Subgingival Human Cytomegalovirus and Epstein Barr Virus in Patients with Aggressive Periodontitis in Ahwaz, Iran

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Abstract:
Objective: Assessment of subgingival human cytomegalovirus (HCMV) and Epstein Barr virus (EBV-1) in subjects with aggressive periodontitis.
Materials and Methods: Samples were obtained from plaques formed in subgingival regions of 26 aggressive periodontitis patients. All specimens were submitted to polymerase chain reaction in order to detect HCMV and EBV-1.
Results: HCMV and EBV-1 were observed in 27% and 25% of the participants respectively. Coinfection with both viruses was found in 52% of the patients.
Conclusion: Within the limitations of the present study, it can be suggested that HCMV and EBV-1 in subgingival plaques may be associated with aggressive periodontitis. Additionally, concomitant occurrence of these viruses may negatively affect the wellbeing of periodontal tissues.

Key Words: Herpesviridae; Aggressive Periodontitis; Prevalence

INTRODUCTION
Aggressive periodontitis is a separate entity from chronic periodontitis with distinct clinical and laboratory characteristics. Both localized and generalized forms of this disease occur before 35 years of age and induce rapid attachment loss along with bone destruction in an otherwise healthy appearing oral environment [1]. Specific herpesviruses can cause gingival infection followed by a reduction in the resistance of periodontal tissues leading to an increased overgrowth of pathogenic bacteria [2]. Epstein Barr virus (EBV-1) and human cytomegalovirus (HCMV) have been suggested as possible sources of human periodontal disease [3,4]. It has been proposed that transient suppression of the immune system following reactivation of these infectious agents may, to some extent, clarify the intermittent nature and progressive quality of aggressive periodontitis [2].

The aim of the current investigation was to evaluate HCMV and EBV-1 in subgingival plaque samples of individuals with aggressive periodontitis from those referred to the School of Dentistry, Ahwaz Jondishapour University of Medical Sciences (AJUMS), Iran.

MATERIALS AND METHODS
A total of 26 systemically healthy patients (13 women and 13 men; mean age, 35.2 years) with aggressive periodontitis were selected from those referred to the School of Dentistry, AJUMS. Subjects who had undergone periodontal treatment or received antibiotics within the past six months were excluded from the study sample. The protocol of the investigation
was approved by the ethics committee of our University and informed consents were obtained from all participants after the procedures were fully explained. A diagnosis of aggressive periodontitis was rendered if the subjects were less than 35 years of age, exhibited severe destruction of periodontal tissues and displayed attachment loss exceeding 5 mm at two or three areas in more than 14 teeth (at least three of which were not first molars or incisors). After isolating the region with cotton rolls, the three deepest pocket sites (5 to 10 mm) were selected, dried and subgingival plaque sampling was performed with a sterile curette by inserting it into the base of the pocket and removing the plaque with a single stroke. Amplicon size and positive samples were assessed and reexamined in order to confirm the specificity of the test. All plaque samples were collected in Pronase-E buffer (20 mM tris-hydrochloride, 10 mM EDTA, 0.2% SDS) and kept at -70°C prior to PCR. For each sample, DNA extraction was followed by high pure viral DNA extraction, using the corresponding kit (Roch Co.). Diagnosis of HCMV was performed utilizing 10 µl of each extracted plaque template in the PCR mixture (50 µl), which was composed of 1xPCR buffer, 2 U Tag-polymerase, 5 mM MgCl₂, 0.1 mM dNTP, 10 pmol primers and 0.8 mM betaine in each microtube. The HCMV sequence primer was 5´-GAGCGGTCCACAAAGTCTA-3´, 5´-GTGATCCGACTGGCGAAAA-3´ in the PCR. All tubes containing PCR mixture were then placed in a thermocycler (Techno company, UK) and subjected to 35 rounds, including denaturation, 94°C for 45s; annealing, 59°C for 30s and extension, 72°C for 45s.

RESULTS
Table 1 provides a statistical comparison of the study samples in both sexes. HCMV and EBV-1 were detected in 25% (6 cases) and 27% (7 cases) of the patients, respectively. Coinfection with both viruses was found in 52% of the subjects (Fig 1). Positivity was based on the observation of at least one area containing discernable levels of bacteria. PCR findings including HCMV DNA and its amplification in addition to the viral sequence of EBV-1 are demonstrated in Fig 1.

DISCUSSION
We studied the presence of HCMV and EBV-1 in subjects with aggressive periodontitis and evaluated their possible role in the pathogenesis of this disease. According to previous investigations, HCMV and EBV are considered as common findings in advanced periodontal lesions [3]. The possibility of coinfection with HCMV, EBV-1, EBV-2, HSV, or HIV in deep and shallow periodontal defects has shown the presence of at least one of these viruses in subgingival samples, especially in those occurring in deeper pockets [4]. Similar results have been reported in patients with localized aggressive periodontitis [5]. Kamma et al [6] in a study on aggressive periodontitis found significantly higher percentages of HCMV, EBV-1 and HSV in active periodontal sites compared to stable areas. Yapar et al [1] also confirmed the relationship between aggressive periodontitis and HCMV and EBV-1. Contreras et al [7] reported a significant amount of herpesvirus inflammatory cells in adult periodontal defects, but not in cell fractions from non-lesional gingival tissues.

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<tr>
<th>Viruses</th>
<th>Prevalence in Women</th>
<th>Prevalence in Men</th>
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<tbody>
<tr>
<td>EBV-1</td>
<td>14.5%</td>
<td>12.5%</td>
</tr>
<tr>
<td>HCMV</td>
<td>14.5%</td>
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In the current study, we detected an average prevalence of HCMV and EBV-1 among the studied patients but coinfections of CMV and EBV-1 viruses were high. Anything that can induce the passage of inflammatory cells from blood vessels into the surrounding connective tissues, i.e. bacterial infections, can potentially cause an HCMV infection in that site [7,8]. Therefore, the presence of gingivitis can promote a herpesvirus infection in periodontal tissues [9]. Mononuclear inflammatory cells are implicated as etiologic factors in the development of destructive periodontitis and could be infected with HCMV [9]. Human macrophages are active for a maximum of 2 to 4 months in connective tissues [10]. Therefore after this period, the cells should either be replaced by new infected monocytes or live longer as a result of inhibited apoptosis promoted by HCMV [11]. A decrease in HCMV-infected monocytes may explain the fact that there was no activation of HCMV in patients that were treated for aggressive periodontitis [5].

CONCLUSION
In summary, our findings suggest an association between HCMV and EBV-1 coinfection and aggressive periodontitis. Further evaluation of this relationship using a larger sample size and advanced microbiologic techniques is required to confirm the present results.

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