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## **Multiple apical radiolucencies and external cervical resorption associated with Varicella Zoster Virus: A case report**

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### **Abstract**

Varicella zoster virus (VZV) is responsible for the primary infection chicken pox. Following 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 virus has not been investigated. 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 demonstrated the presence of VZV DNA in the root canal system in absence of bacteria. This confirms that VZV can cause localised pulp necrosis and apical periodontitis. The lesions healed following endodontic treatment implying chemomechanical debridement using sodium hypochlorite irrigation and a calcium hydroxide interim dressing may be effective against the virus.

### **Introduction**

Apical periodontitis is a localised 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 caries, 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 hypothesised that bacteria within the blood circulation could enter and infect necrotic pulps (anachoresis). However, this has been demonstrated 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 double-stranded DNA molecule enclosed in a viral envelope. Eight human herpes viruses have previously been identified: herpes simplex 1 & 2 (HSV-1, -2), VZV, EBV, HCMV, human herpes virus 6, 7 & 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 HIV (human immunodeficiency virus) infected patients (20).

VZV is responsible for the primary infection chicken pox. The virions enter from the skin or T-lymphocyte viraemia and travel in a retrograde manner to the sensory nerve ganglia (21). Following the initial infection, the virus remains latent in the long lived, non-dividing perineural satellite cells of the sensory ganglia (22, 23). In 20% of cases the virus can reactivate, either spontaneously or as a result of impaired host immune defence 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 PCR analysis or immunofluorescence is sometimes required. Complications include Bell's palsy, ocular involvement, hearing impairment, Ramsay Hunt syndrome and vasculopathy (25). Reported sequelae of dental relevance include devitalisation of teeth (26, 27), post herpetic neuralgia, osteonecrosis, dental resorption (internal, 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 demonstrated the presence of VZV in the root canal systems in 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 radiolucencies associated with #25, #26, #27, #28, #29, #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 hospitalised for 10 days and experienced severe pain and vesicles localised to this distribution. He subsequently suffered from post-herpetic neuralgia and reported mild anaesthesia 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 #25-29 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 respond to pulp vitality testing using EPT and cold testing. Radiographic examination confirmed periapical radiolucencies associated with #25-30. Tooth #27 also had external cervical resorption (ECR) (Fig. 2). A CBCT 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 was reached for #25, #26, #28, #29
- 2) Pulp necrosis and symptomatic apical periodontitis was reached for #31
- 3) Previously treated and asymptomatic apical periodontitis was reached for #30

- 4) Pulp necrosis, asymptomatic apical periodontitis and external cervical resorption was 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  $4.0-11 \times 10^9$ ) 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  $1.2-3.3 \times 10^9$ ) with a low subset of CD4  $257/\mu\text{L}$  (normal range  $300-1400/\mu\text{L}$ ), CD3  $575/\mu\text{L}$  (normal range  $700-2000/\mu\text{L}$ ) and NK lymphocytes  $66/\mu\text{L}$  (normal range  $90-600/\mu\text{L}$ ). A HIV test gave a negative result and the IgG for VZV was positive. The persistent moderate chronic leukopenia indicates that these levels are likely to be normal for the patient.

### **Treatment**

Non-surgical endodontic treatment was carried out on #25, 26, 28, 29, 30. The teeth were all necrotic and when accessed under microscope magnification had an unusual odourless, black, pigmented substance in the pulp chamber and canals.

Chemo-mechanical 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 carried out over two 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 rubber dam and the field was cleaned with 30% (vol/vol) hydrogen peroxide and decontaminated with 2% sodium hypochlorite followed by sodium thiosulphate. 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 (1.0M Tris-HCl containing 0.1M EDTA; pH 8.0 prepared in UHQ water) (31).
- For viral sampling, they were placed into universal transport medium. Following the extraction of #27, the root canal was sampled using the same technique. Both samples from #27 and #31 were immediately immersed in ice and transported to the laboratory, following which endodontic treatment of #31 was completed.

Transmission electron (TEM) and histological analysis was also carried out according to the following protocol:

- For TEM analysis, samples of #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 a clean slide and left to dry.
- For histological analysis of #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, United Kingdom) for 30 seconds, serially diluted in fastidious anaerobe broth (FAB, Lab M, United Kingdom) and plated onto non-selective media; duplicate plates of fastidious anaerobe agar (FAA) supplemented with 5% horse blood (Lab M, United Kingdom). The FAA plates were incubated anaerobically for 7 days and aerobically for 3 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 the both samples taken from #31. An identical culturing technique gave a positive bacterial result for #27. An additional qPCR analysis was carried out for both samples, confirming the presence of bacteria in LR3 and the absence of bacteria in #31.

### **Viral analysis of samples**

Qualitative PCR testing of two samples from both #31 and #27 gave a positive result for VZV DNA. Tooth #31 had a cycle threshold (CT) value 29 and #27 had a CT value 28. The cut off CT value is 39 and anything below this is classified as a positive result. Qualitative PCR gave a negative result for HSV-1/-2.

### **Histology**

Histological analysis was only conducted on #27. After fixation, the tooth was decalcified, embedded in paraffin and serially sectioned through the ECR lesion. Haematoxylin and 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. 3).

### **TEM analysis for samples**

TEM negative stain analysis was conducted only on #27. The dried smear was rehydrated in water and applied to formvar/carbon coated TEM grids. 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 TEM fitted with an AMT XR60 digital camera. 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 one 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 #30. The mesiolingual and 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 #30 was subsequently carried out (Fig. 4).

## **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 non-viable genetic material and their use has significantly improved our understanding of endodontic infections. Recently, it has been reported that other microorganisms such as fungi, archaea and viruses can co-inhabit 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 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 hypothesised; 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 demonstrate that apical periodontitis can develop in the absence of bacteria. The root canal space of #31 gave two 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 #27 was positive for VZV and cultured positive for bacteria. We expected to detect bacteria in #27 because of the communication between the root canal system and oral cavity caused by the ECR.

PCR is highly sensitive technique and requires a few DNA segments/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 polymerase chain reaction. 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 (i.e. lower cycle threshold) it indicated a higher viral load. The necrotic tissue samples taken from #27 and #31 demonstrated a low cycle threshold thus showing 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, TEM can only identify virus particles if they are relatively intact. Furthermore, TEM analysis has a relatively low sensitivity ( $10^5$ - $10^6$ particles/ml) compared to most other detection methods (36).

Endodontic treatment was carried out for all the restorable affected teeth. A chemo-mechanical preparation technique and interim dressing of calcium hydroxide was used to disinfect the root canal system. Numerous studies have shown that sodium hypochlorite is effective against bacteria during root canal treatment due to 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 has not been proven. In this case, one-year review radiographs confirmed a significant reduction in size of the periapical radiolucencies which may indicate that chemo-mechanical 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 emphasises 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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*The authors disclose no conflicts of interest*

## Figures legend

Figure 1: Preoperative photographs. #25-29 were unrestored; #30 was root treated in 2004 by his dentist and restored with a large composite restoration; #31 had a small occlusal composite restoration present.

Figure 2: Preoperative radiographs. (A) OPG reveals multiple periapical radiolucencies associated only with the teeth in the lower right quadrant; (B-E) preoperative periapical radiographs, demonstrating periapical radiolucencies associated with #25-30 and external cervical resorption associated with #27; (F) periapical radiograph taken a few months later demonstrating #31 had developed a large radiolucency; (G-I) Preoperative CBCT of #27; (G) axial slices demonstrate the external cervical resorption is buccal and extends distally. It communicates with the root canal system; (H) Sagittal view; (I) Coronal view

Figure 3: (A) Histology of #27. Vertical section demonstrating the external cervical resorption communicates with the root canal system; (i) External cervical resorption; (ii) Root canal system; (B) Transmission electron microscope analysis of root canal contents of #27. Different bacterial types could be seen but no intact viral particles were detected.

Figure 4: 1-year review. (A-C) Postoperative radiographs demonstrating healing of all the teeth in the lower right quadrant following root canal treatment except the mesial root of #30; (D) apical surgery postoperative radiograph; (E) postoperative photograph after restorative treatment had been carried out