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# Viral serological and molecular data on possible involvement of herpes viruses in periodontal disease

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ABSTRACT

*Background:* Recent studies have suggested that latent herpes virus infections can be associated with chronic periodontal sites that exhibit a predisposition to disease progression.

The aim of this study was to identify the possible relationship between infections with CMV and EBV and the severity of periodontal disease.

*Materials and methods:* Fifty two patients aged between 27 and 70 years, diagnosed with periodontal disease were enrolled in the study after giving informed consent. Quantitative immunoenzymatic assays were used to determine the concentration of anti CMV and EBV antibodies. The presence of CMV and EBV DNA was tested in biopsies from periodontal tissues using an in-house PCR adapted after a method described previously.

*Results and conclusions:* Higher titers of the anti CMV antibodies appear to be correlated with the severity of the periodontal lesions ( $p<0$, 05). These correlations have not been found for anti EBV antibodies. Higher titers of specific anti CMV and EBV antibodies were correlated with a history of periodontal treatment ($p<0$, 05). Only two samples were positive for the viral genome. Both samples were collected from female patients diagnosed with very advanced forms of periodontal disease.

Although the molecular biology data from the present study do not support the pathogenic involvement of EBV or CMV in the development of chronic periodontitis lesions, the serological data might be important markers for the evolution and severity of the periodontal disease



## Introduction

Periodontal disease represents a group of clinical entities of uncertain etiology, characterized by the periodontal junction breakdown, loss of alveolar substance up to teeth loss. Various factors can promote the onset and evolution of this disease (environmental, behavioral, and genetic risk factors). The possible involvement of viral infections, especially with herpes viruses, in the pathogenesis of periodontal disease has been reported in recent studies [1–4].

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Herpes viruses produce systemic infections following the reactivation of latent infections especially during immunosuppression, inflammatory disorders or after a trauma and have been isolated from saliva and gingival tissues in patients affected by periodontal disease [5–9]. Socioeconomic status, racial and educational factors may be contributing to the seroprevalence of herpes viruses infection and its impact on the evolution of the periodontal disease [5].

Recent studies [9] have suggested that latent herpesvirus infections (especially cytomegalovirus– CMV and Epstein Barr virus- EBV) can be associated with chronic periodontal sites that exhibit a predisposition to disease progression [9]. A large number of copies of the DNA genomes of both CMV and EBV were isolated from some cases of progressive marginal periodontitis, a fact that has important implications in understanding the disease etiology.

In this context, the aim of this study was to identify the possible relationship between infections with CMV and EBV and the severity of periodontal disease by causing the specific immune responses and the viral replication in the periodontal lesions.

## Materials and methods

Fifty two patients aged between 27 and 70 years, diagnosed with periodontal disease, undergoing treatment in different dental clinics were enrolled in the study after giving informed consent. The eligibility criteria were: age over 18 years, suggestive clinical manifestations of periodontitis (gingival inflammation, gingival bleeding and mobility or loss of dental units), without subtotal dentition, absence of antibiotics or periodontal treatments during the last 6 months, without any uncompensated serious systemic disease, and without major modifications in terms of diet or lifestyle.

A data sheet was prepared for the registered patients, which included their personal data and personal history. Intraoral clinical examination was carried out by inspection and palpation, the appearance of the oral mucosa, changes in the gum aspect, the oral hygiene index (OHI)(Green and Vermillion 1960), the papillary bleeding index, gingival retraction, tooth mobility, existing periodontal pockets depth (21 with depth >5 mm) were noted. The study was approved by the local Ethics Committee. Venous blood samples and biopsies from the affected periodontal tissue were collected during the same visit.

Quantitative immnunoenzymatic assays (DIA.PRO, Italy) were used to determine the concentration of anti CMV IgG and IgM antibodies and of anti VCA IgM, anti VCA IgG and anti-EBNA IgG specific antibodies. The presence of CMV and EBV DNA was tested in biopsies from periodontal tissues using an in-house PCR adapted after the method described previously (Jankovic) [10]. Briefly, the total cellular DNA was isolated from periodontal tissue in maximum two days after harvest, using a High Pure PCR template preparation commercial kit (Roche, Germany). The integrity of the isolated DNA's was verified by amplification of a 110 bp β -globin gene fragment using specific primers synthetized by Invitrogen. Amplification of a specific fragments of 264 bp and 256 bp were performed using CMV primers: (Fw: 5′-GAGCGCGTCCACAAAGTCTA-3′; R: 5′-GTGATCCGACTGGGCGAAAA-3′) and EBV primers (Fw: 5′-AGGGATGCCTGGACACAAGA-3′; R: 5′-GCCTCGGTTGTGACAGAG-3′) PCR products were detected following electrophoresis in a 1.5% agarose gel containing 0.5 mg/ml ethidium bromide.

## Results

The socio-demographic and epidemiological characteristics of patients are shown in Table 1. Half of the patients (26/52, 50%) had one or more risk factors for periodontal disease, the most common being: poor dental hygiene, local causes (overflowing fillings, prosthodontic bridges or crowns which have lost their adaptation, family factors, smoking); 56% of the subjects did not experience any periodontal treatment. Out of 52 patients, 27 (52%) were diagnosed with deep chronic marginal periodontitis (PMCP), of whom 78% (21/27 patients), experienced periodontal pockets with depth >5 mm and 96%

**Table 1 – Studied patients characteristics.**

| | Chronic periodontitis N=27 | Gingivitis N=25 |
|---|---|---|
| Sex ratio M:F | 9/18 | 11/14 |
| Mean age ±SD | 51,81(±13,28) | 35,84(±13,22) |
| Urban vs rural medium | 21/6 | 24/1 |
| Education highschool vs University | 18/9 | 9/16 |
| Comorbidities *n*(%) | 12 (45) | 0 |
| Smoking *n*(%) | 9 (33) | 5 (20) |
| Poor dental hygiene *n*(%) (OHI Average) | 20 (74)(3,27) | 10(40)(2,94) |

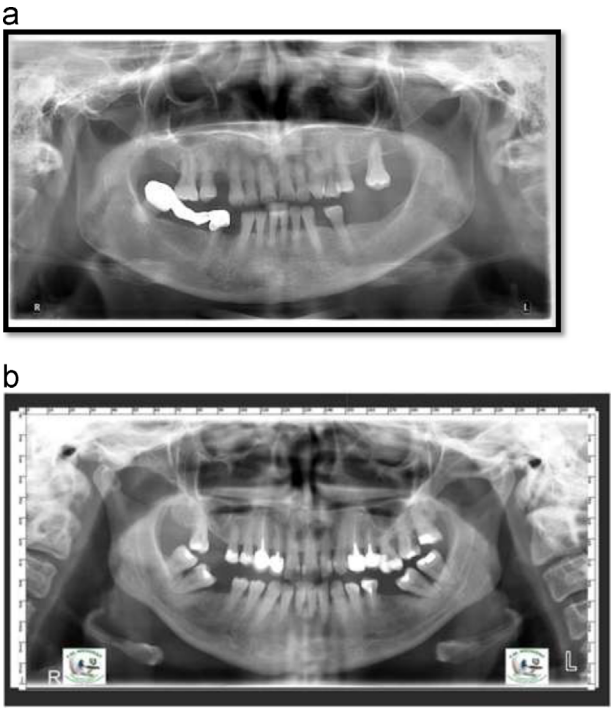


**Fig. 1 – a. Panoramic radiograph of a patient aged 70 with chronic periodontitis. b. Panoramic radiograph of a patient aged 45 years with chronic periodontitis.**