

Original Research

Detection of Herpes Simplex Virus (Hsv-1 & 2) In Gingival Tissue of Chronic Periodontitis Patients Using Polymerase Chain Reaction (PCR) Technique

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ABSTRACT

Background: Recent studies have suggested the role of herpes viruses in the etiopathogenesis of periodontal disease. Very few studies have explored the role of herpes virus in periodontal disease. The occurrence of herpes viruses may also vary depending on the race of the population studied. Herpes Simplex Virus (HSV 1&2) is frequently detected in gingival crevicular fluid of periodontal pockets, but only a few studies have been carried out on gingival tissue to detect the presence of herpes virus. Thus, the aim of the present study is to detect HSV 1&2 in gingival tissue of chronic periodontitis patients and compare their result with those having clinically healthy gingiva.

Method: Gingival tissue samples were collected from 10 subjects with clinically healthy gingiva and 10 subjects with chronic periodontitis. Clinically healthy gingival specimens were obtained from the patients undergoing crown lengthening procedures. Gingival tissue samples for chronic

periodontitis subjects were obtained from patients undergoing periodontal surgery. HSV 1&2 were detected using multiplex polymerase chain reaction technique. The data collected was analysed statistically using descriptive analysis (SPSS Version 20).

Result: Chronic periodontitis patients revealed presence of HSV-1 in 8 samples (80%) and HSV-2 in 2 samples (20%).

Conclusion: This study demonstrated that human viruses may occur in periodontitis lesions with relatively high prevalence.

Keywords: Herpesvirus; periodontitis; Herpes Simplex Virus 1 & 2; pathogenesis; polymerase chain reaction

INTRODUCTION

Periodontitis is an infectious disease caused by bacterial pathogen and modulated by characteristic humoral and cellular host responses.^[1,2]The composition of subgingival microbiota varies in periodontal health and various forms of

periodontal diseases.^[3]

Recently, it was suggested that certain viruses might also influence the development and severity of periodontal disease.^[4] Various Human herpes viruses (HHVs) have emerged as putative pathogens in destructive periodontal disease.^[5] Until now, eight herpes virus species have identified and oral disease has been attributed to four viruses with distinct biologic and clinical characteristics: Herpes Simplex Virus (HSV Type 1 & 2), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), Human cytomegalovirus (HCMV) and Human Herpes virus 8 (HHV-8).^[6]

There is little data present on role of herpes viruses in periodontal disease. Primary infection by herpes virus hominis (HSV), overt or asymptomatic, transmitted from person to person^[8], is prevalent in early childhood.^[9] This can be evidenced by the acquisition of HSV antibodies in about 70% to 80% of the population from the first year of life to adolescence.^[9] Primary infection is followed by spontaneous recovery, and the virus enters a latent state within 2 weeks.^[9] Periodically, the latent virus can become overt and infective in spite of the presence of specific humoral antibodies.^[9] The balance between latency and activation involves the regulation of gene expression and reactivation may be triggered by various factors such as stress, hormonal changes, infection and immunosuppressive medication.^[8]

Herpes viruses may enhance periodontal pathogenicity through a number of mechanisms operating either alone or in combination with other infectious agents.^[10] They exert direct cytopathic effects on fibroblasts, keratinocytes, endothelial cells, inflammatory cells such as polymorphonuclear leukocyte, lymphocytes and macrophages and on bone cells.^[10]

The prevalence and number of herpes viruses in periodontal pockets may vary according to a number of factors such as ethnicity^[11], type of periodontal disease^[12], immune status^[4] and genetic predisposition^[13] of patients.

Thus, further studies in other races from different parts of the world are deemed essential to establish herpes viruses as a recognized etiologic (or co-etiological) agent for periodontitis. Accordingly, this study was conducted to detect the presence of HSV 1 & 2 in subjects with clinically healthy gingiva and gingival tissue of chronic periodontitis patients using polymerase chain reaction technique (PCR).

MATERIALS AND METHODS

The study subjects were selected from the selected from the patients attending the Out Patient Department of Periodontics, Swami Devi Dyal Hospital and Dental College, Barwala (Panchkula) Haryana, India. Each patient was given a detailed verbal and written description of the study and all the selected patients were required to sign an informed consent form prior to commencement of the study.

The study was carried out on clinically healthy subjects and those suffering from chronic periodontitis. The screening and diagnosis of subjects like healthy and chronic periodontitis was made based on the clinical criteria proposed by the 1999 AAP International World Workshop for a Classification of Periodontal Diseases and Conditions.^[14]

Samples were collected from 10 subjects with clinically healthy gingiva and 10 patients with chronic periodontitis, with an age range 18-60. Subjects with clinically healthy gingiva showed absence of gingival inflammation when examined clinically, absence of loss of attachment and absence of sites with probing depth greater than 3mm. Chronic periodontitis patients showed gingival inflammation when examined clinically and had periodontal pocket between 4-7 mm with attachment loss greater than 3 mm in more than 30% of sites.

All patients were systemically healthy and had not taken antibiotic therapy for at least 3 months and also not undergone any periodontal therapy for 6 months prior to clinical examination and sampling. Patients on immunosuppressive therapy, pregnant or lactating mothers, and patients with a

history of smoking were excluded from the study.

The pre-study records included detailed past and present medical and dental history and periodontal assessment was done using clinical parameters like Probing Depth (PD), Plaque Index(PI)^[15], Gingival Index (GI)^[16]and Russell's Periodontal Index (RPI).^[17]All the clinical parameters were recorded prior to the commencement of the study.

METHODOLOGY

A total of 20 gingival tissue samples were collected from selected sites of 10 subjects with clinically healthy gingiva and 10 from chronic periodontitis subjects. After obtaining informed consent, baseline examination of patients and recording of all clinical parameters, gingival tissue samples

for subjects with clinically healthy gingiva were obtained during crown lengthening procedure and tissue samples for chronic periodontitis patients were obtained during periodontal surgery.

Immediately after removal, all tissue specimens were transferred in TE buffer (Tris (hydroxymethyl)aminomethane - Ethylenediaminetetraacetic acid). The collected samples were sent to the laboratory on the same day for further processing. Multiplex Polymerase chain reaction analysis was done to estimate levels of Herpes viruses (HSV-1, HSV-2) in the sample.

TABLE 1: Nucleotide Sequences of the Primers used for the detection of viruses:

HSV-1	Forward	5'-CGTACCTGCGGCTCGTGAAGT-3'
	Reverse	5'-AGCAGGGTGCTCGTGTATGGGC-3'
HSV-2	Forward	5'-TGGTATCGCATGGGAGACAAT-3'
	Reverse	5'-CTCCGTCCAGTCGTTTATCTTG-3'
HCMV	Forward	5'-ACGTGTTACTGGCGGAGTCG-3'
	Reverse	5'-TTGAGTGTGGCCAGACTGAG-3'
EBV	Forward	5'-AGCACTGGCCAGCTCATATC-3'
	Reverse	5'-TTGACGTCATGCCAAGGCAA-3'

Multiplex PCR is a very sensitive technique that can detect microorganisms (quantitative analysis) in small samples than conventional and basic microbiological techniques. Viral identification was done using this technique with which microorganisms of different species may be detected simultaneously. Primer pairs specific to each intended organisms are engaged in a single tube amplification process.

RESULTS

Chronic periodontitis sites revealed HSV-1 in 8(80%) samples, HSV-2 in 2(20%) samples. Subjects with clinically

healthy gingiva revealed HSV-1 in 2(20%) samples. HSV-2 was not detected in any specimen.

A combination of herpesviruses (HSV 1 &2)was seen in 2 (20%) samples (out of 10) from chronic periodontitis group.

The result was subjected to statistical analysis using SPSS Version 20.0. (Armonk, NY: IBM Corp.). Descriptive data were reported in frequency and percentage for categorical variable. Student t-test was used for comparison between subjects.

TABLE 2 Frequency of virus detection in chronic periodontitis patients and healthy subjects

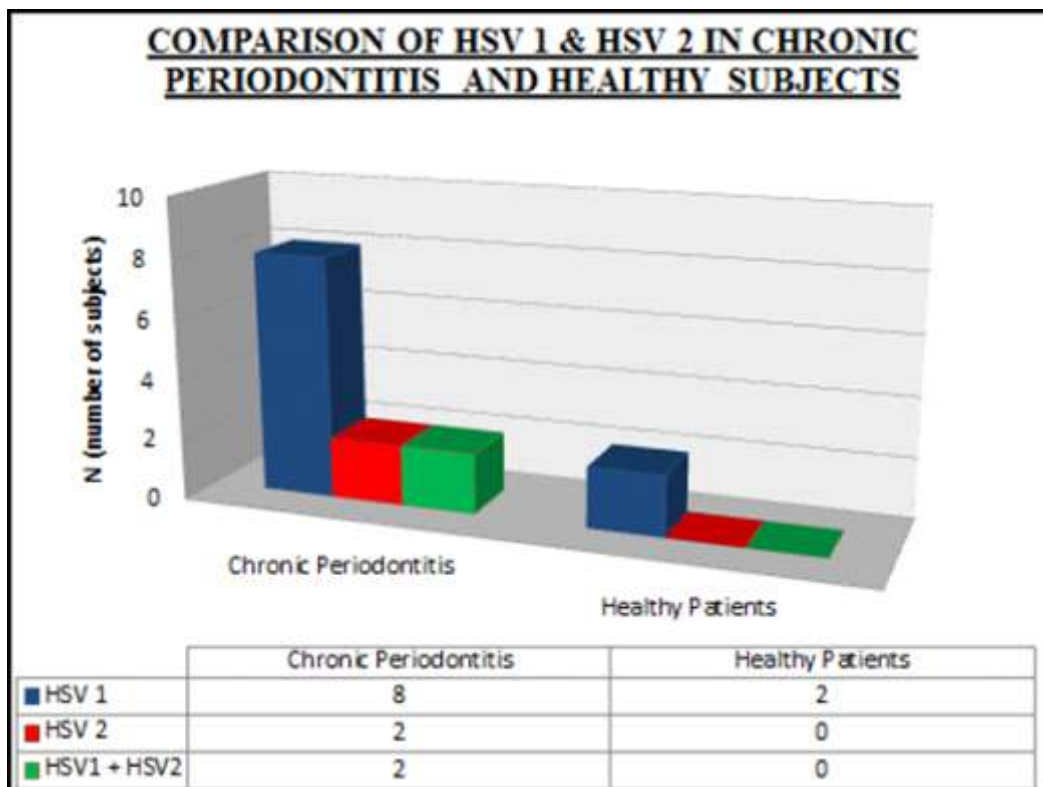
VIRUSES	C H R O N I C PERIODONTITIS (N=10)	HEALTHY SUBJECTS (N=10)
HSV-1	8 (80%)	2 (20%)
HSV-2	2 (20%)	0

TABLE 3 Comparison of HSV-1 & HSV-2 in chronic periodontitis patients and healthy subjects

	HSV-1	HSV-2	BOTH (H S V 1 +	ABSENT (H S V 1 ,
Chronic periodontitis	8 (80) (P = 0.001) (S)	2 (20)	2 (20)	0 (0)
Healthy subjects	2 (20)	0 (0)	0 (0)	8 (80)

S= statistically significant (p<0.05)

GRAPH 1: Comparison Of HSV 1 & HSV 2 In Chronic Periodontitis And Healthy Subjects



DISCUSSION

Not many studies have been done to detect the presence of HSV-1 & 2 in gingival tissue samples. Present study detected significantly higher prevalence of herpes viruses in gingival tissue samples of chronic periodontitis patients (HSV1 80% & HSV2 20%) than that in healthy controls (HSV1 20% & HSV2 0%).

Similar study was done by Para & Slots (1996) in which HSV1 was present in 20% of gingival tissue samples of chronic periodontitis patients.^[18] Contreras et al. (1999) demonstrated HSV 1 in 35% of gingival tissue samples of chronic periodontitis patients.^[19] Contreras et al (2000) in a later study demonstrated HSV 1 in 57% specimens of chronic periodontitis patients.^[20]

In another study, by Contreras and Slots (2001), HSV-1 was seen in all the samples but HSV-2 was not detected in any of the samples.^[21] HSV-2 is usually transmitted through genital infection and is rarely found in the oral cavity. HSV 1 was present in 20% of the specimens of clinically healthy gingiva.^[21]

This is in contrast to a study by Amit R (1992) where HSV was seen in 39.3 % specimens of clinically healthy gingiva.^[9] This variation in results are perhaps due to the age range of the patients, ethnic differences within the study population or limited sample size or methodological approach used to detect herpesviruses.^[13]

This finding may suggest that gingival tissue may be a reservoir for herpes viruses and that it can infect periodontal tissues and thereby serve as a cofactor in destructive periodontal disease. Genetic predisposition of the patient for disease susceptibility and severity should be contemplated when considering viral etiopathogenesis as a patient may be genetically predisposed to severe periodontal breakdown, as shown in cases with genetic pleomorphism of numerous interleukins.^[13]

CONCLUSION

HSV1 & 2 is associated more with chronic periodontitis patients than subjects with clinically healthy gingiva and may vary according to age, ethnicity, immune status, genetic predisposition and methodology used to detect HSV. It may be certain that gingival tissue may be a reservoir for herpes virus.

The present pilot study showed presence of significantly higher number of HSV 1 in chronic periodontitis patients as compared to subjects with clinically healthy gingiva and the finding of an abundance of mammalian viruses in periodontitis lesions may suggest a role for viruses in more oral diseases than previously recognized. Thus, further studies in other races may be reckoned essential to establish HSV as a recognized etiologic factor for periodontitis and thereby aid in prevention and treatment of disease.

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