontitis lesions at significantly higher frequencies than in healthy pulp controls (P = .020 and P = .020, respectively). HCMV gB type II was significantly more frequent compared with gB type I in the examined groups (P = .036). No HCMV gB type III or IV products were found. In both periapical lesions and controls, EBV-1 occurred more often compared with EBV-2 (P = .001). Dual EBV and HCMV coinfection was more frequently detected in large-size periapical lesions (P = .038). **Conclusions:** Both HCMV and EBV are associated with inflammatory processes of periapical bone destruction. HCMV gB type II and EBV-1 are the most prevalent genotypes in apical periodontitis lesions. *(J Endod 2015;41:1847–1851)*

Key Words

Apical periodontitis, EBNA-2 protein, Epstein-Barr virus, genotype, glycoprotein B, human cytomegalovirus

From the *School of Dental Medicine, Clinic for Oral Surgery and Implantology; †Faculty of Medicine, Institute of Microbiology and Immunology, Department of Virology; ‡Faculty of Medicine; §School of Dental Medicine, Institute of Biology and Human Genetics, University of Belgrade, Belgrade, Serbia.

Address requests for reprints to Dr Aleksandar Jakovljevic, University of Belgrade, School of Dental Medicine, Clinic for Oral Surgery and Implantology, Dr Subotica 4, 11000 Belgrade, Serbia. E-mail address: dr.sasuli@hotmail.com

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Apical periodontitis represents an inflammatory process in the periapical tissue. It is characterized by chronic inflammation and destruction of tooth-supporting structures (1, 2). Although various factors are able to induce periapical inflammation, overwhelming evidence indicates that polymicrobial infection of endodontic origin is essential for its establishment (3).

Sabeti et al (4, 5) detected herpesviruses DNA in periapical tissue by using polymerase chain reaction (PCR) and concluded that herpes viral infection, in particular infection with human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV), may contribute to the pathogenesis of apical periodontitis. Although numerous studies have analyzed the relationship between herpesvirus presence in periapical tissue and clinical features of apical periodontitis lesions (6–10), its role in the etiopathogenesis of periapical lesions has not yet been elucidated (11). One hypothesis is that herpesviruses are implicated in the pathogenesis of apical periodontitis as a direct result of viral infection or as the result of a virally induced impairment of local host defense that favors bacterial overgrowth (12). On the other hand, Ferreira et al (13) hypothesized that herpesviruses might be just an epiphenomenon to bacterial infection that caused inflammation of periapical tissue and consequent influx of virus-infected inflammatory cells to the periapical area.

The increasing knowledge about HCMV and EBV indicates that there is a great diversity of viral strains that may influence the course and severity of diseases in different ways. It has been pointed out that different genotypes of HCMV and EBV possess specific pathogenic abilities that are due to various interactions with the host's immune system and differences in cell tropism (14).

Subtype classification of HCMV is mostly based on the glycoprotein B (gB) gene that encodes a glycoprotein with high immune reactivity incorporated in the viral envelope. This glycoprotein is required for HCMV infectivity because it regulates viral penetration into cells, transmission of infection, and modulation of cellular transcription (15, 16). On the basis of the nucleotide sequence of gB gene, all HCMV genotypes belong to 1 of 4 variants (gB I–gB IV) (17) with evidently different geographical distribution (18).

Depending on the sequence of EBV nuclear antigen 2 (EBNA-2) gene, 2 types of EBV can be distinguished, type I (EBV-1) and EBV-2. EBNA-2 acts as a transcription factor inducing the expression of viral latent membrane protein genes. EBNA-2 is also required for continued proliferation of lymphoblastoid cell lines in which the latent viral genome is maintained (19).

It was demonstrated that the distribution of HCMV and EBV genotypes varies among patients and depends on their health status. The most prevalent genotype in congenitally infected newborns (20) and human immunodeficiency virus (HIV)–positive patients was gB-I (21). On the other hand, gB-II and gB-III occurred more often than other genotypes among organ-transplant recipients (22). Previous reports indicate that EBV-1 was predominant in healthy patients (23), whereas EBV-2 was widespread among HIV-positive patients (24).

Wu et al (25) demonstrated that different genotypes of HCMV and EBV are associated with clinical features of gingivitis and generalized chronic periodontitis. Similarly,

Jankovic et al (26) found a significant correlation between the presence of HCMV-2 and EBV-1 genotypes and clinical parameters of peri-implantitis.

Because of these results and the fact that EBV and HCMV genotypes in periapical lesions were not previously analyzed, the aim of this study was to determine the distribution of EBV and HCMV genotypes in apical periodontitis lesions in relation to their clinical and histopathologic features.

Materials and Methods

Patient Data and Sample Collection

One hundred patients (age, 10–79 years; mean age, 40.6 years; 47 males and 53 females) with clinical and radiographic findings suggestive of apical periodontitis were enrolled in the study. The protocol of the study was reviewed and approved by the Ethical Committee of the School of Dental Medicine, University of Belgrade (approval number: 34/2013). Informed consent was obtained from all patients, and all procedures were fully explained to them.

Individuals in good general health (American Society of Anesthesia I or II) (27) who required surgical apicoectomy because of the failure of conventional root canal treatment were included in the study. At the moment of surgery, all involved teeth had either definitive coronal restorations and root canal fillings or calcium hydroxide–based temporary root canal fillings and light-curing composite–based temporary restorations. Patients with periodontally involved teeth (probing depth > 4 mm, with periodontal bone loss), vertical root fracture, immunocompromised, or patients treated with antibiotics, antiviral or immunosuppressive therapy 3 months before the examination were excluded from the study.

Routine clinical examination was performed immediately before surgery (28). On the basis of clinical data and history of disease, apical periodontitis lesions were categorized as either symptomatic or asymptomatic. Symptomatic lesions were characterized by acute pain, discomfort on biting, and sensitivity by percussion or palpation. The lesions that did not have any clinical symptoms, except the radiolucent area on radiographs, were noted as asymptomatic. On the basis of the radiographic size of the lesions, samples were divided into 2 subgroups, >5 mm and ≤5 mm diameter of lesions. According to histopathologic examination, all apical periodontitis lesions were divided into periapical granulomas and radicular cysts.

Samples of periapical lesions were collected following standard apicoectomy procedure as described previously (8). Briefly, before administering local anesthesia, operative area was treated with 0.12% chlorhexidine. By using a sterile no. 15 blade, an appropriate gingival incision was extended 1 or 2 teeth mesially and distally from the involved tooth, followed by a vertical releasing incision. A full-thickness mucoperiosteal flap was then reflected, and periapical lesion was exposed with a sterile round burr by using sterile saline as a coolant. A sterile curette was used to obtain the periapical specimen. After harvesting, tissue samples were divided into 2 parts by using a sterile no. 11 blade. One portion of each lesion was used for isolation of DNA. It was placed into sterile Eppendorf tube with saline solution and immediately frozen at −70°. The other part of the lesion was fixed in 10% formalin for histopathologic evaluation. Once full clinical, radiographic, and histopathologic examinations were performed, final diagnosis was made for all lesions.

The pulp tissues of 25 patients scheduled for surgical removal of impacted third molars (age, 16–29 years; mean age, 21.6 years; 16 males and 9 females) were used as healthy controls. All control teeth were mature impacted third molars with no evidence of caries, restoration, cracking, or pulpal inflammation. Samples of the control group were collected following standard surgical procedure as described previously (29). Briefly, before administering local anesthesia, operative area was treated with 0.12% chlorhexidine. By using a sterile no. 15 blade, gingival incision was made, full-thickness mucoperiosteal flap was reflected, and the impacted third molar was exposed with a sterile round burr by using sterile saline as a coolant. Each removed tooth was placed in a sterile plastic vial with sterile saline solution. The pulp tissues from the impacted third molars were obtained after disinfecting the crown of a tooth with 0.12% chlorhexidine. A sterile burn was used to gain access to the pulp chamber. Immediately before entering the pulp chamber, the cavity was rinsed with 0.12% chlorhexidine once again. After that, the dental pulp was retrieved by means of sterile endodontic files. The harvested tissues were placed into sterile Eppendorf tubes with sterile saline solution and immediately frozen at −70°. During the entire surgical procedure, by using meticulous high-volume suction, care was taken to minimize the risk of salivary contamination of samples.

Viral Detection–Extraction of DNA and Nested PCR

All samples of experimental and control groups were homogenized by using tissue disrupter (UP50H; Hielscher Ultrasonics, Teltow, Germany). DNA was isolated by KAPA Express Extract DNA Extraction Kit (Kapa Biosystems, Wilmington, MA) according to the manufacturer's instructions.

A nested PCR method was used to detect HCMV, EBV-1, and EBV-2. The sequences of primers and nested PCR conditions are given in Table 1. Primers were designed to amplify sequences from the HCMV gB and EBNA-2 genes. The first round of PCR amplification was performed in a total volume of 25-μL mixture that contained 5 μL sample, 1 × KAPA *Taq* buffer A with 1.5 mmol/L $MgCl_2$, 1.5 mmol/L $MgCl_2$, 0.4 mmol/L deoxynucleoside triphosphates (dNTP Set; Qiagen, Venlo, The Netherlands), 400 nmol/L of each primer, and 1 U Taq DNA polymerase (KAPA Taq DNA Polymerase; Kapa Biosystems). The second round of PCR amplification was performed in a total volume of 25-μL mixture that contained 3 μL of the first-round PCR product, 1 mmol/L $MgCl_2$ (Kapa Biosystems), and all other components from the first round with identical concentrations.

Lymphoid cell lines containing HCMV and EBV were used as positive controls, whereas a PCR mixture containing 3 μL distilled water instead of sample was used as a negative control. Specific EBV and HCMV PCR amplicons of expected size were detected by electrophoresis in 8% polyacrylamide gel stained with ethidium bromide under an ultraviolet transilluminator. Details on the sensitivity and specificity of the sets of primers have been described previously (30–32).

DNA sequencing according to the Sanger method was used to confirm the specificity of the PCR methods for the detection of EBV and HCMV used in this study. PCR products of the randomly selected 1 EBV-positive and 2 HCMV-positive samples were sequenced by using Big Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA) by using inner PCR primers as sequencing primers. Sequencing reactions were obtained and analyzed on the ABI Prism 310 Genetic Analyzer at the Institute of Microbiology and Immunology, School of Medicine, University of Belgrade, Serbia. The obtained nucleotide sequences were aligned and compared with documented virus sequences available in the GenBank database by using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Restriction Fragment Length Polymorphisms Analysis

The HCMV gB genotypes were further determined by restriction fragment length polymorphisms analysis. The amplicons were digested with fast digest endonuclease RsaI (Fermentas, ThermoScientific, Vilnius, Lithuania). After RsaI digestion the HCMV products were split