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Human cytomegalovirus is present in odontogenic cysts

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Introduction: Recent studies suggest that some viruses, including human cytomegalovirus (CMV), may be involved in the pathogenesis of periapical lesions. Since periapical cysts (PCs) represent the next stage in the evolution of periapical granuloma, it seemed reasonable to investigate the presence of CMV in PCs and any possible relationship between its presence and the clinical features of those cysts, as well as to compare the results obtained with corresponding findings in non-inflammatory lesions, like odontogenic keratocysts (OKCs).
Methods: Samples of 33 PCs and 10 OKCs, obtained at the time of surgery, were used for the detection of CMV DNA by polymerase chain reaction. Presence of the virus was correlated with clinical and radiographic features of the cysts.
Results: CMV was detected in 18 PCs (54.5%) and six OKCs (60%). The presence of CMV was more frequent in cyst samples collected from patients who reported previous episodes of acute infection. The presence of sinus tract was more frequent in CMV-positive cysts and CMV presence was less frequent in a group of cysts showing signs of acute inflammation at the time of sample collection. The mean sizes of CMV-positive and CMV-negative PCs were almost the same; CMV-positive OKCs were slightly larger than CMV-negative OKCs. None of these results proved to be statistically significant.
Conclusion: The presence of CMV in the cystic wall is a common feature of both inflammatory and non-inflammatory odontogenic cysts. Although this study has not proved that CMV affects pathogenesis of odontogenic cysts, such a possibility could not be ruled out.

Key words: cytomegalovirus; odontogenic keratocyst; periapical cyst

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Odontogenic cysts are common lesions of the jaws. Among them, periapical (radicular) cysts (PCs) and odontogenic keratocysts (OKCs) draw special attention; PCs because of their frequent occurrence and OKCs because they have special biological features. Essentially, PCs represent an inflammatory response to chronic irritation from the root canal of a tooth with necrotic pulp (20). Furthermore, proinflammatory cytokines and inflammation-induced expression of some growth factors are crucial for the development of PCs (5, 8). On the other hand, OKCs are non-inflammatory lesions, and genetic factors are thought to play a major role in their etiology (27). However, secondary inflammation in their walls is very frequent and results in changes of the characteristic histopathological features of OKCs towards non-specific findings, similar to those of PCs (23).

The role of pathogenic oral bacteria in the initiation of periodontal inflammation, including periapical tissues, is well recognized (16). Moreover, it is suggested that some viruses, particularly human cytomegalovirus (CMV) and Epstein–Barr virus type 1, may be involved because they were detected in inflammatory cells in periodontal lesions (2). It was also shown that an increase in herpes virus copy-counts is related to more severe forms of periodontitis (14).

Several studies provided substantial information on the involvement of CMV in periapical inflammation. Active CMV infection was detected in specimens of periapical lesions obtained at the time of apicectomy (26). Moreover, such infections were more frequent in specimens from the periapical region of teeth that showed signs of acute inflammation (25), even reaching 100% of CMV-positive symptomatic specimens in a study by Slots et al. (29). Active CMV infection was also detected in periapical lesions of teeth with intact crowns (24). This

suggests that CMV in periapical tissues does not originate from the mouth, although it is frequently excreted through saliva. All these studies imply the possible participation of CMV in the etiopathogenesis of periapical inflammation – initiation of cytotoxic reactions, release of tissue-destructive cytokines, and immune impairment favoring bacterial infection, were denoted as possible mechanisms of that influence (30).

As CMV is detected in periapical lesions and PCs actually represent the next step in the evolution of periapical granulomas, it seems reasonable to believe that the virus is also present in PCs. However, there are no available references on that topic. The immune response to CMV infection includes the activation of cytotoxic T lymphocytes and natural killer cells (1, 33), which represent a significant part of the inflammatory cell infiltrate in periapical granuloma (11, 13). It is possible that the destruction of infected cells in periapical granuloma favors its cystic transformation. Furthermore, CMV induces the production of proinflammatory cytokines interleukin-1β (IL-1β), IL-6, IL-12, and tumor necrosis factor-α (19), which are known to be mediators of bone resorption in periapical lesions (16) and are present in odontogenic cysts (5, 17). Accordingly, CMV, if present, might contribute to bone resorption and growth of the cyst.

Having in mind the possible involvement of CMV in the etiopathogenesis of PCs, and the frequent secondary inflammation of OKCs, the aim of this study was to investigate if CMV is present in the walls of both PCs and OKCs and to correlate its presence to the type of cyst and to their clinical and radiographic features.

Material and methods

Patients and clinical data

Forty-nine patients with clinical and radiographic findings suggestive of cystic lesions of the jaws enrolled in the study. Immunocompromised patients or patients who were receiving antiviral or immunosuppressive therapy were excluded from the study. The Ethical Committee of the School of Dentistry, University of Belgrade, approved the study protocol. Informed consent was obtained from all patients and all procedures were fully explained to them before the study.

Data on patients' complaints, duration of symptoms and previous treatment were recorded for each patient. Particular attention was paid to the history of acute infection of the cyst, number of previous episodes of infection and treatment needed to resolve them. Clinical examination was performed immediately before surgery, and presence of pain in the previous 48 h, inflammatory swelling, and sensitivity to palpation or percussion were noted. Lesions exhibiting any of those signs were considered to be symptomatic. Otherwise, lesions were noted as asymptomatic. Also, presence of a sinus tract was recorded. Cystic fluid was aspirated by inserting a needle through the mucosa in an area of bony expansion, and the appearance of cystic fluid was noted as either typical or purulent.

To assess the size of the cyst, the greatest mesio-distal and cranio-caudal diameters of the lesion were measured on standard panoramic images, and cyst size was calculated as the arithmetic mean of those values.

Collection of samples

Before the surgical incision, the operative area was treated with 0.12% chlorhexidine. After that, an appropriate incision was made and a full-thickness mucoperiosteal flap was reflected, to expose the area of the cyst. Thinned bone was removed using rotary instruments to gain access to the cyst. Part of the cystic wall was excised, placed in an empty plastic vial and immediately frozen until the virological examination. The remaining, larger portion of the cyst was enucleated and used for histopathological examination.

Regarding the size of the lesions, at the time of the surgery it was possible to demonstrate the presence of a sac-like cystic lesion of the jaw in all but two cases (these two were then excluded from further study). Criteria for establishing diagnosis of PC were ability to demonstrate the presence of a cystic lumen at the time of surgery, presence of endodontically treated tooth or a tooth with necrotic pulp in connection with the cyst, and histopathological findings of epithelium-lined cavity with features characteristic of PCs. Diagnosis of OKC was made if typical histopathological features of OKCs, including parakeratosis of the epithelial lining, were present in at least some parts of the cystic wall and if neighboring teeth demonstrated no pulpal or periapical disease. The presence of epithelium-lined cavity was required to distinguish inflamed cysts with purulent fluid from periapical abscesses.

During the entire surgical procedure, using meticulous high-volume evacuation, care was taken to minimize the risk of salivary contamination of samples.

Once full clinical, radiographic and histopathological examinations were performed, final diagnosis was made for all lesions. Specimens from lesions that fulfilled criteria for the diagnosis of PCs or OKCs were used for the detection of CMV DNA.

Viral detection

Polymerase chain reaction (PCR) was used to detect CMV. Samples of cystic wall were processed using a standard proteinase K and phenol–chloroform nucleic acid extraction method. Primers for viral detection were CMV F: 5′-CCACCCGTGGTG CCAGCTCC-3′ and CMV R: 5′-CCC GCTCCTCCTGAGCACCC-3′ (Fermentas, Burlington, Canada). Details on the sensitivity and specificity of the set of primers have been described previously (28). The PCR product was 159 bp in size. PCR amplification was performed in a total volume of 25 μl mixture that contained 3 μl sample, 1× *Taq* buffer (Promega, Madison, WI, USA), 3 mM $MgCl_2$ (Promega), 0.2 mM deoxynucleoside triphosphates, and 0.20 μM of each primer.

Human fibroblast monolayers containing CMV were used as a positive control, while a PCR mixture containing 3 μl distilled water instead of cyst sample was used as a negative control. Initial denaturation of DNA was performed at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C; annealing at 56°C; and elongation at 72°C, 30 s each. Final extension was performed at 72°C for 5 min.

Ten microliters of amplification mixture were subjected to electrophoresis in 8% polyacrylamide gel (20 V/cm in Tris–borate ethylenediaminetetraacetic acid buffer), containing 0.5 μg/ml ethidium bromide. Amplified fragments were visualized under an ultraviolet transilluminator. The result was considered to be positive if a band of the expected size was present.

Statistical evaluation

Statistical analysis was carried out using chi-squared test, Fisher's exact test and Student's *t*-test (SPSS 10.0 statistical software; SPSS Inc, Chicago, IL, USA); *P*-values of 0.05 or less were considered to be statistically significant.

Results

After histopathological examination, final diagnosis was made for all patients and out of 49 enucleated lesions, 43 samples fulfilled the criteria of the study – 33 were

Table 1. Clinical features of cytomegalovirus (CMV)-positive and CMV-negative cysts

	Periapical cysts (PCs)		Odontogenic keratocysts (OKCs)	
	CMV-positive	CMV-negative	CMV-positive	CMV-negative
Without episodes of infection	2 (11.1)	3 (20)	4 (66.6)	3 (75)
One episode of infection	7 (38.9)	8 (53.3)	1 (16.7)	1 (25)
Repeated infections	9 (50)	4 (26.7)	1 (16.7)	0 (0)
With sinus tract	7 (38.9)	3 (20)	3 (50)	0 (0)
Without sinus tract	11 (61.1)	12 (80)	3 (50)	4 (100)
Symptomatic cysts	7 (38.9)	9 (60)	0 (0)	1 (25)
Asymptomatic cysts	11 (61.1)	6 (40)	6 (100)	3 (75)
Typical cystic fluid	7 (38.9)	3 (20)	4 (66.7)	3 (75)
Purulent cystic fluid	7 (38.9)	10 (66.7)	0 (0)	1 (25)
N/A	4 (22.2)	2 (13.3)	2 (33.3)	0 (0)

Values are given as *n* (%).

PCs and 10 were OKCs. Three dentigerous cysts, one orthokeratinized keratocyst, and two cases in which it was not possible to demonstrate the presence of a cystic lumen were excluded from the study. There were 18 male and 25 female patients; mean age was 43.3 years (SD 17.7). Of the 43 cysts, 14 were located in the lower jaw and 29 in the upper jaw. Mean size of the cysts was 30.12 mm (SD 15.07 mm). The smallest and the largest cysts were 11 and 80 mm in diameter, respectively.

Cytomegalovirus DNA was detected in more than half of the collected samples. Eighteen of 33 PCs (54.5%) and six of 10 OKCs (60%) harbored CMV in their walls. As can be seen, the frequency of CMV detection was similar for both PCs and OKCs (chi-squared test: $s = 0.76$).

Cytomegalovirus was more frequently present in cysts from patients who reported previous episodes of acute infection (Table 1) In the group of PCs, twice as many CMV-negative cysts were without episodes of infection, compared with CMV-positive cysts (20% vs. 11.1%). Moreover, two or more previous episodes of infection (repeated infections) were seen more often in CMV-positive PCs. However, according to chi-squared tests, these differences were not statistically significant. Although most of the patients with OKCs had not reported episodes of infection, it is interesting to point out that the only patient with OKC who had repeated infections had a CMV-positive cyst (Table 1).

The presence of sinus tract (which might be regarded as a consequence of more pronounced inflammatory reactions) was more frequent in PCs that harbored CMV. In the group of OKCs, cysts with sinus tract always harbored CMV in their walls, while none of the CMV-negative OKCs showed the presence of sinus tract (Table 1). Again, according to Fisher's exact test, those differences were statistically insignificant.

Surprisingly, the presence of CMV was less frequent in a group of cysts that showed clinical signs of acute inflammation at the time of sample collection (symptomatic cysts). This was especially so in a group of PCs, although Fisher's exact test had not shown statistical significance (Table 1). Similarly, frequency of CMV detection was lower in cysts that had purulent cystic fluid, although some cysts were empty because of previous drainage or tooth extraction (Table 1).

Mean sizes of CMV-positive and CMV-negative PCs were practically the same: 25.8 mm (SD 12.3 mm) for CMV-positive PCs and 28.7 mm (SD 15.2 mm) for CMV-negative PCs. On the other hand, CMV-positive OKCs were somewhat larger (mean size 45 mm, SD 18.8 mm) than cysts in which CMV was not detected (mean size 32.5 mm, SD 8.9 mm). Again, according to the Student's *t*-test, these differences were statistically insignificant.

Discussion

This study has shown the presence of CMV in the walls of odontogenic cysts. This came as an expected result for PCs because CMV infection of periapical granulomas has already been demonstrated. However, previous studies were unable to demonstrate viral infection of odontogenic cysts (7, 22). Moreover, CMV was implicated in the pathogenesis of lymphoepithelial cysts of the parotid gland, which have histological features similar to odontogenic cysts (32). Still, in a study of Yen et al. CMV was detected in 20% of lymphoepithelial cysts and 23% of normal parotid tissue specimens (34). From that point of view, >50% of CMV-positive odontogenic cysts in our study represents a relatively high frequency of virus detection.

Bearing in mind the substantial etiological and pathogeneic differences between PCs and OKCs, almost the same frequency of CMV detection in both cyst types was surely the most surprising result of this study. Although frequent inflammation of OKCs results in changes of their histopathological features (23), an inflamed OKC is still a lesion with very different embryogenic origin and biological behavior from a PC. This opinion is supported by studies showing that inflammation of OKCs does not reduce (10), or even enhance (3) the expression of cell proliferation markers in the epithelial lining of OKCs. Increased expression of those markers (compared with other odontogenic cysts) is known to be typical of OKCs (27). Furthermore, common recurrences after surgical removal are characteristic of OKCs, and it was shown that infected OKCs might recur even more frequently than their non-infected counterparts (6).

Considering this, the similar percentage of CMV-positive PCs and OKCs suggests that CMV probably does not play a significant role in the etiology of these cysts, but rather in their behavior related to inflammation. It was shown that monocytes and peripheral macrophages are major sites of latent CMV infection (21, 31). As macrophages represent a significant part of the inflammatory cells' infiltrate in periapical lesions (15, 18), it might be assumed that the presence of CMV in the cyst wall is a result of its infiltration by infected macrophages. This opinion is reinforced by Sabeti et al. who showed that CMV in periapical lesions probably does not originate from the mouth (24). However, it is not known how often reactivation of latent CMV in cyst walls takes place and what are its proportions.

Cytomegalovirus was detected more frequently in samples collected from patients who reported previous episodes of infection. The same is true for the presence of sinus tract, a feature that also reflects a pronounced inflammatory reaction in the cyst. Although those results lack statistical verification it is noticeable that similar findings were present in both cyst types. This could indicate a probable contribution of CMV to inflammatory processes in odontogenic cysts.

It was unexpected that CMV was more readily detected in cysts that had not shown signs of acute inflammation at the time of surgery (asymptomatic cysts). Since proinflammatory cytokines have an important role in the reactivation of latent CMV (9), one should expect that exacerbation of local inflammation results in virus reactivation and an increased number of viral particles that will facilitate its

detection. Studies by Sabeti and by Slots et al. showed increased detection of active CMV infection in symptomatic periapical lesions (25, 29). However, those studies investigated the active replication of CMV, making comparison with our results difficult. A lower frequency of CMV detection in cysts that had purulent fluid might partially explain our results: it is possible that severe purulent inflammation causes transitory change in the inflammatory cell infiltrate in the cystic wall, from a relative predomination of infected macrophages to an increased number of non-infected neutrophils, which results in a decrease in viral detection.

Practically, the same mean size of CMV-positive and CMV-negative PCs corresponds to common detection of CMV in periapical granulomas and indicates that virus contaminates the PC at an early stage of its development. It is particularly interesting that the OKCs that harbored CMV were larger than CMV-negative cysts. Although there is no statistical verification of this result, it suggests that OKCs need more time to be infected by CMV and the longer evolution of the cyst also facilitates secondary inflammation in its wall. Furthermore, the similar size of CMV-positive and CMV-negative PCs might be understood as an indicator that CMV does not contribute to their pathogenesis; however, a viral contribution to the growth of cysts, as slowly growing lesions, cannot be substantiated. Furthermore, it could be hypothesized that CMV-positive cysts are smaller because they are diagnosed earlier as a result of the intensive inflammation in the cystic wall.

Finally, it has to be stated that PCR detection of CMV has some limitations that have to be taken into account when discussing the results of this study. It has already been shown that high sensitivity of PCR might be misleading, when diagnosing clinically significant CMV infection (4, 12). Consequently, the results of our study could, possibly, reflect only the presence of latently infected macrophages in the cystic wall, instead of productive CMV infection. On the other hand, reverse-transcriptase PCR detection of active viral replication overcomes a possible problem of detecting latently infected cells. However, sole detection of active replication of the virus could not be regarded as proof of its contribution to the pathogenesis of odontogenic cysts. Such findings might only reflect transient differentiation of circulating monocytes into peripheral macrophages, a process that initiates reactivation of the latent virus (21) and that should be expected in the exacerbation of inflammatory reactions. Still, such reactivation might be limited (both in quantity and duration) and without significant influence on the pathological processes connected with pathogenesis of the cyst.

This study has demonstrated the presence of CMV in a significant proportion of odontogenic cysts. Although there is a lack of evidence that CMV affects the pathological processes in those lesions, its results do not rule out such a possibility. It is likely that the introduction of more sophisticated methods, such as reverse-transcriptase and real-time PCR, will improve our understanding of the possible role of CMV in the pathogenesis of jaw cysts.

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References

1. Borysiewicz LK, Rodgers B, Morris S, Graham S, Sissons JG. Lysis of human cytomegalovirus infected fibroblasts by natural killer cells: demonstration of an interferon-independent component requiring expression of early viral proteins and characterization of effector cells. J Immunol 1985: **134**: 2695–2701.
2. Contreras A, Zadeh HH, Nowzari H, Slots J. Herpesvirus infection of inflammatory cells in human periodontitis. Oral Microbiol Immunol 1999: **14**: 206–212.
3. de Paula AM, Carvalhais JN, Domingues MG, Barreto DC, Mesquita RA. Cell proliferation markers in the odontogenic keratocyst: effect of inflammation. J Oral Pathol Med 2000: **29**: 477–482.
4. Delgado R, Lumbreras C, Alba C et al. Low predictive value of polymerase chain reaction for diagnosis of cytomegalovirus disease in liver transplant recipients. J Clin Microbiol 1992: **30**: 1876–1878.
5. Formigli L, Orlandini SZ, Tonelli P et al. Osteolytic processes in human radicular cysts: morphological and biochemical results. J Oral Pathol Med 1995: **24**: 216–220.
6. Forssell K, Forssell H, Kahnberg KE. Recurrence of keratocysts. A long-term follow-up study. Int J Oral Maxillofac Surg 1988: **17**: 25–28.
7. Fujita S, Shibata Y, Takahashi H, Tsuda N, Okabe H. Latent infection with Epstein–Barr virus in odontogenic disorders: comparison among ameloblastoma, dentigerous cyst and odontogenic keratocyst. Pathol Int 1997: **47**: 449–453.
8. Gao Z, Flaitz CM, Mackenzie IC. Expression of keratinocyte growth factor in periapical lesions. J Dent Res 1996: **75**: 1658–1663.
9. Hahn G, Jores R, Mocarski ES. Cytomegalovirus remains latent in a common precursor of dendritic and myeloid cells. Proc Natl Acad Sci U S A 1998: **95**: 3937–3942.
10. Kaplan I, Hirshberg A. The correlation between epithelial cell proliferation and inflammation in odontogenic keratocyst. Oral Oncol 2004: **40**: 985–991.
11. Kettering JD, Torabinejad M. Presence of natural killer cells in human chronic periapical lesions. Int Endod J 1993: **26**: 344–347.
12. Kidd IM, Fox JC, Pillay D, Charman H, Griffiths PD, Emery VC. Provision of prognostic information in immunocompromised patients by routine application of the polymerase chain reaction for cytomegalovirus. Transplantation 1993: **56**: 867–871.
13. Kontiainen S, Ranta H, Lautenschlager I. Cells infiltrating human periapical inflammatory lesions. J Oral Pathol 1986: **15**: 544–546.
14. Kubar A, Saygun I, Ozdemir A, Yapar M, Slots J. Real-time polymerase chain reaction quantification of human cytomegalovirus and Epstein–Barr virus in periodontal pockets and the adjacent gingiva of periodontitis lesions. J Periodontal Res 2005: **40**: 97–104.
15. Marton IJ, Kiss C. Characterization of inflammatory cell infiltrate in dental periapical lesions. Int Endod J 1993: **26**: 131–136.
16. Marton IJ, Kiss C. Protective and destructive immune reactions in apical periodontitis. Oral Microbiol Immunol 2000: **15**: 139–150.
17. Meghji S, Qureshi W, Henderson B, Harris M. The role of endotoxin and cytokines in the pathogenesis of odontogenic cysts. Arch Oral Biol 1996: **41**: 523–531.
18. Metzger Z. Macrophages in periapical lesions. Endod Dent Traumatol 2000: **16**: 1–8.
19. Mogensen TH, Paludan SR. Molecular pathways in virus-induced cytokine production. Microbiol Mol Biol Rev 2001: **65**: 131–150.
20. Nair PN. New perspectives on radicular cysts: do they heal? Int Endod J 1998: **31**: 155–160.
21. Pass RF. Cytomegalovirus. In: Knipe D, Howley P ed. Fields Virology. Philadelphia, PA: Lippincott Williams & Wilkins, 2001: 2675–2705.
22. Rider CA, Rupkalvis R, Miller AS, Chen SY. Search for evidence of three viral agents in radicular (periapical) cysts with immunohistochemistry. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995: **80**: 87–91.
23. Rodu B, Tate AL, Martinez MG Jr. The implications of inflammation in odontogenic keratocysts. J Oral Pathol 1987: **16**: 518–521.
24. Sabeti M, Simon JH, Nowzari H, Slots J. Cytomegalovirus and Epstein–Barr virus active infection in periapical lesions of teeth with intact crowns. J Endod 2003: **29**: 321–323.
25. Sabeti M, Simon JH, Slots J. Cytomegalovirus and Epstein–Barr virus are associated with symptomatic periapical pathosis. Oral Microbiol Immunol 2003: **18**: 327–328.
26. Sabeti M, Valles Y, Nowzari H, Simon JH, Kermani-Arab V, Slots J. Cytomegalovirus and Epstein–Barr virus DNA transcription

in endodontic symptomatic lesions. Oral Microbiol Immunol 2003: **18**: 104–108.
27. Shear M. The aggressive nature of the odontogenic keratocyst: is it a benign cystic neoplasm? Part 2. Proliferation and genetic studies. Oral Oncol. 2002: **38**: 323–331.
28. Shibata D, Martin WJ, Appleman MD, Causey DM, Leedom JM, Arnheim N. Detection of cytomegalovirus DNA in peripheral blood of patients infected with human immunodeficiency virus. J Infect Dis 1988: **158**: 1185–1192.
29. Slots J, Nowzari H, Sabeti M. Cytomegalovirus infection in symptomatic periapical pathosis. Int Endod J 2004: **37**: 519–524.
30. Slots J, Sabeti M, Simon JH. Herpesviruses in periapical pathosis: an etiopathogenic relationship? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003: **96**: 327–331.
31. Taylor-Wiedeman J, Sissons JG, Borysiewicz LK, Sinclair JH. Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. J Gen Virol 1991: **72**: 2059–2064.
32. Vargas PA, Villalba H, Passos AP et al. Simultaneous occurrence of lymphoepithelial cysts, cytomegalovirus and mycobacterial infections in the intraparotid lymph nodes of a patient with AIDS. J Oral Pathol Med 2001: **30**: 507–509.
33. Walter EA, Greenberg PD, Gilbert MJ et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med 1995: **333**: 1038–1044.
34. Yen TL, Murr AH, Rabin J, Mhatre AN, Lalwani AK. Role of cytomegalovirus, Epstein–Barr virus, and human herpes virus-8 in benign lymphoepithelial cysts of the parotid gland. Laryngoscope 2004: **114**: 1500–1505.