Multiple Apical Radiolucencies and External Cervical Resorption Associated with Varicella Zoster Virus: A Case Report

Kreena Patel, BDS(Hons), MJDF RCS, Elia Schirru, DDS, Sadia Niazi, BDS, MSc, PhD, Philip Mitchell, BDS, MSc, MRD, and Francesco Mannocci, MD, DDS, PhD, FHEA

Abstract
Varicella zoster virus (VZV) is responsible for the primary infection chickenpox. After the initial infection, it remains latent but can reactivate, resulting in shingles (herpes zoster). Previous reports have implicated VZV in the pathogenesis of apical periodontitis, but the involvement of the virus has not been investigated fully. The present case describes a patient who suffered from a severe episode of shingles and subsequently developed periapical radiolucencies of all the teeth in the affected nerve distribution. Molecular and culture techniques showed the presence of VZV DNA in the root canal system in the absence of bacteria. This confirms that VZV can cause localized pulp necrosis and apical periodontitis. The lesions healed after endodontic treatment, implying chemomechanical debridement using sodium hypochlorite irrigation and a calcium hydroxide interim dressing may be effective against the virus. (J Endod 2016;42:978–983)

Key Words
Apical periodontitis, external cervical resorption, herpes zoster, shingles, sodium hypochlorite, varicella zoster, virus

A pical periodontitis is a localized immune-modulated inflammatory disease caused by an infection of the dental pulp. Numerous studies have revealed the essential role of bacteria in the etiology of the disease (1–4). Microorganisms normally enter the pulp via canals, clinical procedures, or cracks (5, 6). Bacteria have frequently been isolated from teeth with necrotic pulps but clinically intact crowns (4, 7–9). It was also hypothesized that bacteria within the blood circulation could enter and infect necrotic pulps (anachoresis). However, this has been shown to be highly unlikely (10).

Several studies have suggested that other microorganisms are associated with the pathogenesis of apical periodontitis, including fungi and viruses (11–15). Among the latter, human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and varicella-zoster virus (VZV) have been most commonly isolated (14, 16, 17). These viruses belong to the family of Herpesviridae. A common feature within the family is a single-stranded DNA molecule enclosed in a viral envelope. Eight human herpes viruses have previously been identified: herpes simplex virus 1 and 2; VZV; EBV; HCMV; and human herpes viruses 6, 7, and 8. There appears to be a higher occurrence of HCMV, EBV, and VZV in symptomatic cases and larger lesions (18, 19) and a higher prevalence in human immunodeficiency virus (HIV)-infected patients (20). VZV is responsible for the primary infection chickenpox. The virions enter from the skin or T-lymphocyte viremia and travel in a retrograde manner to the sensory nerve ganglia (21). After the initial infection, the virus remains latent in the long-lived, nondividing perineural satellite cells of the sensory ganglia (22, 23). In 20% of cases, the virus can reactivate, either spontaneously or as a result of an impaired host immune defense, resulting in shingles (herpes zoster). The virus begins to replicate and reaches the skin by anterograde nerve transport (21). Prodromal symptoms include tingling, itching, and pain in the affected dermatome. This is followed by a maculopapular rash in the region, which evolves into vesicles and pustules.

The trigeminal nerve is affected in only 13% of patients (24). The clinical diagnosis of VZV infection is sufficient most of the time although polymerase chain reaction (PCR) analysis or immunofluorescence is sometimes required. Complications include Bell palsy, ocular involvement, hearing impairment, Ramsay Hunt syndrome, and vasculopathy (25). Reported sequelae of dental relevance include devitalization of teeth (26, 27), postherpetic neuralgia, osteonecrosis, dental resorption (internal and external), and tooth exfoliation (28–30). However, these are case reports or case series, and the involvement of VZV has not been fully investigated.

This case report describes a patient who suffered from a severe episode of trigeminal herpes zoster and subsequently developed periapical radiolucencies of all the teeth in the affected nerve distribution area and external cervical resorption of #27. Molecular and culture techniques showed the presence of VZV in the root canal systems in the absence of bacteria.

Examination

A 52-year-old Asian man was referred to Guy’s Dental Hospital, London, UK, in August 2014. The patient presented with periapical radioluencies associated with #25, #26, #27, #28, #29, and #30. There was no history of trauma, metabolic bone disease, or orthodontic treatment.
The patient was medically fit and healthy at the initial consultation. In 1987, he suffered from an episode of herpes zoster affecting the right trigeminal nerve branch V3 (lower right quadrant). He was hospitalized for 10 days and experienced severe pain and vesicles localized to this distribution. He subsequently suffered from postherpetic neuralgia and reported mild anesthesia in this area.

Clinical examination revealed a minimally restored and well-maintained dentition (Fig. 1). Tooth #30 had been root treated by his dentist in 2004. Teeth #25 to 29 were sound, unrestored, and asymptomatic. The teeth in the lower right quadrant were not tender to percussion or palpation and had no mobility or pathological probing associated. None of the teeth in this quadrant responded to pulp vitality testing using electronic pulp testing and cold testing. Radiographic examination confirmed periapical radiolucencies associated with #25 to 30. Tooth #27 also had external cervical resorption (ECR) (Fig. 2–I). A cone-beam computed tomographic (CBCT) scan confirmed that the resorption on #27 communicated with the root canal space and extended circumferentially and apically down the root surface (Fig. 2).

Tooth #31 had been restored with a small occlusal composite restoration. Although the tooth did not have a periapical radiolucency present on the initial radiographs or CBCT scan, it became symptomatic and developed a large radiolucency in the following few months (Fig. 2).

The following provisional diagnoses were made:

1. Pulp necrosis and asymptomatic apical periodontitis were reached for #25, #26, #28, and #29.
2. Pulp necrosis and symptomatic apical periodontitis were reached for #31.
3. Previously treated and asymptomatic apical periodontitis was reached for #30.
4. Pulp necrosis, asymptomatic apical periodontitis, and external cervical resorption were reached for #27.

The patient had routine blood tests on November 2010, which revealed a slight leukopenia of $3.3 \times 10^9$ cells/L (normal range, 4.0–11 x $10^9$) with normal cellular morphology. Routine blood tests run in May and October 2015 revealed the same result. Particularly, lymphopenia $1.1 \times 10^9$ cells/L (normal range, 1.2–3.3 x $10^9$) with a low subset of CD4 $257/\mu$L (normal range, 300–1400/µL), CD3 $575/\mu$L (normal range, 700–2000/µL), and natural killer lymphocytes $66/\mu$L (normal range, 90–600/µL). An HIV test gave a negative result, and the immunoglobulin G for VZV was positive. The persistent moderate chronic leukopenia indicates that these levels are likely to be normal for the patient.

**Treatment**

Nonsurgical endodontic treatment was performed on teeth #25, 26, 28, 29, 30, and 31. The teeth were all necrotic and when accessed under microscope magnification had an unusual odorless, black, pigmented substance in the pulp chamber and canals.

Chemomechanical debridement of the canals was completed using a combination of hand and rotary instruments while irrigating with 1% sodium hypochlorite and 17% EDTA. The endodontic treatment was performed over 2 visits using an interim calcium hydroxide dressing placed using a spiral filler. The teeth were obturated with gutta-percha and a zinc oxide eugenol–based sealer using a warm vertical condensation technique.

The extension and position of the resorptive lesion on #27 was not amenable to treatment and the tooth was extracted.

**Sampling**

During the endodontic treatment of #31, samples of the pulp chamber and canal contents were taken. All sampling was undertaken under strict aseptic conditions. The tooth was isolated using a rubber dam, and the field was cleaned with 30% (vol/vol) hydrogen peroxide and decontaminated with 2% sodium hypochlorite followed by sodium thiosulfate. After decontamination, the isolated tooth and surrounding dam were swabbed to check for contamination. The access cavity was initially prepared with a sterile round bur without water cooling and using sterile saline. This bur was replaced, and only saline irrigation was used when approaching the pulp chamber. On gaining access to the pulp, sterile files and paper points were inserted into the pulp chamber and contents obtained for testing. This was repeated with the distal root canal up to the apical foramina. Surgical sterile gloves were used and replaced regularly throughout the procedure. For bacterial sampling, all the tissues removed were transferred into 1 mL Tris-EDTA buffer (1.0 mol/L Tris-HCl containing 0.1 mol/L EDTA, pH = 8.0 prepared in ultra high quality water) (31). For viral sampling, they were placed into universal transport medium. After the extraction of #27, the root canal was sampled using the same technique. Both samples from teeth

*Figure 1.* Preoperative photographs. Teeth #25 to 29 were unrestored; tooth #30 was root treated in 2004 by his dentist and restored with a large composite restoration; and tooth #31 had a small occlusal composite restoration present.
#27 and #31 were immediately immersed in ice and transported to the laboratory, after which endodontic treatment of #31 was completed. Transmission electron microscopic (TEM) and histologic analyses were also carried out according to the following protocol:

1. For TEM analysis, samples of tooth #27 were collected from the root canal space using the previously described protocol and the resorptive lesion using a sterile excavator. The samples were smeared on clean slides and left to dry.
2. For histologic analysis of tooth #27, the tooth was placed into 10% formalin solution.

**Microbial Analysis of Samples**

Each sample was dispersed by vortexing with sterile 3.5- to 4.5-mm-diameter glass beads (BDH; Lutterworth, Leicester, UK) for 30 seconds, serially diluted in fastidious anaerobe broth (Lab M, Heywood, UK), and plated onto nonselective media (duplicate plates of fastidious anaerobe agar [FAA] supplemented with 5% horse blood [Lab M]). The FAA plates were incubated anaerobically for 7 days and aerobically for 5 days. The swabs taken from the tooth for sterility check were plated directly onto FAA and incubated anaerobically for 7 days.

Aerobic and anaerobic bacterial culturing gave a negative result for both samples taken from tooth #31. An identical culturing technique gave a positive bacterial result for tooth #27. An additional quantitative PCR (qPCR) analysis was performed for both samples, confirming the presence of bacteria in tooth #27 and the absence of bacteria in #31.

**Viral Analysis of Samples**

Qualitative PCR testing of 2 samples from teeth #31 and #27 gave a positive result for VZV DNA. Tooth #31 had a cycle threshold (CT) value

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**Figure 2.** Preoperative radiographs. (A) Orthopantomogram reveals multiple periapical radiolucencies associated only with the teeth in the lower right quadrant. (B–E) Preoperative periapical radiographs showing periapical radiolucencies associated with teeth #25 to 30 and external cervical resorption associated with tooth #27. (F) A periapical radiograph taken a few months later showing tooth #31 had developed a large radiolucency. (G–I) A preoperative CBCT scan of tooth #27. (G) Axial slices show the external cervical resorption is buccal and extends distally. It communicates with the root canal system. (H) Sagittal view. (I) Coronal view.
of 29 and #27 had a CT value of 28. The cutoff CT value is 39, and anything below this is classified as a positive result. Qualitative PCR gave a negative result for herpes simplex virus 1 and 2.

**Histology**

Histologic analysis was only conducted on tooth #27. After fixation, the tooth was decalcified, embedded in paraffin, and serially sectioned through the ECR lesion. Hematoxylin-eosin was used to stain the sample. The ECR lesion was shown to communicate directly with the pulp space and showed ingrowth of inflamed periodontal tissue with epithelium. Neutrophils and bacteria were detected in the pulp space. No caries was detected (Fig. 3A and B).

**TEM Analysis for Samples**

TEM negative stain analysis was conducted only on tooth #27. The dried smear was rehydrated in water and applied to formvar/carbon-coated TEM grids (Agar Scientific Limited, Essex, UK). 1% alcian blue was used as a wetting agent for the grids, and 1.5% (w/v) phosphotungstic acid was used as the negative stain. The negatively stained grids were viewed in a JEOL JEM-1400 (JEOL UK, Welwyn Garden City, UK) TEM fitted with an AMT XR60 digital camera (Advanced Microscopy Techniques, Woburn, MA). It was not possible to detect a clear image of VZV particles in the specimens. Nevertheless, small bacteria aggregates were obvious inside the canal space and within the resorptive cavity (Fig. 3).

**Review**

The patient was reviewed 1 year postoperatively. There were no clinical signs or symptoms. Radiographs showed a reduction in size of the periapical radiolucencies in all the treated teeth except for the mesial root of tooth #30. The mesiobuccal canals were blocked or ledged from the previous root canal treatment and could not be negotiated to the full working length during the root canal retreatment. Therefore, apical surgery of the mesial root of tooth #30 was subsequently performed (Fig. 4A–E).

**Discussion**

The role of viruses in the development of apical periodontitis and endodontic disease in general has not been fully investigated. Molecular techniques detect the presence of viable and nonviable genetic material, and their use has significantly improved our understanding of endodontic infections. Recently, it has been reported that other microorganisms such as fungi, archea, and viruses can cohabit the root canal and periapical tissues with bacteria (11, 12, 14, 15, 32, 33).

Herpesviruses have been isolated from symptomatic and asymptomatic periapical tissues. Several viral pathogenicity mechanisms have been proposed in the past, but the most common is via an indirect route. The primary bacterial infection of the root canal results in periapical inflammation and the recruitment of immune cells infected with latent herpes virus. The herpes virus is subsequently reactivated and results in a local immunosuppression that allows overgrowth of the pathogenic bacteria apically (14).

A direct link has also been hypothesized; the virus infects the trigeminal nerve endings in the dental pulp leading to infection, infarction, and necrosis of the pulpal vasculature (34). It may also have cytopathic effects on periapical tissue, resulting in impaired turnover and repair. This could result in loss of bone and potentially result in the formation of a radiolucency (35). This case report suggests a direct mechanism of pulp necrosis and periapical inflammation is credible.

To our knowledge, this is the first case to use molecular methods to detect VZV in the root canal system and show that apical periodontitis can develop in the absence of bacteria. The root canal space of tooth #31 gave 2 positive qPCR tests for the presence of VZV, negative aerobic and anaerobic cultures, and qPCR for the presence of bacteria. This confirms that a direct link may exist and that VZV can cause apical periodontitis without the presence of bacteria. The root canal system of tooth #27 was positive for VZV and cultured positive for bacteria. We expected to detect bacteria in tooth #27 because of the communication between the root canal system and the oral cavity caused by the ECR.

PCR is highly sensitive technique and requires a few DNA segments per mL to give a positive result. A threshold value is required to ensure false-positive results are rejected. The detection of VZV DNA is based on a real-time PCR. Therefore, this technique monitors the amplification of specific VZV genomic DNA sequences during the PCR process.

A qualitative PCR cannot precisely quantify the amount of viral DNA present. However, if the viral DNA is detected earlier in the replication process (ie, lower CT), it indicates a higher viral load. The necrotic tissue samples taken from teeth #27 and #31 showed a low CT, indicating a strong positive result for the presence of VZV.

The apparent discrepancy between PCR molecular analysis and electron microscopy may be explained by the physical “state” of the virus particles. PCR can detect viral nucleic acid even if it is no longer in a virus particle. However, transmission electron microscopy can only identify virus particles if they are relatively intact. Furthermore, TEM analysis has a relatively low sensitivity (10^2–10^6 particles/mL) compared with most other detection methods (36).
Endodontic treatment was performed for all the restorable affected teeth. A chemomechanical preparation technique and interim dressing of calcium hydroxide were used to disinfect the root canal system. Numerous studies have shown that sodium hypochlorite is effective against bacteria during root canal treatment because of its broad antibacterial spectrum and tissue-dissolving properties (37). It has also been shown to kill herpesviruses and HIV on environmental surfaces (38–41). However, conclusive data on eliminating VZV inside the root canal have not been proven. In this case, 1-year review radiographs confirmed a significant reduction in size of the periapical radiolucencies which may indicate that chemomechanical debridement is effective against VZV.

Conclusion

This case highlights that VZV infection may result in pulp necrosis and formation of apical periodontitis in the absence of bacteria. It emphasizes the need for regular dental review in any quadrant affected by herpes zoster. Patients may present with scarring in this area, which should lead the dentist to enquire about a past infection. Further studies are required to clarify the role of VZV in the pathogenesis of apical periodontitis.

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