Viruses as Culprits of Periodontal Disease: A Hypothesis or a Possibility?

Abstract: Recent studies have identified various herpes viruses in human periodontal disease. Epstein–Barr virus type 1 (EBV-1) infects periodontal B-lymphocytes and human cytomegalovirus (HCMV) infects periodontal monocytes/macrophages and T-lymphocytes. EBV-1, HCMV and other herpes viruses are present more frequently in periodontitis lesions and acute necrotizing ulcerative gingivitis-lesions than in gingivitis or periodontally healthy sites. The purpose of this review was to evaluate the evidence supporting the hypothesis that viral infection plays a role in the development of periodontitis.

Keywords: Periodontitis; Virus; Herpes; Pathogenesis; Periodontopathogens; Inflammation.

INTRODUCTION
The task of determining the periodontopathic importance of suspected disease determinants is hampered by difficulty in identifying the initial stage of periodontitis and in distinguishing between progressive and stable phases of the disease. Differences of case definitions and diagnostic methods also complicate the interpretation of epidemiological findings in periodontal research studies. Periodontitis typically occurs in otherwise healthy individuals and is statistically associated with various environmental and demographic factors. It is not clear if some of the proposed risk factors for periodontal disease reflect true genetic or immunological variations, or merely poor health seeking behaviour related to socioeconomic factors, lifestyles or cultural differences.

Microbiological culture and culture-independent molecular studies have identified more than 1,200 bacterial species and 19,000 phylotype in the oral cavity. The shift in the periodontal microbiota with disease development is the result of a multifaceted interaction of microbial-specific traits, host immune responses and ecosystem-based factors.

Fitting that concept, various herpes viruses have been associated with severe types of periodontal disease. Studies on a viral cause for periodontitis mark a turning point in periodontal research, which until recently was centred almost exclusively on a bacterial etiology. Epstein–Barr virus and cytomegalovirus are the most commonly researched viruses in periodontology, and more than one million herpes virus genome-copies can be present in a single periodontitis site.

The herpes viral–bacterial hypothesis of periodontitis proposes that an active herpes virus infection initiates periodontal tissue breakdown and that host immune responses against the herpes virus infection are an important component of the etiopathology of the disease. The herpes virus infection triggers a release of proinflammatory cytokines that have the potential to activate osteoclasts and matrix metalloproteinases and to impair antibacterial immune mechanisms, causing an up-growth of periodontopathic bacteria. Herpes viruses and bacteria in aggregate seem capable of explaining several of the clinical features of periodontitis.

Periodontal diseases in immunocompetent subjects:
Contemporary studies of periodontal viruses have employed high-performance polymerase chain reaction (PCR) techniques to determine the frequency of the viral genome (single step and nested end-point detection PCR), to quantify viral genome-copies (real-time PCR), or to indicate active viral multiplication (reverse-transcription PCR).

PCR-based studies of periodontal herpes viruses have targeted different genomic regions and used methods of different efficiency to extract the target nucleic acid. The presence of herpes virus in the periodontium has also been confirmed using labelled DNA probes, flow cytometry, immunofluorescence staining and culture.
Periodontal health and gingivitis:
However, healthy gingival may also be used to describe sites that reveal attachment loss but demonstrate shallow pocket depth and no current inflammation, and normal periodontium may apply to sites that exhibit no attachment loss but show some degree of inflammation. As herpes viruses may have been involved in previous attachment loss and may be present in even slightly inflamed periodontium, ultra-sensitive PCR techniques may identify herpes viruses in unhealthy control sites. Also, healthy periodontal sites of periodontitis patients may harbour more herpes viruses than healthy periodontal sites of individuals with a generally healthy periodontium.11

Periodontitis:
The detection rate of periodontal herpes viruses depends on the type of periodontal lesion studied, the viral identification method employed and ethnic/geographical factors.6 As an etiological agent typically peaks during advancing disease and may only occur at low level or be absent in a disease state of remission, studies of periodontal herpes viruses face the difficult task of identifying disease-active and disease-stable periodontitis. One of the most challenging decisions in periodontal classification is to allocate borderline cases to either aggressive periodontitis or chronic periodontitis.

Probably because of diagnostic difficulties and a natural fluctuation of periodontal herpes viruses, periodontitis studies have reported a wide variation in the occurrence of herpes simplex virus (13–100%), Epstein–Barr virus (3–89%) and cytomegalovirus (0.3–83%).13 Most studies found higher levels of Epstein–Barr virus and cytomegalovirus in sites of aggressive/progressive periodontitis than in sites of chronic periodontitis, but some studies describe a similar occurrence,13 or even a lower occurrence,14 of the two viruses in aggressive periodontitis.

Aggressive periodontitis:
Localized aggressive (juvenile) periodontitis is a distinct disease entity that has served as a model for studying periodontal diseases. Localized aggressive periodontitis debuts at puberty and attachment loss occurs at the approximal surfaces of permanent incisors and first molars. The disease can appear in up to half of the children in an affected family. The presence of subgingival herpes viruses was studied in Afro-Caribbean adolescents with classical localized aggressive periodontitis, with incidental periodontal attachment loss, or with a normal periodontium.15 Localized aggressive periodontitis was associated with cytomegalovirus with an odds ratio of 6.6, and with P. gingivalis with an odds ratio of 8.7. The odds of having localized aggressive periodontitis increased multiplicatively in individuals with a co-infection of cytomegalovirus and P. gingivalis (odds ratio, 51.4), compared with the odds of harbouring neither of the two infectious agents.15 Ting et al. hypothesized that a primary cytomegalovirus infection at the time of root formation of permanent incisors and first molars can give rise to a defective periodontium.16 Viruses infecting odontogenic cells of developing hamster teeth can disrupt normal cell differentiation,17 and an active cytomegalovirus infection can change the morphology of developing teeth.18 Perhaps because of a cytomegalovirus infection early in life, teeth affected by localized aggressive periodontitis often show cemental hypoplasia.19 Profound hormonal changes at the onset of puberty may re-activate a periodontal cytomegalovirus infection, resulting in suppression of antibacterial immune defenses and overgrowth of exogenous-like bacteria, such as specific genotypes of A. actinomycetemcomitans,6 a major pathogenic species in the early phases of localized aggressive periodontitis.6 Localized aggressive periodontitis lesions harbouring an active cytomegalovirus infection tend to be more heavily infected with A. actinomycetemcomitans than sites showing a latent cytomegalovirus infection.

Chronic periodontitis:
Herpes viruses can multiply in gingival tissue20 and tend to reach higher copy-counts in gingival tissue than in subgingival sites.21 However, the great majority of chronic
periodontitis sites, which have a low probability of disease progression, show a latent rather than an active cytomegalovirus infection. Antibodies against the herpes viruses were predominantly of the immunoglobulin A (IgA) isotype in the gingival crevice fluid and of the immunoglobulin G (IgG) isotype in serum samples. These antibody findings suggest a local synthesis by plasma cells rather than passive transudation from the blood, and thus provide further evidence of a close relationship between herpes viruses and periodontal disease.

The Epstein–Barr virus nuclear antigen 2 (EBNA2) genotype 1 occurs more frequently in periodontitis lesions than the EBNA2 genotype 2. In the study of Wu and colleagues, EBNA2 genotype 1 was detected in 45%, and EBNA2 genotype 2 was detected in 20% of Chinese patients with chronic periodontitis; however, EBNA2 genotypes 1 and 2 were associated with chronic periodontitis with odds ratios of 2.0 and 8.2, respectively.

Wu et al. also identified cytomegalovirus in 79% of patients with chronic periodontitis, moreover, they found the cytomegalovirus gB-I genotype in 20% and the cytomegalovirus gB-II genotype in 87% of cytomegalovirus-positive subjects with periodontitis, and the cytomegalovirus gB-I genotype in 57–59% and the cytomegalovirus gB-II genotype in 47–49% of infected subjects with gingivitis or a normal periodontium. Co-infection with Epstein–Barr virus type 1 and the cytomegalovirus gB-II genotype was associated with periodontitis with an odds ratio of 28.9, compared to an odds ratio of 11.0 for a co-infection with all genotypes of Epstein Barr virus and cytomegalovirus.

Periodontitis lesions can also harbour papillomaviruses, Human immunodeficiency virus (HIV), human T-lymphotropic virus type 1, hepatitis B virus, hepatitis C virus and torquetenovirus. Linkages have been established between human T-lymphotropic virus type 1 infection and gingivitis (odds ratio 3.8) and periodontitis (odds ratio 10.0), between hepatitis B and C viruses and periodontal disease, and between torquetenovirus in gingival biopsies and periodontitis.

**Periodontal abscesses:**
The early events in the development of a periodontal abscess include multiplication and tissue invasion of one or more subgingival bacterial species. Bacteria typically recovered from periodontal abscesses are Fusobacterium spp. (75% of abscesses studied), Prevotella intermedia / nigrescens (60%), P. gingivalis (51%) and A. Actinomycetemcomitans (30%).

Epstein–Barr virus was detected in 72%, cytomegalovirus in 67%, and co-infection with the two viruses in 56% of 18 abscesses studied, and the herpes viruses were not identified in healthy periodontium or after treatment of the periodontal abscess. Hypermobility was present in 90% of abscessed teeth showing a herpes viral dual infection. Epstein–Barr virus has also been linked to extra-oral abscesses in children and young adults, and cytomegalovirus has been implicated in periodontal and extra-oral abscesses of HIV-infected individuals. It is suggested that re-activation of a periodontal herpes virus latent infection impairs the periodontal host defense, which permits bacterial pathogens to enter the gingiva and cause abscess formation.

**Periodontal diseases in compromised subjects**

**HIV-associated periodontitis:**
Cytomegalovirus infection in neonates and immunocompromised patients (HIV-infected patients and transplant recipients) has a high rate of morbidity. HIV-induced immunosuppression facilitates herpes virus re-activation, but active herpes viruses may also activate latent HIV. Re-activation of latent periodontal herpes viruses by HIV may start a cascade of tissue-destructive events leading to periodontal breakdown. Periodontitis in HIV-infected patients may resemble periodontitis of non-HIV-infected individuals, or be associated with profuse gingival bleeding or necrotizing gingival tissue.
Neohrotizing ulcerative gingivitis/periodontitis:

Neohrotizing ulcerative gingivitis/periodontitis affects immunocompromised, malnourished and psychosocially stressed individuals. In Europe and the USA, necrotizing ulcerative gingivitis/periodontitis develops primarily in adolescents and young adults and especially in HIV-infected individuals, and almost never in young children. In developing countries, necrotizing gingivitis may expand considerably beyond the periodontium and give rise to the life threatening disease termed noma or cancrum oris. Noma affects primarily children and is sometimes preceded by a viral infection, such as herpetic gingivostomatitis or measles, or HIV, which may impair host defences against resident viruses and pathogenic bacteria.38

Contreras et al. studied necrotizing ulcerative gingivitis in non-HIV-infected malnourished Nigerian children, 3–14 years of age.39 Necrotizing gingivitis lesions of malnourished children yielded herpes simplex virus (23% of study lesions), Epstein–Barr virus (27%) and cytomegalovirus (59%), whereas periodontal sites of malnourished, but periodontally normal children revealed virtually no herpesviruses.40

Herpesviral–bacterial interactions in periodontitis:

A periodontal herpes virus infection is typically associated with an increased occurrence of periodontopathic bacteria.6 A study of adults with gingivitis or periodontitis found statistically significant associations between periodontal Epstein–Barr virus type 1 or cytomegalovirus and the pathogens P. gingivalis, Tannerella forsythia, P. intermedia, P. nigrescens and Treponema denticola.41 Quantitative PCR studies of severe periodontitis have revealed a close relationship between genome copy-counts of Epstein–Barr virus and cytomegalovirus and counts of P. gingivalis and T. forsythia. As discussed earlier, cytomegalovirus-infected localized aggressive periodontitis lesions exhibit an elevated occurrence of P. gingivalis or A. actinomycetemcomitans. Similarly, respiratory tract infections, otitis media and other nonoral diseases that were previously thought to be caused solely by bacteria may actually have a combined viral–bacterial etiology.6

Herpes virus periodontopathic potential:

Herpes viruses can exert direct cytopathic effects on fibroblasts, keratinocytes, endothelial cells and inflammatory cells, including polymorphonuclear leukocytes, lymphocytes, macrophages and possibly bone cells.41 Epstein–Barr virus and cytomegalovirus can also infect and alter the functions of monocytes, macrophages and lymphocytes in periodontitis lesions. Perhaps as result of a herpes virus periodontal infection, aggressive periodontitis lesions contain fewer overall viable cells, more T-suppressor lymphocytes and more B-lymphocytes (Epstein–Barr virus effect) than chronic periodontitis lesions or healthy periodontal sites.32 A periodontal herpes virus infection may increase the pathogenicity of the periodontal microbiota. Herpes virus proteins expressed on eukaryotic cell membranes may act as new bacterial binding sites. Cytomegalovirus can enhance the adherence of A. actinomycetemcomitans to primary periodontal pocket epithelial cells and to HeLa cells.38

Herpesviruses may induce abnormalities in the adherence, chemotaxis, phagocytic and bactericidal activities of polymorphonuclear leukocytes, which are cells of key importance for the control of periodontopathic bacteria.38 Epstein–Barr virus active infection can also generate anti-neutrophilic antibodies and neutropenia, and polyclonally stimulate the proliferation and differentiation of B-lymphocytes. The pathogenic mechanisms of herpes viruses cooperate in exacerbating disease, and probably for that reason, a periodontal dual infection with cytomegalovirus and Epstein–Barr virus, or with cytomegalovirus and herpes simplex virus, tends to occur in severe types of periodontitis.38

The interaction between herpes viruses and bacteria is probably bidirectional, with bacterial enzymes or other inflammation-inducing factors having the potential to
activate periodontal herpesviruses. Epstein–Barr virus and cytomegalovirus infections up-regulate the interleukin-1β and tumor necrosis factor-alpha gene expression of monocytes and macrophages. Increased levels of pro-inflammatory cytokines in periodontal sites are associated with an enhanced risk of periodontal tissue destruction. The herpes virus-associated pro-inflammatory cytokines and chemokines can hamper the antibacterial host defense, stimulate bone-resorbing osteoclasts, up-regulate matrix metalloproteinase and down-regulate tissue inhibitors of metalloproteinase, thereby impeding tissue turnover and repair and increasing the risk of periodontal tissue breakdown. Also, periodontitis tends to be of greater severity in carriers of the HLA-DR4 alloantigen, perhaps because cytomegalovirus-specific CD8+T cells can cross-recognize HLA-DR4 molecules and potentially induce autoimmune reactions.

Papillomaviruses:
Papillomaviruses were previously included in the Papovaviridae family but are now assigned to a separate family, the Papillomaviridae. Most of the papilloma viruses responsible for significant diseases in humans belong to the genus alpha-papilloma virus (genital papilloma viruses), beta-papilloma virus (responsible for epidermodysplasia verruciformis) and gammapapilloma virus (most of the viruses responsible for cutaneous lesions).

Papilloma viruses can induce benign lesions of the skin (warts) and mucous membranes (condylomas). Some papilloma viruses can cause epithelial malignancies, especially cancer of the uterine cervix. Worldwide, cervical cancer is the second most common malignancy among women. Papilloma viruses are also implicated in certain types of anal cancer, vulvar cancer, penile cancer, laryngeal cancer and oral cancer. Based on their association with cervical carcinoma, papilloma viruses are classified as exhibiting high (types 16, 18 and 31) or low (types 6, 11, 42 and 36) oncogenic risk. Papilloma virus type 16 exhibits the highest, and type 18 the second-highest, oncogenicity. Papilloma viruses, especially type 16, have been implicated in one-third of oropharyngeal squamous cell carcinomas and show a particularly strong relationship with cancer of the tonsils. Human papilloma viruses are frequent inhabitants of the oral mucosa of normal adults and have been found to occur in the saliva of 25% of healthy individuals.

Human immunodeficiency virus:
Human immunodeficiency virus is transmitted through sexual contact or by contaminated needles and blood, but only exceptionally rarely through saliva. A recent study provided compelling evidence that three infants acquired HIV / acquired immunodeficiency syndrome (AIDS) after receiving prechewed food. The HIV-infected caregivers had bleeding gingiva while masticating food for the infants, and thus blood, not saliva, was probably the vehicle for HIV transmission in the three cases reported. In fact, submandibular / sublingual gland secretions contain mucin molecules that normally will prevent infection and transmission of HIV by the oral route.

Hepatitis viruses:
Hepatitis viruses (designated A through G) cause the majority of cases of acute and chronic hepatitis and liver damage worldwide. Chronic hepatitis C affects more than 170 million people worldwide, and the hepatitis C virus persists in 80% of the infected individuals, where it can give rise to liver inflammation, liver cirrhosis and hepatocellular carcinoma, and perhaps to periodontitis, Sjogren’s syndrome, oral lichen planus and sialadenitis.

The gingival crevice fluid was identified as the major source for salivary hepatitis C virus. Twenty seven percent of spouses of individuals with chronic hepatitis C revealed antibodies against the virus, pointing to an intra-familial, but not necessarily a sexual, mode of transmission of the virus. Toothbrushes used by hepatitis C patients can contain the virus and should not be utilized by other members of a family. Ebola virus was detected in the saliva of patients with a positive Ebola diagnosis, and transmission of the Ebola virus through oral exposure has been demonstrated in nonhuman primates.
is a highly lethal neuroepithelial tumor of the skin, and at least some Merkel cell carcinomas appear to be caused by a newly discovered polyoma virus. The Merkel cell polyoma virus is found in relatively high numbers in respiratory secretions and in the saliva of patients with Merkel cell carcinoma, possibly exposing close individuals to a risk of infection.

CONCLUSION: A solid understanding of the etiology of periodontitis is critical for developing clinically relevant classification systems and therapies that can ensure long lasting disease control. Co-infection with Epstein–Barr virus and cytomegalovirus shows a particularly close link with progressive periodontitis. Also, specific genotypes of herpes virus species may exhibit increased periodontopathic potential. Because of the high copy-counts of Epstein–Barr virus and cytomegalovirus in aggressive periodontitis, it is unlikely that these pathogenic viruses are acting merely as innocuous bystanders present in proportion to the severity of the underlying periodontal pathosis. A co-infection of active herpes viruses and periodontopathic bacteria may constitute a major cause of periodontitis and explain a number of the clinical characteristics of the disease. Conversely, it is implied that herpes virus negativity is an indicator of a favourable periodontal prognosis.

Papilloma viruses and other mammalian viruses are also frequent inhabitants of periodontitis lesions, but their role, if any, in the pathogenesis of the disease is unknown. The current paradigm of the pathogenesis of periodontitis needs to be revisited based upon the concept of a herpes viral–bacterial co-infection. Periodontitis may develop stepwise in a series of simultaneous or sequential infectious disease events, including (i) A high herpes virus load (gingivitis level) in periodontal sites, (ii) Activation of periodontal herpes viruses, (iii) An insufficient antiviral cytotoxic T-lymphocyte response, (iv) The presence of specific periodontal pathogenic bacteria, and (v) An inadequate antibacterial antibody response. In most individuals, these five suggested pathogenic determinants of periodontitis may collaborate in a detrimental constellation relatively infrequently and mainly during periods of suppressed cellular immunity. Herpes viruses play a major role as activators of the disease process in this model of periodontitis. Indeed, herpes viruses may be a key missing piece of the periodontopathogenetic jigsaw puzzle that would explain the transition from gingivitis to periodontitis or from stable to progressive periodontitis. Herpes virus infections of both periodontal and non-oral sites may also be responsible for some of the relationships observed between periodontitis and various medical diseases.

Author affiliations: 1. Dr. Rohit Gupta, MDS, Reader, 2. Dr. Aakash Tripathi, MDS, PG student, 3. Dr. Ira Gupta, MDS, Reader, 4. Dr. Sanjivini Sharma, MDS, Senior Lecturer, Dept. of Periodontics, Rama Dental College Hospital and Research Centre, Kanpur, UP.

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Corresponding author:
Dr. Sanjivini Sharma,
Senior Lecturer,
Dept. of Periodontics,
Rama Dental College Hospital and Research Centre, Kanpur, UP.
Contact no. 9415878392
Email id: sanj_dentist@yahoo.com


Sources of support: Nil

Conflict of Interest: None declared