

A Correlated Presence of Human Cytomegalovirus and Human Papillomavirus in the Odontogenic Epithelium of Radicular Cyst

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Abstract

Radicular cyst (RC) is a type of odontogenic cysts that thought to be of inflammatory origin. Recently, the role of viruses in the pathogenesis of these cysts was highlighted. The aim of the current study was to evaluate the presence and correlation of Human Cytomegalovirus (HCMV) and Human Papillomavirus (HPV) in samples of large RCs. A total of 16 cases of RCs were immunohistochemically analyzed for the expressions of HCMV-gB and HPV-L1 proteins. A positive HCMV-gB protein expression in the odontogenic epithelium of RC was found in 12 cases (75%) of the total samples. HCMV-gB expression was located mainly in the superior part of the epithelial wall. Meanwhile, HPV-L1 protein was expressed in the odontogenic epithelium of RC in 11 cases (68.8%). The HPV-L1 expression was located throughout the epithelial layers of the studied RCs. Spearman's correlation test revealed a very high significant correlation between HCMV-gB and HPV-L1 expressions in the odontogenic epithelium of RC ($\rho = 0.856$, $p = 0.000$). This study showed a strongly correlated presence of HCMV and HPV in the odontogenic epithelium of RC. However, a proposed role of these viruses in the pathogenesis of RC needs further investigations.

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Introduction

Radicular cyst (RC) is an epithelium-lined cavity, surrounded by a fibrous capsule which believed to evolve from inflamed granulation tissue in which the epithelial rests are stimulated by the inflammatory process. It is classified as an inflammatory odontogenic cyst and it is usually asymptomatic unless it became infected.¹

Human Cytomegalovirus (HCMV) is a double-stranded DNA virus enclosing 165 genes while the viral proteins responsible to the virus latency and virulence. The HCMV proteins are mimic to and interact with the human cellular proteins. HCMV is the most frequent human pathogen in the family of the herpes viruses as it infects 50% - 100% of the general adult

population.² HCMV infects different host cells like monocytes/macrophages, polymorphonuclear leukocytes, T lymphocytes, fibroblasts, endothelial, and epithelial cells.³ The virus replicates inside the nucleus of the host cell and illustrates as enormous intranuclear but tiny cytoplasmic inclusion bodies in the histopathological sections.⁴ Several studies have been conducted to find HCMV in apical periodontitis,⁵⁻⁹ RC,^{3,10} and odontogenic keratocyst (OKC).¹⁰

Human Papillomavirus (HPV) is a double-stranded DNA, non-enveloped virus with a circular genome of ~8000 base-pairs. Its genome codes for six nonstructural early genes (E1, E2, E4, E5, E6, and E7) and two structural late genes (L1 and L2). The HPV-L1 protein, which is studied in our report, is a general protein for all genomes of the HPV and is required for viral assembly. There are 228 HPV types have been identified till now. These HPVs infect and replicate in epithelial cells of the skin and mucosa.¹¹ HPVs are divided into two groups based on their association with neoplasia, namely low-risk types and high-risk types. HPV

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was previously detected in acute apical abscesses,¹² OKC,^{10,13} and ameloblastoma.^{14,15}

There are abundant studies considering the role of some viruses in the benign and malignant tumors. However, the researches dealing with the presence and effect of these viruses in the RC are limited. This study aimed at investigating the presence and correlation of HCMV and HPV in large RCs.

Materials and methods

The current study involved 16 RCs. The samples were obtained from Oral pathology department of Tongji Hospital, the affiliated hospital of the Huazhong University of Science and Technology. All selected samples had a definite diagnosis and an adequate epithelial component. The pathology reports and available original H&E slides of all the cases were reviewed and confirmed. Baseline data including the patients' age and gender as well as the location of the lesions were noted according to the patients' medical files. All participants did not take antiviral or immunosuppressive therapies and were free of acquired immune deficiency syndrome (AIDS). The Institutional Review Board of Tongji Medical College approved this study which followed the protocol of the World Medical Association Declaration of Helsinki.

The greatest cranio-caudal and mesio-distal diameters of the lesion were measured on standard panoramic images to assess the size of the cyst using the arithmetic mean of those values. The degree of chronic inflammation was classified as mild when the proportion of the chronic inflammatory cells were less than 25% of the whole tissue; whereas the proportion of chronic inflammatory cells was between 26% and 50%, and more than 50%, it was categorized as moderate and severe, respectively.

The standard streptavidin-biotin peroxidase complex method was used (Wuhan Boster Biological Technology, Ltd.). The samples of FFPE tissue were cut into 5- μ m sections, dewaxed, rehydrated and their endogenous peroxidase activities were quenched by 3% hydrogen peroxide solution. The antigens of the tissue were unmasked by exposing the slides in heated 0.01 M citrate buffer till the boiling point in a microwave. After that, goat serum (Wuhan Boster Biological Technology, Ltd.) was used to treat the samples at room temperature for 50 min

before incubating them at 4°C overnight in 1:100 dilutions of both primary mouse monoclonal anti-HCMV-gB antibody (bsm-2271M; Clone: 1F11, Beijing Bioss Co, China) and primary rabbit polyclonal anti-HPV-L1 antibody (Beijing Biosynthesis Biotechnology Co, China). Then after, the slides were incubated with 10 μ g/ml biotinylated secondary antibody for 2h at room temperature. This step was followed by staining with 20 μ g/ml streptavidin-biotin-peroxidase complex. Consequently, 3,3'-diaminobenzidine substrate was used to develop the sections which are then counter-stained with Mayer's hematoxylin. The negative controls were passed through all the previous steps, but phosphate-buffered saline was used instead of the primary antibody.

Each slide was investigated totally under high power field microscopy. The positive result of HCMV-gB staining was identified as a yellowish-brown precipitate in the nucleus and cytoplasm of the odontogenic epithelium. Meanwhile, HPV-L1 positivity was interpreted as a yellowish-brown precipitate, predominantly intranuclear, in a focal or diffuse pattern. The absence or questionable intracellular precipitate of both stains was considered as a negative result.

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) 19.0 software (IBM SPSS, Armonk, NY, USA). The correlation of the IHC expressed proteins was analyzed using Spearman's rank correlation coefficient test and p value of ≤ 0.05 was deemed significant.

Results

This retrospective study involved 16 samples of RCs. Of them, there were 12 males and 4 females, and the mean age was 38.56 years. Of the 16 cysts, 13 RCs were located in the upper jaw and three RCs in the lower jaw. The samples comprised large size cysts where the mean size of the cysts was 3.0219 Cm. The most relevant clinical features are summarized in Table 1.

HCMV-gB was immunohistochemically identified as a yellowish to brown precipitate that located mainly in the nucleus and the cytoplasm of the studied odontogenic epithelial cells. HCMV-gB positive cells were located mainly in the superior layers of the epithelium (Figure 1).

A positive HCMV-gB protein expression was found in 12 cases (75%) of odontogenic epithelium of RC. Several HCMV-infected cells like fibroblasts, lymphocytes, macrophages, and endothelial cells were also recognized variably throughout connective tissue (Figure 1).

Variable	No	%
Total	16	
Age (years)		
Mean	38.56	
Range	[12-73]	
Size		
Mean	3.0219	
Range	[1-5 Cm]	
Gender		
Male	12	75%
Female	4	25%
Location		
Maxilla	13	81.3
Mandible	3	18.8

Table 1. Clinical findings of patients with radicular cyst.

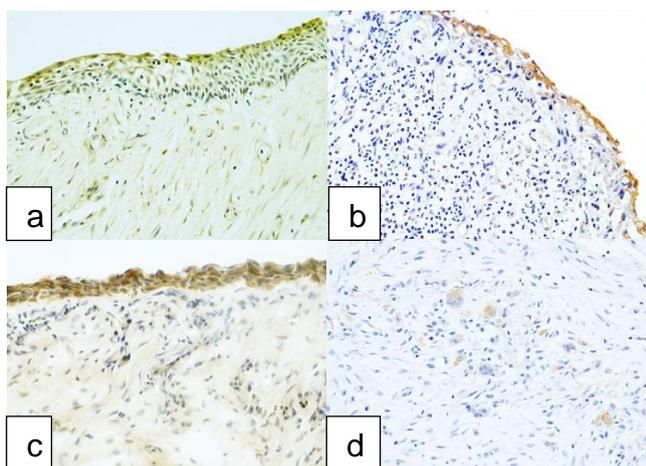


Figure 1. The Immunohistochemical expression of HCMV-gB and HPV-L1 proteins in radicular cysts (RC). HCMV-gB positively stained cells are more in the superior layers of the odontogenic epithelium of RC with some positively stained cells in the connective tissue capsule (a). HCMV-gB positively stained cells in the superior layers of the odontogenic epithelium of RC with negative staining in the connective tissue (b). HPV-L1 protein positively stained epithelial cells were distributed throughout the full thickness of the epithelium (c), and HPV-L1 protein stained mononuclear and multinucleated giant cells in the connective tissue capsule of RC (d) (400 x).

HPV-L1 protein was expressed in the odontogenic epithelium of 11 cases (68.8%). HPV-L1 protein principally expressed as a

yellowish to brown stain of the nucleus of odontogenic epithelial cells. The expression was throughout all epithelial layers of the studied RCs (Figure 1).

Spearman's correlation test revealed a very high significant correlation between HCMV-gB and HPV-L1 expressions in the odontogenic epithelium of RC ($\rho = 0.856$, $p = 0.000$). However, there was no correlation between both studied markers and the degree of inflammatory infiltrate or the size of the RC ($p > 0.5$).

Discussion

There are abundant studies considering the role of some viruses in the benign and malignant tumors. However, the researches dealing with the presence and the effect of these viruses in the cystic lesions like that of the jaws are limited in numbers. This study investigated the presence of HCMV and HPV in samples of RC.

The first raised question in such studies is the pathway of the virus to reach these deep lesions. HPV-DNA was found in the peripheral blood mononuclear cells (PBMCs) in 52% of patients who have urogenital HPV infection, but not in any case of the control group. This presence is proposed to impair the immunological function of the host lymphocytes that ultimately might play a role in the pathogenesis of HPV-induced diseases.¹⁶ Furthermore, patients with human papillomavirus-associated invasive cancers were also found to have circulating HPV-DNA. This level of circulating DNA is related to tumor dynamics and they are present even when the cancer is at the sub-clinical stages.¹⁷ Bodaghi et al. found HPV-DNA positive PBMCs and speculated that PBMCs execute HPV remote transmission and infection of epithelial cells as they do for many other viral infections.¹⁸ Inflammatory cells and giant cells are commonly present in RC.¹⁹ Interestingly, we found several positive HPV-L1 protein giant cells in addition to positive inflammatory cells in the connective tissue capsule of the RC. This way of HPV transmission could justify the detection of HPV in deep tissues like the prostate²⁰ and breast cancers.²¹ More specifically, it could explain the presence of the virus in the cysts and tumors of the jaws which are essentially intra-bony lesions.

On the other hand, the current study

found several inflammatory cells that are HCMV-gB positive in the connective tissue and epithelia of the RC. This finding indicates the role of PBMCs in the transfer of infection to the RC. The latent phase of HCMV infection, which followed its initial infection, occurs in bone marrow-derived myeloid progenitor cells, thereby the viral genome survives in the infected individuals throughout the lifetime. Occasionally, the reactivation of herpesvirus may occur for several reasons, then HCMV can infect monocytes/macrophages, T lymphocytes, fibroblasts epithelial cells, endothelial cells, and other mammalian cells. Periapical granulomas contain numerous T lymphocytes and macrophages which are host cells of cytomegalovirus. Interestingly, a previous study found that HCMV was present in the periapical lesion of teeth with an intact crown, indicating that the infection does not originate from the mouth.⁵ Moreover, HCMV was found in the monocytes/macrophages and T lymphocytes of periapical lesions⁵ and human herpesvirus was not found in both the infected and control pulp tissue samples that related to periapical lesions.²²

Several previous studies used both PCR and IHC for the detection of HCMV^{3,10} and HPV^{23,24} in various tissues. In our study we used IHC as a method for the detection of both HCMV and HPV. The expressions of both HCMV-gB and HPV-L1 proteins were relatively high (75% and 68.8% respectively) in the studied samples. The previous studies found variable presence of HCMV and HPV in different odontogenic lesions. HCMV was detected in (54.5%)¹⁰ and (16.6%)³ of RCs. Besides, HCMV-DNA⁷ and transcript⁵ were highly detected in the apical periodontitis using PCR. Meanwhile, HCMV protein was detected using IHC³ and flow cytometry.⁸ It has been found that HCMV has a higher prevalence in the large periapical lesions than small lesions.⁷ Noteworthy, all the studied samples in the present study were large size RCs. Contrary, other previous studies found that HCMV infection was rare in apical lesions²⁵ and even absent in the periapical abscess.¹² HPV was previously found in 13% of acute apical abscess,¹² (68.4%)¹³ and 60% of OKC,¹⁰ and in (33%)¹⁴ and (18%)¹⁵ of ameloblastoma.

In the current study, the location of HCMV-gB expression was mainly in the superior layers of the epithelial wall, while HPV-L1 protein was distributed throughout the whole epithelial

thickness of RC. Like previous studies, HCMV positive cells revealed both nuclear and cytoplasmic staining,³ while HPV-L1 staining was mainly nuclear.¹³ Double IHC is recommended to show the correlated spatial location of both viruses.

The results of our study revealed a very high significant correlation between HCMV and HPV expressions in the odontogenic epithelium. The concomitant expression of both viruses could reflect immunological deficiency of the affected patients. Interestingly, a recent study found that both HCMV and HPV were higher in the saliva, blood and oral swabs of a patient with type 2 diabetes than those of the non-diabetic patients.²⁶ Further rationalization for this concomitant expressions could be due to the effect of active infection of one virus for the activation of the latent infection of the other one. Similarly, Chalabi et al suggested that the viral coinfection may increase the virulence of herpes virus where an active HCMV infection may activate a latent EBV infection.²⁷

Herpesviruses may cause periapical pathosis whether directly as a result of virus infection and replication or indirectly by jeopardizing the host defense.⁶ In addition, some types of aggressive periapical pathosis could develop as a consequence of a set of interactions among herpesviruses, bacteria, and host immune reactions. Specifically, the activation of herpesvirus plays an important role in case of acute exacerbation of periapical disease. It has been hypothesized that inflammatory cells carrying the herpesvirus enter pulpal tissue through the periapical region after the mechanical trauma or bacterial infection of the pulp. Subsequently, herpesvirus is reactivated, which in turn, enhance cytokine and inflammatory mediator responses in macrophages and other host cells that ultimately lead to periapical bone resorption.⁶ Similarly, one could speculate the sequelae of HCMV periapical infection to stimulate latent infection of the HPV in the odontogenic epithelium.

HPV-L1 capsid protein is important for viral cellular cycle completion and it expressed during the active phase of the HPV infection.²⁸ Nevertheless, HCMV-gB is essential for the infectivity of the HCMV virus.²⁹ Accordingly, the spatial IHC detection of both viruses in the odontogenic epithelium could indicate an active infection and therefore a possible role of the

viruses in the pathogenesis of RC. However, the decision for a specific responsibility of these viruses in the pathogenesis of RC is not that simple and still unclear. Although a previous report suggested an important role of the HCMV in periapical tissue destruction,⁷ the pathological roles of both HCMV and HPV through different pathways should be further investigated. Peculiarly, no correlation was found between the presence of these viruses and the inflammatory infiltrate or the size of the studied lesions. Therefore, the presence of these viruses in RCs could be a superinfection that does not affect the pathogenesis of the affected lesions. Lastly, both current results and the available data about the role of HCMV and HPV in different lesions necessitate conducting further studies that investigate the role of these viruses in RC emphasizing that different viruses could have various behaviors in different pathological lesions. Ultimately, we could reach a non-surgical intervention as a conjugated or solo treatment for the odontogenic lesions which are currently managed just surgically.

Conclusions

The study results showed a strongly correlated presence of HCMV and HPV in the odontogenic epithelium of RC. However, a proposed role of these viruses in the pathogenesis of RC needs further investigations.

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Declaration of Interest

The authors report no conflict of interest.

References

1. Bernardi L, Visioli F, Nör C, Rados PV. Radicular Cyst: An Update of the Biological Factors Related to Lining Epithelium. *J Endod.* 2015;41(12):1951-61.
2. Al-Omari A, Aljamaan F, Alhazzani W, Salih S, Arabi Y. Cytomegalovirus infection in immunocompetent critically ill adults: literature review. *Ann Intensive Care.* 2016;6(1):110.
3. Saboia-Dantas CJ, Coutrin de Toledo LF, Sampaio-Filho HR, Siqueira JF Jr. Herpesviruses in asymptomatic apical periodontitis lesions: an immunohistochemical approach. *Oral Microbiol Immunol.* 2007;22(5):320-25.
4. Lee H, Westerhoff M, Shen B, Liu X. Clinical Aspects of Idiopathic Inflammatory Bowel Disease: A Review for Pathologists. *Arch Pathol Lab Med.* 2016;140(5):413-28.
5. 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(5):321-23.
6. Slots J, Sabeti M, Simon JH. Herpesviruses in periapical pathosis: an etiopathogenic relationship? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2003;96(3):327-31.
7. Popovic J, Gasic J, Zivkovic S, et al. Prevalence of Human Cytomegalovirus and Epstein-Barr Virus in Chronic Periapical Lesions. *Intervirology.* 2015;58(5):271-77.
8. Jakovljevic A, Andric M, Knezevic A, et al. Human Cytomegalovirus and Epstein-Barr Virus Genotypes in Apical Periodontitis Lesions. *J Endod.* 2015;41(11):1847-51.
9. Kazi MMAG, Bharadwaj R. Role of herpesviruses in chronic periodontitis and their association with clinical parameters and in increasing severity of the disease. *Eur J Dent.* 2017;11(3):299-304.
10. Andric M, Milasin J, Jovanovic T, Todorovic L. Human cytomegalovirus is present in odontogenic cysts. *Oral Microbiol Immunol.* 2007;22(5):347-51.
11. Graham SV, Faizo AAA. Control of human papillomavirus gene expression by alternative splicing. *Virus Res.* 2017;231:83-95.
12. Ferreira DC, Paiva SSM, Carmo FL, et al. Identification of herpesviruses types 1 to 8 and human papillomavirus in acute apical abscesses. *J Endod.* 2011;37(1):10-6.
13. Alsaegh MA, Miyashita H, Zhu SR. Expression of human papillomavirus is correlated with Ki-67 and COX-2 expressions in keratocystic odontogenic tumor. *Pathol Oncol Res.* 2015;21(1):65-71.
14. Correnti M, Rossi M, Avila M, Perrone M, Rivera H. Human papillomavirus in ameloblastoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2010;110(3):e20-e24.
15. Ali M, Bukhari MH, Hassan F, Illyas M. Clinicopathological study of ameloblastoma and detection of human papilloma virus by immunohistochemistry. *Pak J Med Sci.* 2019;35(6):1691-6.
16. Pao CC, Lin SS, Lin CY, Maa JS, Lai CH, Hsieh TT. Identification of human papillomavirus DNA sequences in peripheral blood mononuclear cells. *Am J Clin Pathol.* 1991;95(4):540-6.
17. Jeannot E, Becette V, Campitelli M, et al. Circulating human papillomavirus DNA detected using droplet digital PCR in the serum of patients diagnosed with early stage human papillomavirus-associated invasive carcinoma. *J Pathol Clin Res.* 2016;2(4):201-9.
18. Bodaghi S, Wood LV, Roby G, Ryder C, Steinberg SM, Zheng ZM. Could human papillomaviruses be spread through blood?. *J Clin Microbiol.* 2005;43(11):5428-34.
19. Chen JH, Tseng CH, Wang WC, Chen CY, Chuang FH, Chen YK. Clinicopathological analysis of 232 radicular cysts of the jawbone in a population of southern Taiwanese patients. *Kaohsiung J Med Sci.* 2018;34(4):249-54.
20. Sadri Nahand J, Esghaei M, Hamidreza Monavari S, et al. The assessment of a possible link between HPV-mediated inflammation, apoptosis, and angiogenesis in Prostate cancer. *Int Immunopharmacol.* 2020;88:106913.
21. Islam MS, Chakraborty B, Panda CK. Human papilloma virus (HPV) profiles in breast cancer: future management. *Ann Transl Med.* 2020;8(10):650.
22. Rosaline H, Satish ES, Kandaswamy D. 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.* 2009;35(1):9-12.
23. Handayani P, Yudoyono HS, Wardhany II, Wimardhani YS. Detection of Human Papillomavirus 16 and 18 in the Saliva of Indonesian Dental Residents in Jakarta. *J Int Dent Med Res.* 2019;12(3):1139-42.
24. Purwaningsih NMS, Sailan AT, Jalil AA, Sinon SHM. Human Papillomavirus Detection in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma. *J Int Dent Med Res.* 2017;10(2):198-201.
25. Hernádi K, Szalmás A, Mogyórosi R, et al. Prevalence and activity of Epstein-Barr virus and human cytomegalovirus in symptomatic and asymptomatic apical periodontitis lesions. *J Endod.* 2010;36(9):1485-9.

26. Dworzański J, Drop B, Kliszczewska E, Strycharz-Dudziak M, Polz-Dacewicz M. Prevalence of Epstein-Barr virus, human papillomavirus, cytomegalovirus and herpes simplex virus type 1 in patients with diabetes mellitus type 2 in south-eastern Poland. *PLoS One*. 2019;14(9):e0222607.
27. Chalabi M, Moghim S, Mogharehabed A, Najafi F, Rezaie F. EBV and CMV in chronic periodontitis: a prevalence study. *Arch Virol*. 2008;153(10):1917-9.
28. Balan R, Simion N, Giușcă SE, et al. Immunohistochemical assessment of p16, COX-2 and EGFR in HPV-positive cervical squamous intraepithelial lesions. *Rom J Morphol Embryol*. 2011;52(4):1187-94.
29. McVoy MM, Tenorio E, Kauvar LM. A Native Human Monoclonal Antibody Targeting HCMV gB (AD-2 Site I). *Int J Mol Sci*. 2018;19(12):3982.