Journal of Dental Problems and Solutions

Selcuk M Ozbek[1]*, Ahmet Ozbek[2] and Tayfur Demiray[3]

[1]Ministry of Health, Oral and Dental Health Hospital, Department of Endodontics, Eskişehir, Turkey
[2]University of Sakarya, Medical Faculty, Department of Microbiology and Clinical Microbiology, Sakarya, Turkey
[3]Ministry of Health, Sakarya Education and Research Hospital Medical Microbiology Laboratory, Sakarya, Turkey

Dates: Received: 21 December, 2015; **Accepted:** 28 December, 2015; **Published:** 30 December, 2015

***Corresponding author:** Selcuk M. Ozbek, Ministry of Health, Oral and Dental Health Hospital, Department of Endodontics, Eskişehir, Turkey, Tel: +90 546 5476426; Fax: +90 222 2293854; E-mail: selcuk233@yahoo.com

www.peertechz.com

Keywords: Apical Abscesses; EBV; HCMV; Red complex Bacteria; Real-Time PCR

ISSN: 2394-8418

Research Article

Herpesviral and “Red Complex” Bacterial Analysis of Acute Apical Abscesses

Abstract

Introduction: To investigate the possible association between presence of Epstein–Barr virus, human cytomegalovirus and three endodontic bacterial pathogens (*Porphyromonas* gingivalis*, Treponema denticola*, and *Tannerella forsythia*) “red complex” in samples from patients with acute apical abscesses using real-time PCR.

Methods: Periapical samples were collected from 18 acute apical abscess specimens and 6 control samples. The apical area of each study tooth was examined in periapical radiographs obtained using a long cone paralleling technique. The study included only single-rooted teeth that had carious lesions, necrotic pulps, and radiographic evidence of periradicular bone loss. No apparent communication from the abscess to the oral cavity or skin surface was observed. DNA extracts from purulent exudate aspirates of acute apical abscess and six control samples were evaluated for the presence of microbial and viral loads using real-time polymerase chain reaction (PCR) following the kit protocols recommended by the manufacturers. The chi squared test was used for statistical analysis.

Results: HCMV, EBV, *T. denticola, P. gingivalis*, and *T. forsythia* were detected in 16.7 %, 5.6 %, 44.4 %, 27.7 %, and 22.2 % of the samples, respectively. As for the healthy pulps used as noninflamed controls, no control specimens contained bacterial or viral DNA. The pair HCMV/ *T. denticola* and HCMV/ *T. forsythia* was detected in one case. The association between HCMV, *T denticola* and *T. forsythia* was not statistically significant.

Conclusion: Within the limitations of the present study, our PCR based findings revealed that in “red complex” bacteria, *T. denticola* and *T. forsythia* tended to occur in co-infection with HCMV.

Introduction

An acute apical abscess is an inflammatory disease of endodontic origin caused by a mixed bacterial infection, dominated by anaerobic species and characterised by rapid onset, spontaneous pain, tenderness of the tooth to pressure, purulent fluid formation, and associated swelling [1,2]. The microbial diversity in different forms of periapical pathology, including acute apical abscesses, has traditionally been assessed using anaerobic cultures and culture-independent molecular methods. The molecular methods used most often for microbial identification are the polymerase chain reaction (PCR) method and its variations [3,4].

Using molecular methods, Socransky et al. [5], using DNA-DNA hybridization method, identified a consortium of the bacterial species *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, as having the strongest association with periodontal disease severity. They named this microbial consortium the “red complex” [6]. Slots et al. [7], hypothesised that some types of aggressive periapical pathosis develop as a result of a series of interactions among herpesviruses, bacteria, and host immune reactions, essentially the same mechanism as in marginal periodontitis [2,8]. More recently, Ferreira et al. [2], suggested that the same mechanism might be applicable to apical abscesses of endodontic origin [9].

Eight human herpesvirus species with distinct biological and clinical characteristics have been described: herpes simplex viruses 1 and 2, varicella–zoster virus, Epstein–Barr virus (EBV), human cytomegalovirus (HCMV), and human herpesviruses 6, 7, and 8 [8,10,11]. The initial herpesvirus infection is followed by a latent phase in host cells, which ensures the survival of the viral genome throughout the lifetime of infected individuals. The α herpesviruses (herpes simplex and varicella-zoster viruses) establish latency in long-lived nondividing neuronal cells in sensory ganglia. Beta herpesviruses include cytomegalovirus and herpesviruses 6 and 7, which establish latency in bone marrow–derived myeloid progenitor cells. The γ herpesviruses Epstein-Barr virus and herpesvirus 8 are latent in B lymphocytes. Herpesvirus reactivation can occur spontaneously or as a result of concurrent infection, fever, drugs, tissue trauma, emotional stress, and other factors that impair the host immune defences [7]. EBV causes infectious mononucleosis and almost certainly plays a role in the aetiology of nasopharyngeal carcinoma, Burkitt’s lymphoma, and lymphoproliferative disorders in the presence of immunosuppression [7,12]. This virus is usually transmitted by oral secretions or blood [12]. EBV infects relatively long-lived B lymphocytes during both primary and latent infections and can infect the oropharyngeal epithelium [8,9]. HCMV infection is important clinically in pregnant women, immunosuppressed transplant patients, and individuals with acquired immunodeficiency syndrome (AIDS) and is found in the blood and many body secretions, including saliva, urine, semen, and breast milk [11,12]. HCMV infects many different epithelial cells, endothelial cells, smooth muscle cells, mesenchymal cells, hepatocytes, granulocytes, and monocyte-derived macrophages and resides in the bone marrow myeloid progenitor cells during latency [1,9,12].

Studies have shown that the genotype distribution and seroprevalence of EBV and HCMV differ among populations [8,13]. Additionally, molecular studies have directly compared the endodontic microbiota of patients from different geographic locations and found significant differences in the prevalence of some important species [3,14]. Therefore, it is reasonable to postulate that it is important to determine the presence of EBV, HCMV, and red complex bacteria (*P. gingivalis*, *T. forsythia*, and *T. denticola*) in periapical lesions in different populations. Based on the applicability of molecular techniques for bacteria and virus identification, the hypothesis for this current study is that herpesviruses and red complex bacteria associations play major roles in the pathogenesis of acute apical abscesses. Using purulent exudate specimens collected from patients, real-time PCR was conducted to investigate occurrence of EBV, HCMV, and the three endodontic bacterial pathogens (*P. gingivalis*, *T. forsythia*, and *T. denticola*) in acute apical abscess of endodontic origin.

Materials and Methods

Patient selection

Samples were taken from adult patients seeking emergency treatment in the Department of Endodontics, Dental School of Atatürk University [Erzurum, Turkey]. The Ethical Committee in Research of the Dental School of Atatürk University approved the study protocol, and informed consent was obtained from each of the patients. The patient information was coded anonymously.

For each patient, we recorded the age, gender, and clinical symptoms and signs, including pain on occlusion, tenderness to percussion or palpation, swelling, the presence of periapical radiolucency, history of previous dental treatments, probing depth of any periodontal pockets, and the history of previous and present antibiotic therapy. The apical area of each study tooth was examined in periapical radiographs obtained using a long cone paralleling technique. The study included only single-rooted teeth (13 incisors, five canines) from 18 adult patients (13 males, five females) that had carious lesions, necrotic pulps, and radiographic evidence of periradicular bone loss (Table 1). No apparent communication from the abscess to the oral cavity or skin surface was observed.

The diagnostic terminology was based on the guidelines of the American Association of Endodontists consensus conference recommended diagnostic terminology [16]. The diagnosis of an acute apical abscess was based on the presence of spontaneous pain, exacerbated by mastication, and localised swelling, along with fever, lymphadenopathy, or malaise [2]. The inclusion criteria for patients were as follows: individuals in good health (American Society of Anaesthesia I or II) with no severe systemic diseases (such as bleeding disorders, diabetes mellitus, etc.) and who required tooth extraction or endodontic treatment because of the presence of endodontic disease [17]. The patients' ages ranged from 22 to 36 years. None of the individuals reported being human immunodeficiency virus (HIV) positive. The exclusion criteria were periodontal involvement of the teeth [probing depth >4 mm, with periodontal bone loss], vertical root fracture, or immature teeth with open apices. Immunocompromised patients or patients who were receiving antiviral or immunosuppressive therapy or had received antibiotics up to 3 months before the start of the study were also excluded.

Table 1: Clinical features of patients.

	M	F	P	S	F	L	M
Apical abscess (*n=18*)	13	5	18	18	2	2	15

M male, *F* female, *P* pain, *S* swelling, *F* fever, *L* lymphadenopathy, *M* malaise

Study design

Samples were collected using strict asepsis, as described previously [2,9,18]. Abscesses were sampled by aspiration of the purulent exudate from the swollen mucosa over each abscess. After disinfecting the overlying mucosa with 2% chlorhexidine solution, a sterile disposable syringe was used to aspirate the purulent fluid, which was injected immediately into 2-mL CryoTubes containing 0.7 mL of 5% dimethylsulphoxide (DMSO) in tripticase soya broth (TSB). The samples were frozen immediately at −20°C until they were processed. Healthy dental pulp removed from six upper premolars with no tissue inflammation, no signs of caries, restoration or cracking, extracted for orthodontic reasons was used as a non-inflamed control [2,9]. DNA was extracted from the clinical samples using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany), following the protocol recommended by the manufacturer.

DNA extraction

The frozen samples were left at room temperature to thaw and to adjust to room temperature. The samples were vortexed and centrifuged at 14 000 **g** for 5 min, and then, the supernatants were removed the pellets were used for DNA extraction. The precipitates were resuspended in 200 μL of distilled water. For each sample, a new 1.5-mL micro centrifuge tube was prepared, and 25 μL of Qiagen Proteinase K was pipetted into each one, and then, 200 μL of sample was added to each tube. To mix the proteinase K and samples, mixture was pipetted at least five times. Two hundred microliters of buffer AL was added to the 1.5-mL micro centrifuge tube with the proteinase K and sample mixture. To ensure efficient lysis, proteinase K, the sample and buffer AL are mixed thoroughly to yield a homogeneous solution. This homogenous solution was incubated at 56°C for at least 15 min in a heating block. To precipitate DNA, 250 μL of 100% ethanol (molecular grade) was added to the lysate and vortexed for 20 s and left to incubation for 5 min at room temperature 20 (±2°C) (room temperature). The tube containing lysate was centrifuged briefly to remove drops from inside of the lid before tube opening.To collect the DNA, all contents of the lysate in the tube were transferred to QIAamp MinElute column and centrifuged at 6,000× *g* for 1 minute. At this stage, the DNA bound to the silica of the QIAamp MinElute column tube. The DNA bound to the silica was washed twice with washing buffer and then dried at ambient temperature. Finally, the DNA was collected in a 1.5-mL microcentrifuge tube with 60 μL of previously heated (50°C) elution buffer and centrifuged at 14,000× *g*.

PCR amplification

Virus DNA Amplification: To amplify the EBV and HCMV

Citation: *Ozbek SM, Ozbek A, Demiray T (2015) Herpesviral and "Red Complex" Bacterial Analysis of Acute Apical Abscesses. J Dent Probl Solut 2(3): 053-057. DOI: 10.17352/2394-8418.000019*

DNAs, a real-time PCR amplification method was used with commercial Fluorion® EBV QNP 1.0 and Fluorion® CMV 3.0 kits (Iontek, Istanbul, Turkey), respectively. The İCycler İQ Multicolour Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) was used to amplify and detect the DNA of target viruses with virus-specific kits following the manufacturer's recommendations, and all amplifications were repeated twice to confirm the results for EBV and HCMV.

Bacterial DNA Amplifications: Universal primers directed to 16S rDNA (forward/reverse) (5′ TTAAACTCAAAGGAATT-GACGG 3′ / 5′ CTCACGACACGAGCTGACGAC 3′) and species-specific primers for T. denticola (5′ AGAGCAAGCTCTCCCTTAC-CGT 3′ / 5′ TAAGGGCGGCTTGAAATAATGA 3′), P. gingivalis (5′ TACCCATCGTCGCCTTGGT 3′ / 5′ CGGACTAAAACCG-CATACACTTG 3′), and T. forsythia (5′ ATCCTGGCTCAGGAT-GAACG 3′ / 5′ TACGCATACCCATCCGCAA 3′) were chosen based on our previous study [18]. The amplification and detection of DNA with the species-specific and universal primers using real-time PCR were performed with the İCycler İQ Multicolour Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). For each real-time PCR, İQ SYBR Green SuperMix (Bio-Rad Laboratories) supplemented with 4.5% DMSO was used. A 50-μL total PCR amplification volume for each reaction was placed in each well of a 96-well MicroAmp Optical Reaction Plate and covered with Optical-Quality Sealing Tape (Bio-Rad Laboratories). The DNA amplification conditions for PCR with the universal and species-specific primers for P. gingivalis and T. denticola were 15 min initial denaturation at 95°C, followed by 50 cycles of 95°C for 30 s, 65°C for 50 s, and 72°C for 45 s, with a final 45 s at 75°C. The amplification conditions for T. forsythia were the same, except that annealing was performed at 60°C for 50 s. The PCR amplifications with species-specific and universal primers were repeated twice to confirm the results. Positive results were obtained in the melting analysis of each amplicon based on the target region based on the primers specific for each bacterium investigated. From among the positive results, one positive amplicon for each bacterium was selected randomly for sequencing by Iontek (Istanbul, Turkey) using an ABI Prism 310 Genetic Analyzer to verify the positive results.

Statistical analysis

The prevalence of EBV, HCMV, *P. gingivalis*, *T. forsythia*, and *T. denticola* DNA in acute apical abscess lesions was recorded as a percentage of the cases examined. The chi-squared test with Yates's correction or the Fisher's exact test was used to analyse the significance of differences. The latter was used whenever at least one cell of the 2 × 2 contingency table had a value <5. Significance levels were established at 5% (< 0.05).

Results

Eighteen apical abscess specimens were collected from adult patients (average age 29 [range 22–36] years). All of the samples taken from apical abscesses and healthy pulp were positive in the PCR assay targeting the beta-globulin gene. This indicated that the DNA extraction and multiple displacement amplification protocols were effective at making DNA available for detecting viruses and bacteria [18].

Of the 18 abscess samples positive for the beta-globin gene, four (22%) were positive for at least one of the target human viruses. Figure 1 summarises the prevalence of EBV and HCMV DNA in the apical abscesses. HCMV DNA was found in three samples (16.7%), and EBV DNA was found in one (5.6%). Viral coinfection was not observed in any of the 18 apical abscesses studied.

Figure 1 also illustrates the incidence of "red complex" species in the acute apical abscesses. At least one member of the "red complex" was found in 66.6% (12/18) of the cases. *T. denticola*, *P. gingivalis*, and *T. forsythia* were detected in 44.4, 27.7, and 22.2% of the samples, respectively. Five samples (27.7%) yielded two members of the red complex: the pair *P. gingivalis*/*T. denticola* was detected in two cases, *P. gingivalis*/*T. forsythia* in one case, and *T. denticola*/*T. forsythia* in two cases. The complete "red complex", composed of *P. gingivalis*, *T. denticola*, and *T. forsythia*, was not detected in any samples taken from acute apical abscesses.

Viral-bacterial coinfection was observed in two samples (2/18 cases, 11%) the pairs HCMV/*T. denticola* and HCMV/*T. forsythia* were detected in one case each. The association between HCMV, *T denticola* and *T. forsythia* was not statistically significant. ($p > 0.05$). EBV was not associated with any of the red complex bacteria in the acute apical abscess samples (Table 2).

Discussion

The purpose of this study was to investigate the possible associations of EBV, HCMV, and three candidate endodontic

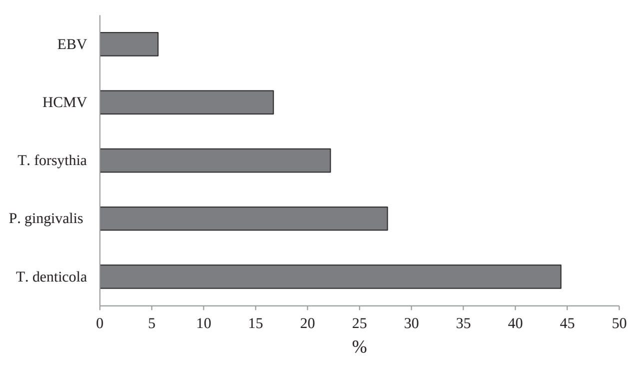

Figure 1: Prevalence of members of red complex and HCMV, EBV DNA in 18 samples from apical abscess lesions.

Table 2: Distribution of bacterial and viral associations in samples from apical abscess lesions.

Bacterial and viral types	*P.gingivalis/ T.denticola* n (%)	*P.gingivalis/ T. forsythia* n (%)	*T.denticola/ T. forsythia* n (%)	HCMV/ *T.denticola* n (%)	HCMV/ *T.forsytia* n (%)
Positive acute apical abscess specimens	2 (11.1%)	1 (5.5%)	2 (11.1%)	1 (5.5%)	1 (5.5%)

bacterial pathogens (*P. gingivalis*, *T. forsythia*, and *T. denticola*) in acute apical abscesses using real-time PCR. The result of this study rejects the null hypothesis and has shown that EBV, HCMV and red complex bacteria associations in abscess lesions were not significant.

Concomitant infection with herpesviruses and specific bacterial species has been suggested as applicable to the aetiology of abscesses [15,18]. Sabeti and Slots [15], reported that most anaerobic bacteria (*P. gingivalis/P. endodontalis*) were isolated from periapical lesions showing HCMV and EBV dual infection where the lesions were symptomatic or large. Ferreira et al. [18], demonstrated that bacterial and viral DNA occurred concomitantly in two-thirds of acute apical abscess samples.

Molecular methods have been used widely to identify microorganisms in samples without the need for culture. In this study, using real-time PCR, *P. gingivalis*, *T. denticola*, and *T. forsythia* were detected in 27.7, 44.4, 22.2 %, respectively, of the acute apical abscess samples. As with any method, molecular methods have their advantages and limitations. The real-time fluorescence-based PCR method used here is a valuable method that is rapid, identifies PCR products directly without the use of agarose gels, and limits contamination of the nucleic acids because post-amplification manipulation is avoided [3,19].

In this real-time PCR study, HCMV and EBV DNA were detected in 16.7% (3/18) and 5.6% (1/18) of the acute apical abscess samples, respectively. Using reverse-transcription PCR, Sabeti and Slots [15], found that all symptomatic periapical lesions contained either HCMV or EBV, and they identified dual infection by the two viruses in 69.6% of symptomatic lesions. Using single and nested PCR, Ferreira et al. [2], did not find HCMV or EBV in their abscess specimens. In another study, Ferreira et al. [18] found EBV in 6% of purulent exudates from acute apical abscesses, whereas they did not identify HCMV in abscess samples using single and nested PCR. The discrepancies in the findings among our study and the three studies mentioned above might arise for several reasons, including differences in the primers used, methods used for DNA extraction, and host and environmental factors such as genetic background, ethnicity, and socioeconomic status [8,20–23]. We used same sampling techniques applied by Chen et al. [1]. This may be the reason of our results consistency with the findings of Chen et al. [1], who found HCMV and EBV in 29 and 6.5% of abscess specimens, respectively.

In this study, when pairs of the target species were evaluated, the pair *P. gingivalis/T. denticola* was detected in two cases (11%), *P. gingivalis/T. forsythia* in one case (5.6%), and *T. denticola/T. forsythia* in two cases (11%). Ferreira et al. [18], reported that several bacterial pairs showed moderately positive associations: *Porphyromonas endodontalis/Filifactor alocis*; *F. alocis/Pyramidobacter piscolens*; *Dialister pneumosintes/P. piscolens*; *Olsenella uli/P. piscolens*; and *P. endodontalis/O. uli*. The differences might occur for several reasons, such as differences in the primers used and methods of DNA extraction, differences in the endodontic microbiota, and several host and environmental factors, such as genetic background, ethnicity, socioeconomic status, psychologic stress, smoking, and the nature of the species colonizing other individuals in the same country [11,24,25].

It has been suggested that the viral-bacterial coinfection observed in abscesses has two basic interpretations: viruses impair local host defences, favouring bacterial overgrowth, or the occurrence of viruses is just an epiphenomenon of the bacterial infection that caused inflammation, with a consequent influx of virus-infected inflammatory cells to the area [18]. In this study, viral–bacterial associations involving the target bacteria and viruses were observed in two samples (11%). The pairs HCMV/*T. denticola* and HCMV/*T. forsythia* were each detected in 1 of 18 (5.6%) abscess specimens. Using single or nested PCR, Ferreira et al. [18], found that bacterial and viral DNA occurred concomitantly in two-thirds of the samples from endodontic abscesses. They found that human herpesvirus-8 and human papillomavirus were the most prevalent viruses showing associations with the target bacterial species. The lower prevalence of viral–bacterial associations in our study may have resulted from the clinical status of the study subjects, viral diagnostic methods used, or geographic differences in herpes viral occurrence [3,8,11,19,24].

Conclusion

Our PCR-based findings revealed that HCMV was the most frequent herpesvirus and *T. denticola* was the most frequent bacterium among the target organisms in acute apical abscesses. Bacterial coinfections were found in four samples (22%). The pairs HCMV/*T. denticola* and HCMV/*T. forsythia* were detected in one case each. Additional studies using *in vitro* systems or animal models are required to elucidate the role of herpesviruses in the pathogenesis of periapical pathosis.

Clinical Significance

The present bacterial and viral findings may have future therapeutic relevance for periapical abscesses and other periapical pathosis.

References

1. Chen V, Chen Y, Li H, Kent K, Baumgartner JC, et al. (2009) Herpesviruses in abscesses and cellulitis of endodontic origin. J Endod 35: 182-188.
2. Ferreira DC, Paiva SS, Carmo FL, Rôças IN, Rosado AS, et al. (2011) Identification of herpesviruses types 1 to 8 and humanpapillomavirus in acute apical abscesses. J Endod 37: 10-16.
3. Siqueira JF Jr, Rôças IN (2011) Microbiology and treatment of endodontic infections. In: Hargreaves KM, Cohen S, eds. Cohen's Pathways of the Pulp, 10th ed. St. Louis, Missouri, USA: Mosby Elsevier 559-600.
4. Rosaline H, Satish ES, Kandaswamy D (2009) Detection of presence or absence of herpes simplex virus, Epstein Barr virus and human cytomegalovirus in infected pulp using a polymerase chain reaction. Aust Endod J 35: 9-12.
5. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr (1998) Microbial complexes in subgingival plaque. J Clin Periodontol 25: 134-144.
6. Siqueira JF Jr, Rôças IN, Souto R, Uzeda M, Colombo AP (2000) Checkerboard DNA-DNA hybridization analysis of endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 89: 744-748.
7. Slots J, Sabeti M, Simon JH (2003) Herpesviruses in periapical pathosis: an etiopathogenic relationship? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 96: 327-331.
8. Slots J. Herpesviral-bacterial interactions in periodontal diseases. Periodontol 2000 2010; 52: 117-140.
9. Ozbek A, Ozbek SM (2015) Detection of herpesviruses and human

papillomavirus in acute apical abscesses by real-time PCR. Clin Oral Investig 19: 343-347.

10. Slots J, Nowzari H, Sabeti M (2004) Cytomegalovirus infection in symptomatic periapical pathosis. Int Endod J 37: 519-524.
11. Ozbek SM, Ozbek A, Yavuz SM (2013) Detection of human cytomegalovirus and Epstein-Barr Virus in symptomatic and asymptomatic apical periodontitis lesions by real-time PCR. Med Oral Patol Oral Cir Bucal 18: 811-816.
12. Cappuyns I, Gugerli P, Mombelli A (2005) Viruses in periodontal disease. Oral Dis 11: 219-229.
13. Rotola A, Cassai E, Farina R, Caselli E, Gentili V, et al. (2008) Human herpesvirus 7, Epstein–Barr virus and human cytomegalovirus in periodontal tissues of periodontally diseased and healthy subjects. J Clin Periodontol 35: 831-837.
14. Rôças IN, Baumgartner JC, Xia T, Siqueira JF, Jr (2006) Prevalence of selected bacterial named species and uncultivated phylotypes in endodontic abscesses from two geographic locations. J Endod 32: 1135-1138.
15. Sabeti M, Slots J (2004) Herpesviral–bacterial coinfection in periapical pathosis. J Endod 30: 69-72.
16. Glickman GN (2009) AAE Consensus Conference on Diagnostic Terminology: background and perspectives. J Endod 35: 1619-1620.
17. Li H, Chen V, Chen Y, Baumgartner JC, Machida CA (2009) Herpesviruses in endodontic pathoses: association of Epstein-Barr Virus with irreversible pulpitis and apical periodontitis. J Endod 35: 23-29.
18. Ferreira DC, Roças IN, Paiva SS, Carmo FL, Cavalcante FS, et al. (2011) Viral-bacterial associations in acute apical abscesses. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 112: 264-271.
19. Ozbek SM, Ozbek A (2010) Real-time polymerase chain reaction of “red complex” (*Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*) in periradicular abscesses. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 110: 670-674.
20. Sabeti M, Simon JH, Nowzari H, Slots J (2003) Cytomegalovirus and Epstein–Barr virus active infection in periapical lesions of teeth with intact crowns. J Endod 29: 321-323.
21. Sabeti M, Simon JH, Slots J (2003) Cytomegalovirus and Epstein–Barr virus are associated with symptomatic periapical pathosis. Oral Microbiol Immunol 18: 327-328.
22. Sabeti M, Valles Y, Nowzari H, Simon JH, Kermani-Arab V, et al. (2003) Cytomegalovirus and Epstein-Barr virus DNA transcription in endodontic symptomatic lesions. Oral Microbiol Immunol 18: 104-108.
23. Hernádi K, Szalmás A, Mogyorósi R, Czompa L, Veress G, et al. (2010) Prevalence and activity of Epstein-Barr virus and human cytomegalovirus in symptomatic and asymptomatic apical periodontitis lesions. J Endod 36: 1485-1489.
24. Rôças IN, Baumgartner JC, Xia T, Siqueira JF Jr (2006) Prevalence of selected bacterial named species and uncultivated phylotypes in endodontic abscesses from two geographic locations. J Endod 32: 1135-1138.
25. Ozbek SM, Ozbek A, Demiray T (2015) Prevalence of several herpesviruses and human papillomavirus in acute apical abscesses. Int Endod J.

Citation: *Ozbek SM, Ozbek A, Demiray T (2015) Herpesviral and “Red Complex” Bacterial Analysis of Acute Apical Abscesses. J Dent Probl Solut 2(3): 053-057. DOI: 10.17352/2394-8418.000019*