Role of Viruses in Determining Pathogenesis of Periodontal Diseases: A Meta-Analytic Review

Tushar Jiyani, Lisa Chacko, *Sathish Abraham, **Ninad Moon and ***Fareedi Mukram Ali

Dept of Periodontics, SMBT Dental College & Hospital, Sangamner Taluka, Maharashtra State, India
*Dept of Conservative Dentistry & Endodontics, SMBT Dental College & Hospital, Sangamner Taluka, Maharashtra State, India
**Dept of Periodontics, RKDF Dental College, Bhopal, India
***Dept of Oral & Maxillofacial Surgery, SMBT Dental College & Hospital, Sangamner Taluka Maharashtra State, India

ABSTRACT

Periodontal diseases are multifactorial, and many etiological agents are suggested to play a role in their etio-pathogenesis. Various risk factors are also suggested to influence the progression of periodontal disease. Periodontal disease consists of episodes of exacerbations and remissions. Evidence strongly suggests the presence of many strains of viruses in the periodontal environment, and possible mechanisms have also been suggested. Periodontal disease as a risk factor for other systemic diseases can also be better explained based on this viral etiology. Maybe virus infection of the periodontium, depending on the latent or active phase of infection, can partly explain the episodic progressive nature of periodontal disease. Virus infection impairs periodontal defense and permits overgrowth of periodontopathogenic bacteria. Pathogenesis of periodontal disease due to these microorganisms is being reviewed in this study.

Key Words: Periodontitis, Viruses, Human Herpes Virus, HIV and PCR.
INTRODUCTION
Viruses lie around our environment all of the time just waiting for a host cell to come along. They can enter us through the nose, mouth or breaks in the skin. Once inside, they find a host cell to infect. For example, cold and flu viruses will attack cells that line the respiratory or digestive tracts. The human immunodeficiency virus (HIV), which causes AIDS, attacks the T-cells of the immune system. Regardless of the type of host cell, all viruses follow the same basic steps in what is known as the lytic cycle.
1. A virus particle attaches to a host cell.
2. The particle releases its genetic instructions into the host cell.
3. The injected genetic material recruits the host cell's enzymes.
4. The enzymes make parts for more new virus particles.
5. The new particles assemble the parts into new viruses.
6. The new particles break free from the host cell.

All viruses have some type of protein on the outside coat or envelope that "feels" or "recognizes" the proper host cells. This protein attaches the virus to the membrane of the host cell. Some enveloped viruses can dissolve right through the cell membrane of the host because both the virus envelope and the cell membrane are made of lipids. Those viruses that do not enter the cell must inject their contents (genetic instructions, enzymes) into the host cell. Those viruses that dissolve into a cell simply release their contents once inside the host. In either case, the results are the same.

AIMS AND OBJECTIVES
The main characteristic of periodontal disease is chronic inflammation that leads to progressive destruction of the connective tissues and bone, which results in tooth mobility and finally tooth loss. Traditionally, the pathogenesis of periodontitis was based on the infection caused by bacteria that colonize tooth surface and gingival sulcus. It has also been observed that host response factors, such as inflammatory reaction and activation of the innate immune system are critical to the pathogenesis of periodontal disease. Periodontal disease has been widely recognized as a chronic disease but the nature of chronicity remains unclear (Beader and Ivić-Kardum, 2011).
Recent microbiological researches have revealed the possible role of Human cytomegalovirus (HCMV), Epstein - Barr virus (EBV), and Herpes simplex virus (HSV-1 and HSV-2) in the etiopathogenesis of periodontal diseases. An association has been demonstrated between HIV infection and some distinct forms of periodontal, infection i.e. necrotizing lesions. Active human cytomegalovirus (HCMV) replication in periodontal sites may suggest that HCMV re-activation triggers periodontal disease activity. The purpose of this review is to evaluate the evidence supporting the hypothesis that viral infection plays a role in the development of periodontitis. An involvement in periodontal diseases has been suspected specifically for human immunodeficiency virus (HIV) and herpes viruses.

PATHO-PHYSIOLOGY
It has been shown that herpesvirus infections generally involve a mild or asymptomatic primary phase followed by an asymptomatic latent phase interrupted sporadically by periods of activation, where viral replication and possibly clinical disease become
manifest. Herpesvirus reactivation is triggered by a number of immune-suppressing factors, some of which have also been shown to be risk indicators of periodontal disease. Available evidence suggests for the involvement of active cytomegalovirus infection in the initiation and progression of localized aggressive periodontitis and possibly other types of periodontal diseases.

In periodontal disease, herpesviruses may cause release of tissue-destructive cytokines, overgrowth of pathogenic periodontal bacteria, and initiation of cytotoxic or immunopathogenic events. Understanding the significance of herpesviruses in the causation and pathogenesis of destructive periodontal diseases may have important implications in future prevention and treatment of the diseases (Slots and Contreras, 2000).

Although recent studies focused on the role of human herpesviruses in various types of periodontal disease, there was a lack of information in these reports regarding the role of pregnancy gingivitis. One study had been conducted to determine the correlation between pregnancy and the subgingival virus presence. Many pregnant and non-pregnant women were examined for gingival and plaque indices, bleeding on probing, and clinical probing depths from the whole dentition. Subgingival plaque samples were obtained from sites showing signs of gingivitis and healthy sites. The polymerase chain reaction methodology was used to detect Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) from plaque samples. Their data concluded that pregnancy increased the risk of the presence of subgingival EBV in pregnant women by 3.647 times more than in non-pregnant women (Eres et. al., 2011).

**REVIEW OF LITERATURE**

A study was done to evaluate the role in human cytomegalovirus in destructive periodontal diseases. HCMV genomic sequences (detected by PCR identification) occur with elevated frequency in severe adult periodontitis, localized and generalized aggressive periodontitis, ANUG and periodontal abscesses. HCMV periodontal infection may cause initiation of cytotoxic or immunopathologic events. HCMV infection of the periodontium may alter the immune control of resident microorganisms and be an important factor in the pathogenesis of periodontitis (Slots, 2004).

There was also a study, which did not suggest any contribution of HSV-1, EBV or HCMV to aggressive periodontitis in a German population. Patients with aggressive periodontitis and unmatched controls from Germany were investigated in the study. Subgingival plaque samples were analyzed for the presence of HSV-1, EBV and HCMV by quantitative real-time polymerase chain reaction (PCR) assays. Viral antibody titres were determined quantitatively by immunosorbent assays. DNA of HSV-1 and HCMV were detected in 1.5% of the patients and controls, whereas EBV DNA was present in 10.8% and 13.9% respectively. Ethnic and methodological aspects might have caused conflicting results of the earlier studies (Stein et al., 2013).

Another study evaluated the prevalence of Human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) in peri-implantitis and mucositis sites and the correlation between herpes virus and clinical parameters. The clinical parameters assessed were: visible plaque index (PI), bleeding on probing (BOP), suppuration (SUP), probing depth (PD). A polymerase
chain reaction assay identified HCMV and EBV in subgingival plaque samples. The number of sites with plaque and BOP were significantly higher around mucositis and peri-implantitis compared to healthy implants. The mean PD around the implants was significantly higher in peri-implantitis, followed by mucositis and healthy implants. A statistically significant correlation was found between the presence of HCMV and EBV subgingivally and clinical parameters of peri-implantitis and healthy sites. These results confirm the high prevalence of HCMV and EBV in subgingival plaque of peri-implantitis sites and suggest the viruses have a possible active pathogenic role in peri-implantitis (Jankovic et al., 2011).

Another study investigated the associations between putative bacterial pathogens, herpesviruses, and chronic periodontitis. Subgingival samples were collected from healthy individuals and from chronic periodontitis patients with probing depths between 3 mm and 6 mm. Polymerase chain reactions were used to identify bacterial pathogens and herpesviruses. Porphyromonas gingivalis, Tannerella forsythia, Epstein-Barr virus (EBV) type 1 & type 2, cytomegalovirus (CMV), Aggregatibacter actinomycetemcomitans were detected. Their results confirm an association between P. gingivalis, T. forsythia, EBV-1 and CMV, and chronic periodontitis. These infectious agents may play an important synergistic role in the pathogenesis of chronic periodontitis (Chalabi et al., 2010).

Dendritic cells are critical components of the host defense system that play a pivotal role in linking innate immunity to adaptive immune responses. Dendritic cells are a potential target for retroviral infection and latency. Dendritic cells are a long-lived reservoir of latent virus in HIV-infected patients. It is hypothesized that HIV-latently infected dendritic cells would be stimulated by oral bacteria leading to reactivation of HIV. In a HIV-latently infected dendritic cell model, significant differences were observed among the bacterial species in their ability to stimulate HIV reactivation. The experimental data support the hypothesis that oral bacteria related to periodontal infections could trigger latently infected dendritic cells in gingival tissues and contribute to HIV recrudescence and undermining anti-retroviral therapy (Huang et al., 2011).

Another study was conducted to determine the prevalence of human cytomegalovirus (HCMV), Epstein-Barr virus (EBV) and herpes simplex virus (HSV) in GCF samples obtained from periodontally healthy, gingivitis, and periodontitis patients. The effect of periodontal treatment (SRP) on the persistence of herpetic viruses was evaluated in a sub-group of patients suffering from chronic periodontitis. The presence of viruses in GCF samples was assessed by a nested PCR amplification technique. The persistence sites were evaluated following a scaling and root planing. This study showed that the prevalence of HCMV and HSV viruses in GCF is higher in periodontitis patients as compared to periodontally healthy subjects and the prevalence of HCMV is higher in deep periodontal pockets. It also brought evidences that periodontal therapy may be associated with virus elimination in diseased sites (Grenier et al., 2009).

It has also been shown that the occurrence of herpes viruses may vary depending upon the age of the patient and the race of the population studied. So, one study aimed at detecting HSV-1 and HSV-2, EBV and HCMV...
in periodontal pockets of Indian patients, having chronic and aggressive periodontitis. Subgingival plaque samples were collected from chronic periodontitis and aggressive periodontitis patients randomly. Herpes viruses were detected using multiplex PCR technique. Chronic periodontitis patients revealed presence of HSV-1 in 100% samples, HSV-2 in 15.7% samples and EBV in 78.9% and HCMV in 26.31% patients’ samples. Samples from aggressive periodontitis patients showed the presence of HSV-1 in 57.14%, EBV in 28.57% and HCMV in 7.14%, whereas HSV-2 was not detected in any specimen. In this population, herpes viruses were found more frequently in chronic periodontitis than in aggressive periodontitis patients and their prevalence may vary according to the age and race of the patients (Bilichodmath et al., 2009).

Other study sought to further define this relationship by determining the prevalence of these viruses at individual disease and healthy sites of patients with periodontal disease and to determine whether the presence and amount of viral DNA correlate with disease severity. Subgingival plaque from 3 healthy and 3 disease sites of 65 patients who had chronic periodontitis were evaluated for the presence and amount of EBV, CMV, and Fusobacterium nucleatum DNA using real-time polymerase chain reaction. Patient serum was evaluated for antibodies against EBV and CMV using enzyme-linked immune sorbent assays. It was concluded that EBV was infrequent and CMV was rarely present in individual subgingival sites affected by chronic periodontitis (Dawson et al., 2009).

Another study was initiated to evaluate the role of these viruses in the pathogenesis of periodontitis. HCMV and EBV were quantified in apical and marginal periodontitis samples, using real time PCR. In situ hybridization or immuno histochemistry was carried out on apical samples to detect presence of virus within the cells. A possible association with relevant bacteria was examined. Among the apical periodontitis samples, 50% contained EBV, while none contained HCMV. Of the marginal periodontitis samples, 40% were positive for EBV and 12% for HCMV. With one exception, however, the amount of virus was close to the detection limits. EBV was only detected in 1 out of 15 healthy periodontium samples. Immunohistochemistry and in situ hybridization were all negative. Significant associations were found between periodontal EBV and the presence of Aggregatibacter actinomycetemcomitans and Porphyromonasgingivalis.

Although there was an obvious association of the virus within the clinical samples, it seems unlikely that these viruses play a major role in the pathogenesis of periodontitis of the average patient. Their presence may reflect that the clinical samples contain more blood or saliva compared to controls, or an accumulation of lymphoid cells harboring virus in the inflamed tissue (Sunde et al., 2008). Another study proposes an infectious disease model for periodontitis, in which herpes virus & bacterial interactions assume a major etiopathogenic role. EBV type 1, HCMV and other herpesviruses occur at a high frequency in aggressive periodontitis lesions. Also, herpesvirus-infected periodontitis lesions tend to harbor elevated levels of classic periodontopathic bacteria, including Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Campylobacter rectus, Treponema denticola and Aggregatibacter actinomycetemcomitans. So the study suggests a herpesvirus active infection in the periodontium impairs local
defenses, thereby permitting overgrowth and increased aggressiveness of periodontopathic bacteria. In turn, periodontal pathogenic bacteria may augment the virulence of periodontal herpesviruses. It is suggested that interactions among herpes viruses and specific bacterial species constitute an important pathogenic feature of periodontitis and maybe also of various non-oral infections (Slots, 2007). Epstein-Barr virus (EBV), causes infectious mononucleosis and oral hairy leukoplakia, and is associated with various types of lymphoid and epithelial malignancies. Saliva is the main vehicle for EBV transmission from individual to individual. Recent studies have also implicated role of EBV in the pathogenesis of advanced types of periodontal disease. EBV DNA is detected in 60-80% of aggressive periodontitis lesions and in 15-20% of gingivitis lesions. The periodontal presence of EBV is associated with an elevated occurrence of periodontopathic anaerobic bacteria. Moreover, EBV active infection occurs in approximately 70% of symptomatic and large-size periapical lesions. EBV and CMV often co-exist in marginal and apical periodontitis. Periodontal therapy can markedly suppress the EBV load in periodontal pockets as well as in saliva, which has the potential to reduce the risk of viral transmission between close individuals. Further research is needed to identify the full range of EBV-related diseases in the human oral cavity (Slots et al., 2006).

Another study aimed to determine the prevalence of Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), human herpesvirus 8 (HHV-8) and human cytomegalovirus (HCMV) in GCF and, eventually, to find the correlation between specific type of viruses and clinical parameters like plaque index (PI), gingival index (GI) and probing depth (PD), which are important in periodontal diseases. A polymerase chain reaction (PCR) and digestion of PCR products with restriction endonuclease were employed to identify the presence of EBV, HHV-6, HHV-8 and HCMV. Their findings confirmed some type of association between herpesviruses and human periodontitis (Klemenc et al., 2005).

DISCUSSION
HCMV, EBV and HCMV-EBV co-infection are closely associated with disease-active periodontitis in juveniles and adults, with acute necrotizing ulcerative gingivitis in children, and with periodontal abscesses. In particular, HCMV reactivation in periodontitis lesions seems to be linked to advancing disease. HCMV infects periodontal monocytes/macrophages and T-lymphocytes, and EBV infects periodontal B-lymphocytes. Herpesvirus-infected inflammatory cells generate a great variety of pro-inflammatory cytokines and may possess diminished ability to defend against bacterial challenge. Herpesvirus-associated periodontal sites tend to harbor elevated levels of periodontopathic bacteria including P. gingivalis, T. forsythia, P. intermedia, P. nigrescens, T. denticola, C. rectus and A. actinomycetemcomitans. In summary, the available data suggest that periodontitis occurs more frequently and progresses more rapidly in herpesvirus-infected than in non infected - periodontal sites.
An infectious disease model based on herpesvirus-bacteria-host immune response interactions is presented to explain how a gingivitis lesion or a periodontal disease site may convert into a stable tissue-destroying periodontitis lesion (Slots, J. 2004). Herpesviruses are implicated in the pathogenesis of human periodontitis. However, the quantity of herpesviruses in periodontal sites remains unknown. One study was done to compare levels of subgingival human cytomegalovirus (HCMV) in aggressive periodontitis patients and in periodontally healthy subjects. Their findings confirmed the frequent presence of HCMV in aggressive periodontitis lesions. Determining HCMV levels in different types of periodontitis may help to determine the periodontopathic role of the virus and advance our understanding of the disease pathogenesis (Kubar et al., 2004).

It has been shown that reactivation of HCMV in periodontitis lesions may be related to progressing periodontal disease. Several possible mechanisms by which HCMV exerts periodontopathic potential have been previously proposed. These are reviewed and include the upregulation of bone resorptive cytokines such as interleukin-1beta and tumor necrosis factor-alpha by active HCMV infection at the periodontitis site. A novel hypothesis is also described, where HCMV plays a significant role in the pathogenesis of periodontal disease by the ability of its immediate early proteins to strongly transactivate IL-1beta gene expression. More studies are needed to further explore this hypothesis and clarify the association between HCMV and periodontitis (Wara-Aswapati at al., 2003).

A prior investigation has demonstrated a higher prevalence of HCMV and EBV in subgingival specimens from periodontitis patients than from gingivitis patients. A study was aimed to determine the frequency of HCMV, EBV-1, EBV-2, HSV and HIV in subgingival samples from those adults, who each had contributed both a periodontitis and a gingivitis site. Viral detection was performed using a nested-polymerase chain reaction method. Results showed that viral co-infection occurred more frequently in deep than in shallow periodontal sites. HCMV was detected with higher frequency in deep than in shallow periodontal sites. The possible periodontopathogenic mechanisms of mammalian viruses in human periodontitis are highly suspected (Contreras and Slots, 1996).

CONCLUSION
Many studies had been undertaken to detect the presence of these viruses in chronic periodontitis, aggressive periodontitis, and healthy individuals and to determine the relationship between these viruses and the clinical parameters. Among these viruses HSV-1 and EBV were found to be significantly associated with destructive periodontal disease, including chronic and aggressive periodontitis. Furthermore, HSV-1 was found to be associated with severity and progression of destructive periodontal disease (Das et al., 2012). Several studies, have demonstrated an association of herpes viruses with periodontal disease. Viral DNA has been detected in gingival tissue, GCF and subgingival plaque from periodontally diseased sites. In addition markers of herpes virus activation have been demonstrated in the GCF from periodontal lesions. Further investigations having substantial sample size from different geographical
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Corresponding Author: Dr. Fareedi Mukram Ali, Reader, Dept of Oral & Maxillofacial Surgery, SMBT Dental College & Hospital, Sangamner Taluka, Maharashtra State, India
Email: faridi17@rediffmail.com