Journal of
Periodontology
&
Implant Dentistry

Research Article

Detection of Herpes Simplex Virus in Chronic Generalized Periodontitis via Polymerase Chain Reaction: A Pilot Study

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Received: 6 February 2017; Accepted: 19 May 2017
J Periodontal Implant Dent 2017;9(1):7–11 | doi:10.15171/jpid.2017.002
This article is available from: http://dentistry.tbzmed.ac.ir/jpid

Abstract

Background. As bacterial etiology could not support and explain various aspects of periodontal disease, herpes virus is now proposed to be one of the factors responsible for the periodontal destruction. The aim of the present study was to detect the presence of Herpes simplex virus (HSV) in patients with chronic generalized periodontitis.

Methods. Eleven patients were consecutively selected for the study, of which 3 were diagnosed with moderate chronic generalized periodontitis and 8 with severe chronic generalized periodontitis. Subgingival material was taken from the deepest pocket of the dentition from every study subject, before the commencement of any procedure and polymerase chain reaction (PCR) assay was used to detect the presence of HSV-1 and -2.

Results. HSV-1 and -2 DNA was not detected in any of the samples.

Conclusion. This study is in contrast with previous studies and questions the proposed pathogenic role and clinical relevance of herpes virus in periodontitis.

Key words: **Chronic generalized periodontitis, herpes viruses, polymerase chain reaction.**

Introduction

According to American Academy of Periodontology (AAP) – International Workshop for Classification of Periodontal Diseases, 1999 – chronic periodontitis is defined as an inflammatory disease of the supporting tissue of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both.[1] The term 'microorganisms' in its full sense includes not only bacteria but also viruses, fungi, protozoa, etc. Currently, the bacterial etiology is the only approved and accepted concept. Until now, researchers have not been able to substantiate various aspects of periodontal diseases such as site specificity,[2] rapid periodontal tissue breakdown with minimal plaque,[3] phases of disease activity and quiescence,[4] and the reason for progression to advanced periodontal destruction in some and not in others in a given population.[5]

It is assumed that periodontitis is prevalent in those individuals who have a genetic or environmental predisposition with distinct immune responses, are infected with virulent infectious agents and exhibit persistent gingival inflammation. Based on this concept, various herpes viruses are found to be associated with severe types of periodontal disease. It is

suggested that herpes viruses may exert their deleterious effects on periodontal tissues via one of the following mechanism: 1) by direct cytopathic effect on fibroblasts, keratinocytes, epithelial cells and inflammatory cells; 2) by hampering the cells involved in host defense; 3) by upregulating the growth of periodontopathic bacteria; 4) by altering the inflammatory pathway and response to cytokines; or 5) by inhibiting cell-mediated immunity by suppression of MHC Class I molecules.

Many recent studies have isolated herpes viruses from gingival biopsies, gingival crevicular fluid and supragingival and subgingival plaque samples.[6-8] It has also been found that there is increase in herpes virus counts with an increase in the severity of periodontitis. Now it has been demonstrated that herpes simplex virus (HSV-1 and HSV-2), human cytomegalovirus (CMV) and Epstein–Barr virus (EBV) have more association with periodontitis than other herpes viruses.[6,7] Recently, it has been demonstrated that phase 1 therapy leads to short-term elimination of viruses in diseased states.[7] However, the prevalence of herpes viruses in periodontitis can vary in terms of age, ethnicity, type of periodontal disease, immune status and genetic predisposition of patients.[9] Therefore further studies in different region should be undertaken to confirm viruses as contributing factors for periodontitis

Therefore the aim of the present study was to detect the presence of HSV in periodontal pockets of patients with chronic periodontitis in a population of south Kerala, using conventional polymerase chain reaction.

Methods

Study Population

Eleven subjects, 6 females and 5 males aged 25–70 years, were consecutively selected from the outpatient Department of Periodontics, Pushpagiri Dental College, Thiruvalla. All the subjects were given a detailed verbal description of the study before they signed a consent form at the commencement of study. The study was approved by the Ethics Committee of Pushpagiri College of Dental Sciences.

The subjects were diagnosed with chronic generalized periodontitis, and its severity was classified according to Clinical Case Definitions proposed by the CDC Working Group For use in Population-based Surveillance of Periodontitis (Table 1): [10]

Subjects with history of systemic diseases, those having or had taken any antiviral therapy or undergone any form of periodontal treatment in the past 6 months, pregnant or lactating females and smokers were excluded from the study. The clinical parameters and the subgingival samples were taken in the first visit before the start of any procedures. Periodontal evaluation included gingival index (GI), plaque index (PI), clinical attachment loss (CAL) and probing pocket depth (PPD) using a UNC-15 probe.

Subgingival Sample Collection

Supragingival plaque and saliva were gently removed from the sample site adjacent to the deepest pocket of the dentition with sterile cotton pellets and air-dried prior to sampling. Subgingival material was then collected from the bottom of the periodontal pocket using a sterile periodontal curette in a single stroke. The collected specimen was then immediately transferred and suspended in 500 µL of TE buffer (10 mm TrisHydrohloride, 1 mm EDTA, pH=8) and stored at -20°C for further processing.

Nucleic Acid Extraction

DNA was extracted from the sample material using an alkaliphenol-chloroform-isoamyl alcohol method. In brief, 20 gr of the sample was added to 500 µL of lysis buffer and incubated in a water bath at 30°C for 30 min. Following centrifugation, 500 µL of the supernatant was added to 500 µL (1 vol) of phenol choloroformisoamyl alcohol (P:Cl:I-25:24:1), which was again centrifuged. Carefully 400 µL of the supernatant (aqueous layer) was added to 2 vol of ice-cold 100% ethanol (Molecular Biology Grade). It was incubated at -20°C overnight and spinned for 20 min at maximum speed. To it 50 µL of 70% ethanol was added and spinned again. The upernatant was removed and the pellet was dried, then dissolved in 50 µL of sterile MiliQ water and stored at -20°C

Table 1. Clinical definition of diseases as used in the study

Disease Category	Clinical Definition		
	CAL		PD
Severe periodontitis	≥2 interproximal sites with ≥6 mm	and	≥1 interproximal site with PD ≥5 mm
Moderate periodontitis	≥2 interproximal sites with CAL ≥4 mm	or	≥2 mm interproximal sites with PD ≥5 mm
No/Mild periodontitis	Neither 'moderate' nor 'severe' periodontitis		

Conventional PCR Amplification of DNA

It is a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands or millions of copies of a particular DNA sequence.

Reagents standardized: Qiagen Blood DNA kit, Orion X Hot start Taq PCR mix. Primer sequence for:

HSV: 5' – ATGGTGAACATCGACATGTAC – 3'
HSV 1: 5' –CCTCGCGTTCGTCCTCGTCCT – 3'
HSV 2: 5' – CCTCCTTGTCGAGGCCCCGAA – 3'

A single round of PCR was carried out for all the 11 samples, 1 positive control and 1 negative control, each of which contained H Taq-PCR mix, primer for HSV, HSV-1 and HSV-2, sterile water and respective sample DNA. Thirty-five amplification cycles were used along with various annealing temperatures of 96°C for 45 sec, 58°C for 30 sec and 72°C for 30 sec. After the last cycle, the samples were incubated at 72°C for 5 min followed by 4°C. Each amplified product was loaded on a 2% agarose gel with Syber Safe dye (Figure 1) and read under ultraviolet light.

Statistical Analysis

Data were analyzed and categorical variables were expressed as numbers and percentages and quantitative variables as means, standard deviations, minimums, maximums and medians. Comparison between severe periodontitis and moderate periodontitis groups were carried out using Mann-Whitney U test for quantitative variables and Fisher's exact test for categorical variables. P-value of <0.05 was considered as statistically significant.

Results

Demographic Data

The demographic data of the subjects are summarized in Table 2.

Of the 11 subjects, 3 (27.27%) were diagnosed with moderate chronic periodontitis and 8 (72.72%) with severe chronic periodontitis. All the moderate periodontitis patients were females while 62.5% were males and 37.5% were females in severe periodontitis group.

Table 2. Demographic data of the studied subjects

Gender	MChP	SChP	Total
Male	0	5 (62.5%)	5 (45.5 %)
Female	3 (100%)	3 (37.5%)	6 (54.5%)
Total	3	8	11

MChP: moderate chronic periodontitis; SChP: severe chronic periodontitis

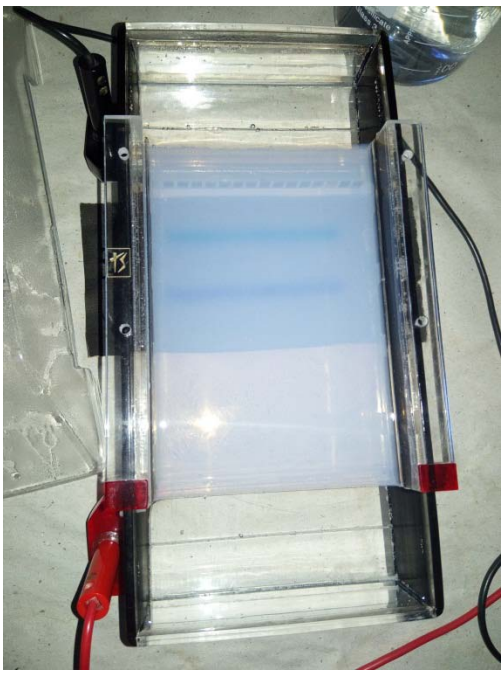

Figure 1. Gel electrophoresis.

There was no significant difference in age between the groups but the total number of teeth present was statistically significant, which shows that there was more tooth loss in severe periodontitis than that in moderate periodontitis, which might be due to increased periodontal destruction present in the former than in the latter group (Table 3).

Clinical Parameters

There was no significant difference in oral hygiene index (OHI), gingival index (GI), pocket depth (PD) and clinical attachment loss (CAL) between the groups. However, there were significant differences in plaque index between the groups, which might indicate that plaque is a more important causative agent inducing periodontal destruction than others or all the other indices are the product of periodontitis rather than the cause for it (Table 4).

Laboratory Investigation

Conventional PCR assay showed no positive findings in terms of HSV-1 and HSV-2 in any of the samples tested (Figure 2).

Discussion

Periodontitis is an inflammatory disease of the periodontium, which is triggered by the presence of microorganisms in the subgingival crevice. Recently, herpes virus has been suspected to be one of the possible periodontopathogens. Evidence for this

Table 3. Age (years old) and number of teeth according to periodontitis condition

	MChP			SChP			Total			
	Mean ± SD	Min-Max	Median	Mean ± SD	Min-Max	Median	Mean ± SD	Min-Max	Median	P-value
Age	42.7+6.0	37-49	42	51.6+12	27-68	52.5	49.2+11.2	27-68	52	0.102
No. of teeth	30.3+1.5	29-32	30	23.0+2.6	20-28	22.5	25.0+4.1	20-32	24	0.014

MChP: moderate chronic periodontitis; SChP: severe chronic periodontitis

Table 4. Studied variables according to periodontitis condition

	MChP			SChP			Total			
	Mean ± SD	Min-Max	Median	Mean ± SD	Min-Max	Median	Mean ± SD	Min-Max	Median	P-value
OHI	1.5+0.4	1.1-1.8	1.7	2.3+0.8	1-.1-3.4	2.3	2.1+0.8	1.1-3.4	2.1	0.125
PI	0.9+0.1	0.8-0.9	0.9	1.7+0.4	0.9-2.2	1.8	1.5+0.5	0.8-2.2	1.7	0.023
GI	1.2+0.3	0.8-1.4	1.3	1.6+0.6	0.7-2.6	1.5	1.5+0.6	0.7-2.6	1.3	0.473
PD	6.0+1.0	5.0-7.0	6.0	6.3+1.3	5-9	6.0	6.2+1.2	5.0-9.0	6.0	0.914
CAL	7.0+1.7	5.0-8.0	8.0	7.6+1.6	5-10	8.0	7.5+1.6	5.0-10.0	8.0	0.592

MChP: moderate chronic periodontitis; SChP: severe chronic periodontitis; OHI: oral hygiene index; PI: plaque index; GI: gingival index; PD: pocket depth; CAL: clinical attachment loss

comes from various epidemiological studies, which support the presence of Herpes viruses in patients with periodontitis.

Contreras and Slots in 2000 reported Herpes viruses as emerging putative pathogens in various types of periodontal diseases. In particular, Epstein-Barr Virus (EBV-1) and human Cytomegalovirus (HCMV) seem to play important roles; the presence of HSV-1 and HSV-2, Varicella zoster virus (VZV), Epstein-Barr virus (EBV) and human Herpes virus-8 (HHV-8) were also attributed to oral diseases.[11] Herpes virus co-infections can give rise to severe immunosuppression that might trigger proliferation of periodontopathic bacteria and other pathological events associated with destructive periodontal diseases.[12] In 2005, Slots et al stated that similar to medical infections, in which Herpes virus can reduce host defense and facilitate overgrowth of pathogenic microorganisms, herpes virus-infected periodontal sites seem to be associated with increased levels of periodontal pathogens.[13] Kubar et al reported that herpes viruses contribute to periodontal pathosis by impairing local host defenses resulting in increased virulence of resident bacterial pathogens or by inducing the release of cytokines and chemokines from inflammatory or connective tissue cells.[14] Contrary to all these, Nibali et al in 2009 showed very low prevalence of subgingival herpes viruses in periodontal lesions and concluded that high prevalence of subgingival virus in periodontitis cases is not common but might depend on the study population.[15]

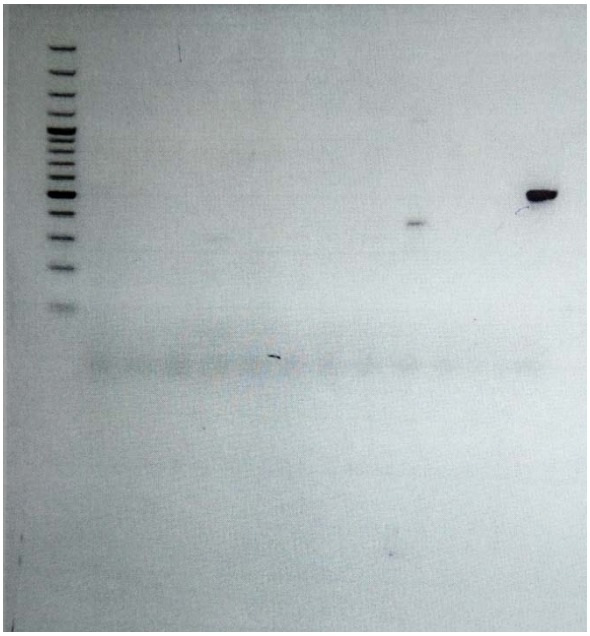

Figure 2. Dark band corresponding to positive control, indicating presence of HSV.

In the present study, we attempted to analyze the presence of HSV-1 and HSV-2 in periodontal phenotypes ranging from severe to moderate generalized chronic periodontitis. Not a single sample was tested positive for the herpes viruses. This study stands out in clear conflict with the results of previous studies. The reason might be the difference in the population studied or differences involved in the study methods. Besides, small sample size might be another possible reason for the negative outcome.

Patients from different geographical area might have differences in the oral microbiota due to differences in the prevalence of infections present in various ethnic groups.[15] Many previous studies have been conducted in populations from South America, China, Turkey or in African Americans, with a lack of study in Indian populations. Also study subjects may not be an exact representation of the population studied as it is impossible to make sure whether periodontal disease was active in the study population at the time of study or whether they were present at an earlier stage of periodontal destruction.

Previous studies using real-time PCR/Nested PCR have yielded positive results with regard to the presence of HSV, which might be due to their higher sensitivity. However, conventional PCR analysis yielded significant results in some of the previous studies, which are different from our study. Parra and Slots, 1996, conducted PCR analysis on the crevicular fluid samples of an American population who were diagnosed with advanced periodontitis and gingivitis; 78% of periodontitis patients and 31% of gingivitis patients were positive for at least one herpes virus.[9] However, our study was carried out in the Indian population which has a different pattern of oral biota. In addition, the sample size for our study was much smaller, which might also be the reason for differences in the results.

Conclusion

Within the limitations of our study, we found a very low prevalence or absence of subgingival presence of Herpes viruses in periodontitis patients in population of south Kerala. Thus we may conclude that the presence of Herpes viruses in the subgingival area is not universal and may depend on the study population and the study methods used.

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