Original Article

Prevalence of Cytomegal and Epstein Barr Viruses Among Patients with Aggressive Periodontitis

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Abstract:

Objective: Recent studies have demonstrated that various human viruses, especially cytomegalovirus (HCMV) and Epstein Barr virus (EBV-1) seem to play a part in the pathogenesis of human periodontitis. The aim of this investigation was to evaluate the subgingival presence of HCMV and EBV in patients with aggressive periodontitis (AgP).

Materials and Methods: A polymerase chain reaction (PCR) method determined the presence of HCMV and EBV. Subgingival Plaque samples from 26 conductive AgP patients were collected.

Results: HCMV was detected in 27% of AgP patients and EBV-1 in 25% of AgP patients. HCMV and EBV-1 co-infection was detected in 52% of AgP patients.

Conclusion: These findings indicated that subgingival presence of EBV-1 and HCMV is associated with aggressive periodontitis, and co-infection with HMCV and EBV appears to be particularly deleterious to periodontal health.

Key Words: Herpes virus, periodontitis, aggressive, prevalence

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INTRODUCTION

Aggressive periodontitis is defined as a specific type of periodontitis below the age of 35 years that has clearly identifiable clinical and laboratory findings, making it significantly different from chronic periodontitis. Localized and generalized forms of aggressive periodontitis are characterized by rapid attachment loss and bone destruction and otherwise appear healthy [1].

Contreras and Slots suggested that gingival infection with certain herpesviruses decreases the resistance of the periodontal tissue, thereby permitting subgingival overgrowth of periodontal pathogenic bacteria [2]. Other investigations have implicated EBV-1 and HCMV in the pathogenesis of human periodontal diseases [3.4]. The reactivation of these viruses in periodontal tissue and resulting transient immunosuppression might partly explain the episodic progressive nature of human periodontitis [2].

The purposes of the present study were to examine the presence of HCMV and EBV-1 in subgingival plaque samples from patients with aggressive periodontitis.

MATERIALS AND METHODS

Patient selection and Clinical Procedures

The present study included 26 patients (13 women; 13 men; 35 2 years) with aggressive periodontitis from patients attending the dental clinic. All patients were systemically healthy and none had received periodontal treatment or antibiotics for at least 6 months prior to sam-

pling and recording. The protocol was approved by the Ethical Committee of Ahwaz jondishapour University of Medical Sciences. Informed consent from all subjects was obtained and all procedures were fully explained before the study.

Patients were diagnosed as having aggressive periodontitis if they were less than 35 years old and exhibited severe periodontal destruction, lost of attachment exceeding 5 mm at two or three sites in more than 14 teeth (at least three of which were not first morals or incisors).

Sample Collection and Preparation

Prior to sampling, supragingival plaque was gently removed with sterile cotton pellets and the sample sites were isolated with cotton rolls and dried. Subgingival plaque samples were taken using a sterile curet. After the curet was gently inserted into the bottom of the pocket, a single stroke was taken to remove the subgingival plaque. In each periodontitis patient, subgingival samples were collected from the three deepest periodontal sites of the dentition (5 to 10 mm PD)

The subgingival specimens were suspended in 250 ul of pronase E buffer (20mM tris-hydrochloride, 10 mM EDTA, 0.2% SDS). Ten ul pronase E (70 mg/ml) was then added to the mixture, which was vortexed and incubated at 42C for 2 hours. The nucleic acid was extracted with phenol-chloroform, then precipitated with ethanol and dissolved in 40 ul of distilled water.

Polymerase Chain Reaction Procedures

A single PCR methos was used to detect viral DNA of HCMV and EBV-1. Primers for the HCMV IE gene region were 5'-ACGTCAGACGATCCGATGAACGTCG-3' and 5'-GTCATGTGGAGAAACCGCCGTTCGG-3'. Amplicon was 231 bp in size. We obtained a 500 bp segment in the EBV-EBVNA-2 region by use of type-1 specific primers 5'-CTCAGCGCAGGGATGCCTGG-3' and 5'-TGGTGCTGCTGGGGCAA-3'.

PCR amplification was performed in total volume of 50ul using a thermal cycler. PCR mixtures consisted of 10 ul sample. 100 pmol of each primer, 0.4 mM deoxynucleosidetriphosphate mix, 5ul of 10X reaction buffer , 1 mM MgCl2, and 2.5 U of Taq DNA polymerase. The tubes were covered with mineral oil and preheated for 4minutes at 94C and amplified by 30 cycles of denaturation at 94C for 30seconds , annealing at 58 to 62C for 30 seconds; and elongation at 72C for 30 seconds.

The PCR detection limit was calculated with serially diluted DNA positive controls and found in 100 viral genom/ml. Specificity was confirmed bye examing the size of amplicons and retesting of positive samples. Positive controls included human fibroblast monolayers containing HCMV and Raji cells containing EBV-1. Negative controls included human placental genomic DNA and without DNA templates.

Amplification mixtures (20 ul) were subjected to electrophoretic separation 4V/cm in Tris EDTA buffer in 2% agarose gel** containing 0.5 ug/ml of ethidium bromide and visualized under a 300nm UV transilluminator.

RESULTS

Statistical comparisons of the study population are shown in Table 1.

HCMV was detected in 27% of AgP patients and EBV-1 in 25% of AgP patients. HCMV and EBV-1 coinfection was detected in 52% of AgP patients. Tables 1 & 2 and Diagram 1.

The PCR assays were carried out on stored subgingival plaque samples. A subject was classified as "positive" for particular species if at least one site had detectable levels of bacteria.

PCR detection of HCMV DNA in agarose gel electrophoresis and amplification and detection of EBV-1 viral sequence are shown in Figure 1.

DISCUSSION

The purpose of this investigation was to evaluate the subgingival presence of HCMV and EBV-1 in aggressive periodontitis patients.

In a previous study, Parra and Slots reported that human viruses may occur in advanced periodontitis lesions with relatively high prevalence, finding HCMV in 60% of their patients and EBV in 30% [3]. Contreras and Slots examined the frequency of HCMV, EBV-1, EBV-2, herpes simplex virus (HSV), and HIV in subgingival samples [2]. They reported that 24 subjects (89%) yielded at least one of five test viruses deep periodontal sites and 15 subjects (56%) yielded from shallow periodontal sites. Ting et al. examined the frequency of HCMV, EBV-1, EBV-2 and HSV in subgingival samples from localized aggressive periodontitis patients [5]. They found that of 11 deep periodontal samples, eight showed HCMV, seven-showed EBV-1, one showed EBV-2, six-showed HSV, and eight showed co-infections. Of 11 shallow samples, two showed HCMV, two showed EBV-1, one showed HSV, and two coinfections[5]. Kamma et al examined the occurrence of human herpesviruses and suspected periodontal bacteria in aggressive periodontitis patients [6]. HCMV was detected in 59.4% of active and in 12.5% of stable sites ($P<0.001$), EBV-1 in 43.8% of active and in 12.5 of stable sites ($P = 0.01$), and HSV in 34.5% of active and in 9.4% of stable sites ($P=0.03$) [6]. Yapar et al, detected a significantly higher prevalence of HCMV (64.7%) and EBV (70.6%) in subgingival plaque samples from aggressive periodontitis patients, and they confirmed the close association between HCMV and EBV-1 and aggressive periodontitis [1].

In this study, we detected average prevalence of HCMV and EBV-1 among the patients but co-infections of CMV and EBV-1 viruses was high. Dual viral infections seem to be particularly pathogenic.

Bacterial infection and other conditions that promote diapedesis of inflammatory cells into a tissue would increase the possibility of initiating an HCMV infection of that tissue [7,8]. Gingival inflammation would facilitate the establishment of a periodontal herpes virus infection [9].

HCMV infects periodontal monocytes/ macrophages and T lymphocytes. These cells have the potential to play an important role in the pathogenesis of destructive periodontal diseases [9].

Since human macrophages have, a limited lifespan of 2 to 4 months [10], HCMV-infected macrophage populations cannot persist in the periodontium unless replenished by infected monocytes or by experiencing an abnormally long survival time due to HCMV-mediated inhibition of apoptosis [11]. The reason why treated aggressive periodontitis patients showed no HCMV activation might be due to the reduced influx of HCMV-infected monocytes [5]. Contreras et al. found a high proportion of herpes virus inflammatory cells in adult periodontitis lesions, but no HCMV, EBV-1, EBV-2 or HSV in cell fractions from healthy gingival specimens [9].

CONCLUSION

In summary, this study provides evidence of positive relationships between subgingival HCMV and EBV-1 presence, particularly herpes virus co-infection, and aggressive periodontitis. However, these conclusions are based on a relatively small sample. Longitudinal clinical and microbiological studies are needed to determine the pathogenetic and therapeutic implications of the present findings.

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