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Research article

ASSOCIATION OF VIRUSES IN CHRONIC PERIODONTITIS - FACT OR HYPE ???

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Abstract

To evaluate the presence of human cytomegalovirus (HCMV), Epstein- Barr virus (EBV) and herpes simplex virus type 1 (HSV-1) in subjects with chronic periodontitis and periodontally healthy subjects.

Key words: human cytomegalovirus, EBV, herpes virus 1, polymerase chain reaction, periodontal disease.

Introduction

Periodontal disease is a microbial infection involving a variety of microbes that trigger inflammation, loss of connective tissue attachment and alveolar bone around the teeth. The primary etiologic factor of periodontitis is bacterial plaque. The bacteria involved are largely gram negative species that express pathogenic factors that elicit host defence responses resulting in inflammation and tissue destruction. In fact the propensity of periodontitis to proceed with periods of exacerbation and remission could suggest that the presence of other organisms contributes to the disease.[1]Non bacterial microorganisms that are found in plaque include viruses, mycoplasma, yeasts and protozoa[2]. The development of human periodontitis may depend upon cooperative interactions among herpes viruses, specific pathogenic bacteria and tissue destructive inflammatory mediators.

Various studies have shown that human viruses, especially Human cytomegalovirus (HCMV), Epstein-Barr virus (EBV) and Herpes simplex virus (HSV-1) seem to play a part in the pathogenesis of periodontal disease[3,4,5] The subgingival presence of both EBV and HCMV was reported to be associated with the major periodontopathic bacteria and the severity of periodontal diseae.[6,7] The hypothesis of a correlation between

HCMV and EBV infection and the pathogenesis and progression of aggressive periodontitis has been proposed by various studies[8,9,10]. Periodontal destruction may be associated with the coexistence of periodontal herpes viruses, especially HCMV, EBV and periodontopathic bacteria.

The herpes viral infection can stimulate the release of cytokines and chemokines from inflammatory and non inflammatory cells and impair the periodontal immune defense, resulting in more virulent resident bacteria[11]. The association of herpes viruses with periodontitis may be important for diagnosis, treatment and monitoring of the disease[12]. Till date several techniques have been employed for the detection of the viruses, which include culture methods, DNA-DNA hybridization technique and various types of polymerase chain reactions (PCR) such as Hot Staurt PCR, multiplex PCR, nested PCR, reverse transcriptase and real time PCR[13,14,15,16]. PCR is a rapid, accurate and sensitive technique for the detection of bacterial and viral DNA sequences. The sensitivity of PCR allows detection of periodontal pathogens below the normal level of detection by culture methods, immunofluorescence, enzyme based tests and DNA probes. PCR is much more sensitive than conventional culture methods for the identification of periodontal pathogens.[17] The aim of this study was to evaluate the presence of Herpes simplex virus -1 (HSV-1), Epstein-Barr virus (EBV) and Human cytomegalovirus (HCMV) in subjects with chronic periodontitis and periodontally healthy subjects.

Materials and methods

In a test group gingival biopsy was taken from the lining of the pocket during the first incision given for flap surgery, and in the control group gingival biopsy was taken during orthodontic tooth extractions. Hot Staurt multiplex PCR method was used to detect the viruses in the biopsy sample.

Methods

Twenty four subjects were included in the study, Patients who were diagnosed with Chronic periodontitis, patients with Probing pocket depth ≥ 5mm, Healthy subjects with Probing pocket depth ≤ 3mm. Subjects who were systemically healthy. Subjects not taking antiviral drugs in the previous six months were included in the study. Study was divided into two groups, test group comprising of twelve subjects with chronic periodontitis, control group comprising of twelve subjects with healthy periodontium.

In a test group gingival biopsy was taken from the lining of the pocket during the first incision given for flap surgery. This methodology was followed from the previous studies, that are study done by Ehrlich et al 1982 and in a study done by Rotalo et al 2008[18,19]. In the control group gingival biopsy was taken during orthodontic tooth extractions. The gingival tissue was then transferred to the tube containing phosphate buffered saline.

DNA Extraction Procedure from tissue sample: Tissue sample was crushed with sterile blade. Then it was transferred to the tube containing Tera ethylene buffer (T.E.) buffer. It was centrifuged at 50,000 rpm for 2 minutes Supernatant was discarded. A fresh 200 micro liter T.E. buffer was added and centrifuged for 3-4 minutes. Above procedure was repeated for 3-4 time with fresh T.E. buffer. Supernatant was discarded and 500 micro liter lysis buffer 1 was added and centrifuged at 5,000 rpm. Supernatant was discarded and 50 micro liter lysis buffer 2 and 5 micro liter proteinase – K was added. It was kept in water bath for 2 hrs then kept in boiling water bath for 10 minutes at 90^0C. DNA was Stored at -20^0C. After DNA extraction viruses were identified by Hot staurt multiplex Polymerase chain reaction.

The primers ۞ used in the study are as follows[20]

1- CMV-5'-ACGTGTTACTGGCGGAGTCG-3'

2 - EBV-5'-AGCACTGGCCAGCTCATATC-3'

3- HSV-1 5'-CGTACCTGCGGCTCGTGAA

۞biosera

Statistical analysis: The collected data was entered in the excel format. It was subjected to statistical analysis using SPSS software. The statistical tests applied were as below

1. Mann whitney U test and

2. Krusskel wallis test

Results

The presence of HSV-1 in the study group is 66.6%, and in the control group is 50% (Table 1)The presence of HCMV in the study group is 41.67%,and in the control group is 25% (Table 2).The presence of EBV in the study group is 25%, and in the control group is 8.33%(Table 3).

Discussion

Our results indicate the presence of HSV-1, HCMV, and EBV is in higher percentage in the test group compared to control group. This was in accordance to previous studies, where in higher proportion of HCMV and EBV was seen in aggressive periodontitis[21] The results here were also in accordance to study done by Kamma et al 2001 where the higher proportion of HCMV, EBV and HSV-1 were seen in the subjects with chronic periodontitis and also the association of P.gingivalis, Bacteroides forsythus and Aggregatibactor actinomycetem comitans were seen along with the viruses[22].

Study done by Contreras et al.,1999 where the sub gingival presence of EBV-1, HCMV, HSV-1 were associated with sub gingival presence of some periodontal pathogens[4]. In all the above studies the percentage of viruses in the control group was very low, where as in our study the percentage of viruses in the control group was a bit high, this difference might be due to the difference in the methodology and the type of PCR used. In all the above studies the type of PCR used was nested PCR which is susceptible to contamination and may give rise to false positive results[23].

Moreover, the mere presence of viral DNA does not necessarily imply an etiologic role of the virus, either as a direct or as a co-infective agent. Previous studies, investigating a potential association between HCMV and periodontitis in an Italian population, reported prevalence values ranging from 9 to 27% in periodontitis patients which was higher compared to control group[24]. The presence of HCMV in this study goes in accordance to study done by Santangelo et al 2004. The presence of EBV in this study goes in accordance to study done by Rotola et al., 2008, In this study biopsy specimens were harvested at the time of periodontal surgery, after multiple sessions of non-surgical periodontal therapy had been performed[19]. It was demonstrated that active periodontal therapy can induce substantial reductions of HSV-1, HCMV counts in subgingival plaque and saliva[25].

The findings of viral genome and protein expression suggest that the virus is present in the latent form in the gingiva.[26.] Detection of viral DNA by PCR has been shown to be a sensitive and specific method for rapid diagnosis.

This study described the rapid and accurate multiplex PCR method for the detection and typing of HSV-1, HCMV and EBV. Large scale amplification by multiplex PCR of viral DNA can lower the cost and time for viral diagnosis[20].

PCR analysis does not discriminate among latent, persistent and productive infections[19].

Table 1: Presence of HSV-1

Group	Negative	%	Positive	%	Grand total
Control	06	50.00	06	50.00	12
Test	04	33.33	08	66.67	12
Total	10	41.67	14	58.33	24
Fisher exact test P-value	P=0.6807 0.4884				

Table 2: Presence of HCMV-1

Group	Negative	%	Positive	%	Grand total
Control	9	75.00	3	25.00	12
Test	7	58.33	5	41.67	12
Total	16	66.67	8	33.33	24
Fisher exact test	P= 0.66				
P-value	P= 0.48				

Table 3: Presence of EBV

Group	Negative	%	Positive	%	Grand total
Control	11	91.67	1	8.33	12
Test	09	75.00	3	25.00	12
Total	20	83.33	4	16.67	24
Fisher exact test	P = 0.59006				
P-value	0.4884				

Conclusions

Prevalence of HSV-1, HCMV, and EBV was more in the chronic periodontitis group, compared to control group. Gingival epithelium may serve as the reservoir for these viruses.

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