Cytomegalovirus in periapical granuloma in routine oral pathology service

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Abstract

Background

Periapical granulomas develop as chronic inflammatory lesions primarily in response to microorganisms from an infected root canal system. Human cytomegalovirus (HCMV) has been identified in some apical lesions and may modify the local inflammatory response, although immunohistochemical evidence remains limited.

Aim

This study aimed to determine the frequency and distribution of HCMV protein expression in histologically confirmed periapical granulomas by immunohistochemistry.

Methods

This cross-sectional study included 27 formalin-fixed, paraffin-embedded periapical granuloma specimens from adults undergoing extraction of teeth with chronic periapical lesions at Taibah University Dental Hospital. Detection of HCMV was performed using a mouse monoclonal antibody cocktail composed of clones 8B1.2, 1G5.2, and 2D4.2. Two experienced pathologists independently reviewed the slides and categorized staining as negative, focal positive, or generalized positive. The frequency of HCMV expression was summarized using counts and percentages, and prevalence was reported with an exact binomial 95% confidence interval.

Results

HCMV expression was detected in 6 of 27 specimens (22.2%; exact 95% confidence interval, 8.6%-42.3%). Five specimens (18.5%) demonstrated generalized positivity, and one specimen (3.7%) demonstrated focal positivity.

Conclusion

The findings show that HCMV antigen can be demonstrated in some periapical granulomas. These results support local HCMV protein expression in some lesions, although they do not clarify whether the virus is causal, reactive, or simply present within infected inflammatory cells. Further controlled studies with larger samples, molecular confirmation, and detailed clinical correlation are needed.

Keywords: cytomegalovirus; HCMV; periapical granuloma; immunohistochemistry; apical lesion; viral infection

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Introduction

Apical periodontitis develops when microorganisms and their products extend from an infected root canal and trigger inflammation in the surrounding periradicular tissues. The lesion begins with an endodontic microbial challenge, but its progression depends on continued interaction between microorganisms and host immunity [1,2]. Histologically, chronic apical lesions can appear as periapical granulomas, radicular cysts, or fibrous scars. A periapical granuloma is composed mainly of vascular granulation tissue infiltrated by lymphocytes, plasma cells, macrophages, and variable numbers of neutrophils, with or without proliferating epithelial rests [3].

Human cytomegalovirus (HCMV), or human herpesvirus 5, is a widespread double-stranded DNA virus that establishes lifelong latency after primary infection. Important reservoirs include cells of the monocyte-macrophage and myeloid lineages, with reactivation occurring under altered local or systemic immune conditions [4,5]. HCMV may alter cytokine responses, leukocyte activity, endothelial behavior, and tissue repair mechanisms. These effects make a modifying role in chronic inflammatory disease biologically plausible, especially when viral reactivation occurs in tissue already exposed to persistent bacterial stimulation.

HCMV DNA, RNA, or antigen has been reported in periapical tissues in earlier studies, sometimes in association with Epstein-Barr virus and endodontic bacteria [6-12]. Detection rates have varied widely across studies owing to differences in lesion selection, symptom status, immune status, geographic population, specimen handling, and laboratory technique. PCR may detect even low-level viral nucleic acid, including latent infection, while IHC demonstrates viral protein in identifiable lesional cells and preserves tissue localization [13-16]. Recent systematic reviews confirm repeated detection of HCMV in apical disease, while emphasizing that detection alone does not prove causation [17,18].

However, limited research has focused on HCMV expression in histologically confirmed periapical granulomas using IHC in routine oral pathology specimens. Therefore, the present study assessed

HCMV expression in a series of periapical granulomas and described the distribution of positive staining. We hypothesized that HCMV protein expression would be demonstrable in a measurable subset of periapical granulomas.

Materials and Methods

Study Design and Setting

This specimen-based, cross-sectional observational study used periapical lesions obtained during routine dental care at the Dental Hospital, College of Dentistry, Taibah University, Saudi Arabia. The Departments of Oral and Maxillofacial Pathology, Oral and Maxillofacial Surgery, Endodontics, and Periodontology contributed to specimen collection. The manuscript was prepared as an observational pathology report emphasizing transparent reporting of specimen selection, laboratory assessment, and outcome classification.

Participants and Eligibility Criteria

The study included 27 adults with clinical and radiographic findings consistent with chronic periapical lesions associated with teeth indicated for extraction. Patients were included when adequate periapical tissue could be obtained and severe systemic disease was absent. Teeth were excluded if they showed vertical root fracture, extensive periodontal destruction, or incomplete root development. Patients were also excluded if they were HIV-positive, otherwise immunocompromised, or had received antiviral, immunosuppressive, or antibiotic therapy during the preceding three months.

Clinical Assessment and Specimen Collection

Available patient and clinical variables were reviewed before extraction, including age, sex, symptoms, clinical signs, radiographic findings, periodontal probing depth, medical history, and recent medication exposure. These variables were used to confirm eligibility and describe the clinical context; patient-level values were not available in the analytical dataset used for this report and therefore were not included in subgroup analyses. After extraction, apical tissue attached to the root tip was carefully retrieved and fixed in 10% neutral-buffered formalin. Routine tissue processing was performed, followed by paraffin embedding, sectioning, and hematoxylin and eosin staining. HCMV analysis was restricted to lesions histopathologically diagnosed as periapical granulomas. Cases with a true cystic cavity or another pathologic diagnosis were excluded.

Immunohistochemical Procedure

HCMV antigen expression was assessed in formalin-fixed, paraffin-embedded tissue sections using a mouse monoclonal antibody cocktail containing clones 8B1.2, 1G5.2, and 2D4.2 (Cell Marque, Sigma-Aldrich; isotype IgG2a). This antibody cocktail recognizes HCMV-associated antigens in fixed tissue and was supplied in Tris buffer at pH 7.3-7.7 containing 1% bovine serum albumin and less than 0.1% sodium azide. Reagents were stored at 2-8 degrees C, and freezing was avoided. Sections underwent deparaffinization, graded alcohol rehydration, and heat-mediated antigen retrieval according to routine laboratory protocol. Endogenous peroxidase activity was blocked before incubation with the primary antibody. Antigen-antibody binding was detected using a horseradish peroxidase-conjugated system and visualized with 3,3-prime-diaminobenzidine as a brown reaction product. Slides were completed by hematoxylin counterstaining, dehydration, clearing, mounting, and light-microscopic examination.

Evaluation of Immunostaining

Two experienced pathologists independently reviewed all IHC slides while blinded to each other's initial interpretation. Staining was considered positive when unequivocal brown nuclear and/or cytoplasmic immunoreactivity was present in morphologically interpretable lesional cells. Staining was not accepted as positive when it represented nonspecific background, folded or damaged tissue, or isolated pigment without compatible cellular localization. Any interpretive disagreement was resolved by consensus after joint microscopic review. Staining was classified according to its distribution. A case was classified as negative when no convincing HCMV immunoreactivity was identified. Focal positivity was defined as immunoreactive cells confined to a limited area of the lesion. Generalized staining indicated widespread HCMV immunoreactivity across the lesion. For the primary analysis, focal positive and generalized positive cases were combined as “HCMV detected.”

Sample Size and Statistical Analysis

The analysis included all 27 eligible, histologically confirmed specimens available for the study. No formal power calculation or between-group hypothesis test was performed because the study was exploratory and lacked a control group. Statistical analysis was performed using IBM SPSS Statistics, version 22.0. Categorical data are presented as frequencies and percentages. A two-sided exact binomial 95% confidence interval was calculated for the proportion of specimens with any HCMV expression. Percentages were calculated with 27 as the denominator, and values were rounded to one decimal place.

Ethical Considerations

The study received institutional ethical approval before specimen analysis, and written informed consent was obtained from the participants. Because tissue was obtained during clinically indicated treatment, participation did not add any surgical procedure or risk. Specimens and study data were handled confidentially and analyzed in de-identified form.

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Results

Specimen Inclusion and Evaluability

Twenty-seven histologically confirmed periapical granuloma specimens met the eligibility criteria and were included in the final analysis. Each specimen was evaluable by IHC, with no exclusions due to inadequate staining or loss of lesional tissue.

HCMV Immunohistochemical Expression

HCMV antigen was identified in 6 of 27 periapical granulomas, corresponding to an overall prevalence of 22.2% (exact 95% confidence interval, 8.6%-42.3%). Generalized positivity was observed in 5 specimens (18.5%), whereas focal positivity was identified in 1 specimen (3.7%). No convincing HCMV immunoreactivity was identified in the remaining 21 specimens (77.8%) (Table 1). Brown immunoreactivity was observed within lesional cells in positive specimens, contrasting with the blue hematoxylin counterstain. Representative photomicrographs illustrate a negative lesion, a lesion with localized focal positivity, and a lesion with broadly distributed generalized positivity (Figure 1).

Table 1. Distribution of HCMV immunohistochemical expression in periapical granulomas

IHC category	n	%	Exact 95% CI
Negative	21	77.8	-
Focal positive	1	3.7	-
Generalized positive	5	18.5	-
Any HCMV expression*	6	22.2	8.6-42.3
Total	**27**	**100.0**	**-**

***Any HCMV expression combines focal and generalized positive cases. CI, confidence interval; HCMV, human cytomegalovirus; IHC, immunohistochemistry.**

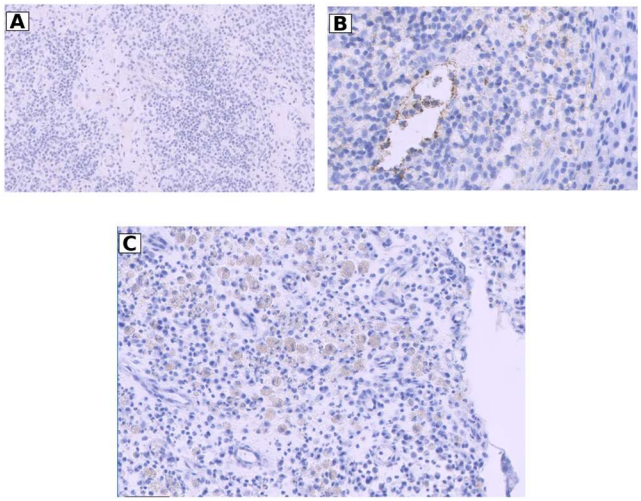

Figure 1. Representative HCMV immunohistochemical patterns in periapical granulomas. (A) Negative granuloma showing hematoxylin-counterstained inflammatory tissue without convincing brown HCMV immunoreactivity. (B) Focal positivity, with immunoreactive cells limited to a localized region. (C) Generalized positivity, with broadly distributed immunoreactive lesional cells. Brown staining represents HCMV-associated antigen detected with the 8B1.2/1G5.2/2D4.2 monoclonal antibody cocktail; hematoxylin counterstain.

Discussion

This study detected HCMV protein expression in 22.2% of histologically confirmed periapical granulomas. Generalized staining was the predominant positive pattern, while focal staining was observed less frequently. This finding demonstrates that HCMV-associated antigen can be localized within the cellular compartment of a subset of routine periapical granulomas. However, most specimens were negative, and the wide confidence interval indicates that HCMV expression is not present in all periapical granulomas.

The prevalence observed in this study is lower than that reported in several molecular studies. Sabeti and Slots found HCMV in 27 of 34 periapical lesions when cases with HCMV alone and HCMV/EBV co-infection were considered together [6]. Slots and colleagues identified active HCMV transcription in all symptomatic lesions and in a smaller proportion of asymptomatic lesions [7]. HCMV has also been reported in symptomatic and asymptomatic apical periodontitis by studies using polymerase chain reaction or transcription-based methods, with considerable variation in estimates [9-12]. In contrast,

IHC-based studies have generally produced lower detection rates because the method requires sufficient viral antigen in the examined tissue section [8].

This difference between immunohistochemical and molecular detection has important clinical and biological implications. Polymerase chain reaction can detect very small quantities of viral DNA, including latent viral genomes within inflammatory cells, but it does not necessarily demonstrate active protein expression or localize the viral signal to a specific cell. Although IHC has lower analytical sensitivity, it provides direct spatial localization and supports the presence of viral antigen in morphologically recognizable cells [15,16]. Therefore, the 22.2% positivity rate may reflect lesions with sufficient antigen burden or active/reactivated infection, whereas low-level or latent infection may not have been detected.

Multiple mechanisms could link HCMV to the inflammatory microenvironment of periapical granulomas. Reactivation within macrophages or other myeloid cells may alter cytokine and chemokine production, disrupt neutrophil and lymphocyte function, stimulate endothelial activation, and prolong local tissue injury [4,5]. Indirectly, HCMV may impair local antimicrobial defense, permitting endodontic bacteria to persist or show greater pathogenic activity. Such herpesviral-bacterial synergy has been proposed in periodontal and periapical disease [6,19]. Cytokine-mediated immune responses are central to the initiation, progression, and persistence of inflammatory periapical lesions [20]. Nevertheless, the direction of this relationship remains unresolved: HCMV may aggravate inflammation, but intense inflammation may itself create the conditions required for viral reactivation.

The current findings are consistent with the broader evidence base but should be interpreted cautiously. In 2014, a systematic review found suggestive but inconclusive evidence connecting HCMV and EBV with clinical features of apical periodontitis [13]. A 2024 systematic review also found frequent viral detection in pulpal and apical disease but emphasized substantial methodological heterogeneity [17]. More recently, a 2026 systematic review and meta-analysis found higher HCMV prevalence in symptomatic and larger periapical lesions and supported a possible role in disease progression [18]. The present study adds morphologic localization of HCMV antigen, but it cannot distinguish primary involvement from secondary reactivation.

Strengths and Limitations

The use of IHC in histologically confirmed periapical granulomas is a principal strength of this study. This method connects viral detection with lesion morphology and allows staining distribution to be classified as negative, focal, or generalized. Independent slide assessment by two pathologists, followed by consensus review, reduced the likelihood that final classification depended on a single observer. In addition, the study focused on immunocompetent adults and excluded recent antiviral, immunosuppressive, and antibiotic therapy, limiting several obvious sources of clinical heterogeneity.

The study also has several important limitations. The sample size was small and came from a single institution, resulting in a wide confidence interval and limited generalizability. No healthy periapical tissue, radicular cyst, or other comparison group was available. The study did not include molecular confirmation using quantitative PCR, reverse-transcription PCR, or in situ hybridization. Patient-level clinical variables were unavailable for correlation with HCMV expression, so relationships with symptoms, radiographic size, tooth type, prior endodontic treatment, and healing could not be examined. Classification of focal and generalized staining was qualitative, and no validated quantitative cutoff or formal interobserver agreement statistic was applied. Finally, antigen preservation and IHC sensitivity may have been affected by fixation, tissue sampling, antigen retrieval, and the phase of viral infection.

Clinical and Research Implications

These findings should be regarded as hypothesis-generating and do not support routine viral testing or antiviral therapy for periapical granulomas. Root canal bacterial infection remains the main therapeutic target, and current endodontic management should not be altered based on this small observational study. The key implication is that HCMV antigen is detectable in some periapical granulomas, supporting the need for better-designed mechanistic studies.

Future studies should use larger, prospectively characterized cohorts and include suitable control tissues. Integration of IHC with quantitative viral DNA and RNA testing would help differentiate latent carriage from active viral expression. Future studies should also profile bacterial communities, inflammatory mediators, lesion size, symptoms, and treatment outcomes to clarify the phenotype associated with HCMV positivity. Digital image analysis may also provide objective measures of staining extent and intensity and improve reproducibility across laboratories.

Conclusion

HCMV antigen was detected by immunohistochemistry in 6 of 27 periapical granulomas (22.2%), with generalized staining in most positive cases. The results confirm that HCMV protein can be locally expressed in some lesions, although causality in chronic apical periodontitis remains unproven. HCMV may function as an inflammatory cofactor, reactivate secondary to the local immune environment, or simply identify infected inflammatory cells. Larger controlled studies that integrate tissue

localization, molecular testing, microbial analysis, and clinical follow-up are needed to define its significance.

Declarations

Ethics approval and consent to participate

Institutional ethical approval was obtained before the study was undertaken. All participants provided written informed consent, and specimen collection occurred during clinically indicated treatment without additional surgical risk.

Consent for Publication

This manuscript contains no directly identifiable patient information. Photomicrographs are presented as anonymized tissue images.

Data Availability

De-identified data supporting the findings are available from the corresponding author upon reasonable request and subject to institutional and ethical requirements.

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Conflict of Interest

The authors report no conflicts of interest.

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