Original Article

Prevalence of herpesviruses in periodontal disease of the North Indian population: A pilot study

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Abstract:
Introduction: Periodontitis is a multifactorial disease of diverse microbiome, predominantly bacterial. Bacterial infection alone may not explain complete pathophysiology and clinical variations in disease pattern. Concept of herpesviruses playing a significant role in periodontal pathogenesis has been explored globally. Studies show varied results and difference may be accounted to variations existing in studied populations. The present study explored the prevalence of herpesviruses in periodontal disease of the North Indian population.

Materials and Methods: In this case–control study, tissue samples were collected from the normal gingiva (control: n = 48) and deepest pocket (cases: n = 48) using a single curette stroke. Periodontal disease status was assessed through the gingival index, pocket depth, and clinical attachment level which were compared to viral marker positivity.

Results: Ninety samples from 48 healthy gingiva and 48 periodontitis patients were assessed between the age range of 17–60 years. The prevalence of cytomegalovirus was 2.083% (cases) and 2.083% (controls) and Epstein–Barr virus (EBV) was 18.75% (cases) and 0% (controls). Odd’s ratio for EBV in patients with periodontitis was calculated (Haldane–Anscombe correction) to be 21.82%. Herpes simplex virus 1/2 and varicella-zoster virus were absent in both groups.

Conclusions: Within the limitation of this study, EBV was found in cases of periodontitis patients. Its role in disease initiation and progression is not clear. Further studies are required to ascertain EBV as risk factor in periodontal disease.

Key words: Epstein–Barr virus, herpesviruses, periodontitis

INTRODUCTION

Periodontitis is a disease of multifactorial etiology with varied clinical features. The most prevalent etiological factor is bacteria; however, they alone fail to explain diverse clinical presentations in periodontal disease patients. Herpesviruses have been isolated from periodontally affected sites through the polymerase chain reaction (PCR).[1] Subgingival colonization and replication of periodontal bacterial pathogens may be modified through the effect on immune regulation exerted by herpesviruses. They reduce host resistance through the direct effect on inflammatory and immune cells.[2]

Humans are infected by eight major types of herpesviruses as follows: herpes simplex virus (HSV) type 1 and 2, Epstein–Barr virus (EBV), human cytomegalovirus ([HCMV]; human herpesvirus-5 [HHV-5]), varicella-zoster virus (VZV), HHV-6, HHV-7, and Kaposi’s sarcoma virus (HHV-8).

Herpesviruses are present in latent or replicating state. The latent phase marks the integration of the viral genome with the host cell’s genome. During the phase of declined herpesvirus-specific cellular immunity, reactivation and re-entry of herpesvirus in productive or replicating phase occur.[3]

Periodontal disease pathophysiology involves interactions among various infectious agents with a fine balance between cellular and humoral host responses. Ongoing research into periodontal disease pathobiology fails to provide definitive reasoning about probable etiology at a molecular level. Essential forerunners for the development of periodontitis are bacterial periodontopathogens. Quantity of bacteria or mere amount of biofilm does not completely explain the important clinicopathological features of the disease. Bacteria as a sole etiological agent also cannot explain gingival
disease conversion to periodontal disease,[41] faster rate of tissue destruction around teeth with less plaque deposition, periodontitis-associated characteristics of periodic exacerbation and remission, and bilaterally symmetrical or localized pattern of periodontal tissue breakdown.[42] Various studies provide evidence toward the role of periodontal herpesviruses in the initiation of periodontal tissue destruction.[14,15] Studies exploring the prevalence and role of herpes virus factor in the development and severity of periodontitis may help explain few unclarified clinical, pathological, and physiological characteristics of the disease.

Concept of herpesviruses playing a role in the pathogenesis of periodontal diseases is questioned in few of these studies.[15,16] It was, thus, concluded that the high prevalence may not be a universal feature of periodontal disease, but it may depend on the type of population studied.

Limiting factor for inconsistency in results associating viruses with periodontal disease, according to a recent review, are variations in the study design, lack of blinding, and limitation in sample size calculations.[17]

The present study was conducted to determine the prevalence of pan-herpes viruses in the pathogenesis of periodontal disease. This study also explored the type of predominant herpes virus associate with periodontitis and its effect on the severity of periodontal disease.

**Objectives**
The objectives of this study are as follows:
1. To assess the prevalence of the pan-herpesvirus in periodontal disease
2. To assess the relationship between the presence of herpesvirus with the severity of periodontal disease.

**MATERIALS AND METHODS**

Ninety-six patients were enrolled in the study from January 2014 to January 2018 from the department of periodontics. The study protocol was approved by the Institutional Ethics Committee (6357/R-cell/13 dated December 19, 2013).

Study design was a case–control study with cases consisting of patients of chronic periodontitis with probing pocket depth of ≥4 mm and controls of patients with healthy gingiva. Smokers, pregnant women, patient with any systemic disease, and on medications or history of dental therapy in the past 1 year before sample collection were excluded from the study.

Written informed consent was obtained from all patients enrolled in the study.

**Sample size**
The prevalence of herpes infection in human beings has been reported to be 20%, and the prevalence of herpes infection in patients with chronic and aggressive periodontitis has been reported to be 80%.[10] In the present study, we are assuming a prevalence of herpes infection in patients with periodontitis (cases) to be between 50% and 80% (middle value 65%) as compared to 20% in controls.[10] With these assumptions in mind, sample size was calculated to be 15 for each group. However, in order to enhance the power and significance of the study, we targeted a sample size of 50 in each group.

**Clinical assessment**
All enrolled patients were assessed for the plaque index,[19] gingival index,[19] bleeding on probing (BOP), probing depth (PD) in millimeters, and clinical attachment level (CAL) in millimeters using a calibrated periodontal probe with William’s markings.[20]

Patients were classified as having chronic periodontitis if the patients had a minimum of 20 remaining teeth with periodontal disease as evidenced by at least four tooth sites with PD ≥4 mm and CAL ≥2 mm.[21]

The patients were classified as healthy, if the patients had a pocket depth <3 mm with no clinical gingival inflammation (no >10% of sites with BOP and the absence of gingival redness/edema).[22]

**Microbiological assessment**
Based on the clinical assessment, tooth with the deepest pocket in each quadrant for cases was selected for sampling. Area for sampling was isolated with cotton rolls, and the tooth was cleaned with sterile cotton pellet. Sterile periodontal curette was inserted gently to the most apical depth of the pocket, and the sample tissue was curetted out with the single vertical stroke. Samples from all isolated tooth in the patients were pooled.

Gingival biopsy was taken from the third molars or from tooth due to extraction for cause other than periodontal disease to prevent unnecessary trauma to the patient in the healthy (control) group.

Tissue sample collected from the normal gingiva (control: n = 48) and deepest pocket (cases: n = 48) was stored at −20°C in microbiology viral laboratory. Samples were subjected to spin column DNA extraction. Extracted DNA underwent real-time PCR with pan herpes primers and probes. Positive samples were typed for HSV 1/2/HCMV/EBV/VZV.

**Statistical analysis**
Data were analyzed using the Statistical Package for the Social Sciences, version 21.0. The Chi-square test, Fisher’s exact test, and Independent samples “t”-test were used to compare the data. “P” < 0.05 was considered as an indicator of statistically significant association.

**RESULTS**

Statistically, there was no significant difference between cases and controls with respect to age and gender. Age of periodontitis group (cases) ranged from 17 to 61 years, with a mean age of 34.96 ± 12.05 years. Majority of cases were males (64.6%). A total of 10 (20.8%) cases were positive for viral markers. Among these, nine (18.8%) were typed for EBV, whereas the remaining one was typed for HCMV.

Age of healthy group (controls) ranged from 17 to 56 years, with a mean age of 31.19 ± 9.90 years. Majority of controls were also males (52.1%). However, only one (2.1%) of the controls...
was positive for viral markers. It was typed for HCMV. None of the controls were EBV positive.

Statistically, the viral positivity rate was significantly higher in cases as compared to controls \( (P = 0.008) \). The positivity rate of EBV was also significantly higher in cases as compared to that in controls \( (P = 0.003) \) [Table 1].

Odd’s ratio for EBV in patients with periodontitis (cases) was calculated (Haldane–Anscombe correction) to be 21.82%. HSV1/2 and VZV were absent in both groups [Table 2].

Mean plaque index, gingival index, BOP, PD, and CAL values of cases were 1.83 ± 0.61, 1.83 ± 0.50, 80.38% ± 27.68%, 7.81 ± 2.35 mm, and 7.98 ± 2.60 mm, respectively. Although the mean value for all these parameters was higher in viral marker-positive cases as compared to that in viral marker-negative cases, this difference was not statistically significant [Table 3].

**DISCUSSION**

The present study on the North Indian population shows a positive association between the presence of EBV and periodontal disease. EBV belongs to the group of HHVs. They are enveloped DNA virus of *herpesviridae* family. These viruses are implicated in modifying host response through the action on immune and inflammatory cells such as polymorphonuclear neutrophils, lymphocytes, and macrophages. Viruses may be an essential link between the conversion of periodontal pocket ecology from Gram-positive cocci and commensals to Gram-negative rods.

Various studies globally have shown variable results in regard to the isolation of herpesvirus and its type from periodontal pockets. The prevalence of viruses in these studies is also quite contradictory. It has been postulated that the differences in the oral microbiota of the studied population and the study methodology used are reasons for these variations. The present study was conducted to generate data for the North Indian population. Our hospital is a tertiary care center with patients coming from Uttar Pradesh, Bihar, and border areas of Nepal, Uttarakhand, certain northeastern regions and northern part of Madhya Pradesh. The studied population can, therefore, be considered the representation of North India.

Most studies use sterile paper points to collect subgingival samples. Paper points are unable to collect samples from the depth of the periodontal pocket. Curettes are found to be more efficient in collecting samples from the apical depth of the pocket. The sample collection in present study was done by sterile curettes.

This study is in agreement with studies by Imbronito et al. (Brazil) and Klemenc et al. (Slovenia) who have found the prevalence of EBV to be 47% and 44%, respectively, in chronic periodontitis samples and 0% in healthy gingiva. The prevalence of EBV genomes in periodontitis patients ranged from 0% to 80%. EBV prevalence in the present study was calculated to be 18.8% in the periodontitis group. Evidence for HCMV prevalence across global studies has been averaged to be 44% in periodontitis patients and 17% in normal healthy gingiva. The present study had an equal prevalence of HCMV in both groups (2.1%).

### Table 1: Comparison of demographic profile, periodontal health parameters, and viral positivity between cases and controls

| Characteristics | Cases \((n=48)\) | Controls \((n=48)\) | Statistical significance \((P)\) |
|-----------------|----------------|-------------------|-------------------|
| Age (years), mean±SD (range) | 34.96±12.05 (17-61) | 31.19±9.90 (17-56) | 0.097* |
| Gender, n (%) | | | |
| Males | 31 (64.6) | 25 (52.1) | 0.214a |
| Females | 17 (35.4) | 23 (47.9) | |
| Viral marker positivity, n (%) | 10 (20.8) | 1 (2.1) | 0.008* |

*aIndependent samples t-test; *Chi-square test; *Fisher’s exact test. \( P < 0.05 \) (statistically significant). SD – Standard deviation; n – Total number.

### Table 2: Prevalence of herpesviruses between cases and controls

| Virus group | Cases \((n=48)\), n (%) | Controls \((n=48)\), n (%) | Statistical significance \((P)\) | OR |
|-------------|----------------|----------------|----------------|---|
| HSV1 | 0 | 0 | - | - |
| HSV2 | 0 | 0 | - | - |
| VZV | 0 | 0 | - | - |
| CMV | 1 (2.083) | 1 (2.083) | 1.000 | - |
| EBV | 9 (18.75) | 0 | 0.003* | 21.82** |

*aFisher’s exact test; **Haldane-Anscombe correction. \( P < 0.05 \) (statistically significant). HSV1 – Herpes simplex virus 1; HSV2 – Herpes simplex virus 2; VZV – Varicella-zoster virus; CMV – Cytomegalovirus; EBV – Epstein–Barr virus; OR – Odds ratio; n – Total number.

### Table 3: Relationship of viral marker positivity with periodontal health parameters in cases \((n=48)\)

| Parameter | Cases \((n=48)\) | Viral marker positive \((n=10)\) | Viral marker negative \((n=38)\) | Statistical significance \((P)\)* |
|-----------|----------------|----------------|----------------|-------------------|
| Plaque index | 1.83±0.61 | 2.10±0.70 | 1.75±0.58 | 0.110 |
| Gingival index | 1.83±0.50 | 1.99±0.48 | 1.79±0.51 | 0.279 |
| BOP (%) | 80.38±27.68 | 87.59±24.72 | 78.48±28.41 | 0.360 |
| PPD (mm) | 7.81±3.35 | 8.70±2.26 | 7.58±2.34 | 0.182 |
| CAL (mm) | 7.98±2.60 | 8.50±2.22 | 7.84±2.70 | 0.482 |

*aIndependent samples t-test. \( P < 0.05 \) (statistically significant). BOP – Bleeding on probing; PPD – Pocket probing depth; CAL – Clinical attachment level; n – Total number.
HSV1, HSV2, and VZV were not detected in any of samples in the present study. The present study is in agreement with a study conducted in Southern Kerala (i.e.,) of the Indian population. They reported the absence of herpesviruses (HSV1 and 2 DNA) in subgingival samples of periodontitis patients.[33]

Michalowicz et al. reported coexistence of EBV and Porphyromonas gingivalis in subgingival microbiota.[41] EBV reside in epithelial cells and seem to have a synergistic effect for P. gingivalis pathogenicity. This might be a probable cause for the association of EBV with more severe periodontal breakdown. The present study lacks in statistically significant data to provide evidence. However, it is relevant to report that individual EBV-positive cases in the present study were of younger patients having severe periodontitis.

**CONCLUSION**

Studies are required with both bacterial and viral profile to explore this association and its effect on periodontal status further. Future research should also focus on the relationship and severity of herpesvirus infection in smokers and cardiovascular disease patients.

**Acknowledgement**

This study is supported by Intramural Faculty Fellowship Seed Grant, Research Cell, King George’s Medical University, Lucknow (Ref No. 5692/R cell 13 dated 03-10-2013). Appreciation of statistical skills and analysis by Mr. Varun Arora of Arun Photostat Lucknow.

**Financial support and sponsorship**

This study is financially supported by Intramural Faculty Fellowship Seed Grant, Research Cell, King George’s Medical University, Lucknow (Ref No. 5692/R cell 13 dated 03-10-2013).

**Conflicts of interest**

There are no conflicts of interest.

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