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Short Communication

Absence of cytomegalovirus in gingivitis and chronic periodontitis in HIV-1 patients in Northern Brazil

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Abstract

Introduction: The influence of cytomegalovirus (CMV) on the progression of chronic periodontitis in HIV patients is poorly investigated. **Methods**: ELISA was used for anti-CMV antibody IgG titer measurements and real-time polymerase chain reaction for qualitative and quantitative CMV detection. Data on the CD_4^+ T lymphocyte count and plasma HIV viral load were obtained from patient records. **Results**: CMV DNA was detected in samples of subgingival biofilm in only three individuals, two of them with chronic periodontitis (4%) and one with gingivitis (3.3%). **Conclusions**: The prevalence of CMV is very low both in HIV-1 patients with gingivitis and chronic periodontitis.

Keywords: HIV. Cytomegalovirus. Periodontal disease.

Human immunodeficiency virus (HIV) infection is characterized by an advanced state of immunosuppression. HIV infects TCD_4^+ lymphocytes ($LTCD_4^+$), also known as T helper lymphocytes (LTh) and the decrease in the number of these cells may contribute to the occurrence of several opportunistic infections and several pathologies. Periodontitis is recognized as an important condition and may be associated with the immunodeficiency caused by HIV. It can also be considered one of the first clinical signs of HIV infection, which can be mitigated by the use of antiretroviral therapy (ART)[1–3].

When chronic periodontitis (CP) occurs in HIV negative patients, the periodontal connective tissue demonstrates a dense inflammatory infiltrate of mononuclear cells, lymphocytes, and macrophages. In addition to the pro-inflammatory and osteoclast cells, recent studies have evidenced an interaction between periodontopathogenic bacteria and CMV, this interaction contributes to severe bone destruction and it is a direct result of the processes of herpesvirus activity and latency[4–6].

In HIV positive patients, herpesvirus reactivation and viral activity frequently occur due to the immunosuppression of antiviral immune cells such as natural killer cells, lymphocytes, macrophages, and interferons caused by HIV-1. Thus, HIV positive patients with active herpesvirus infection present periodontal disease with greater probing depth, spontaneous bleeding and severe loss of the clinical attachment level (CAL)[6–8]. Due to the deficient immune system and secondary infection with herpesvirus, these patients present a more aggressive periodontal disease being recurrent advanced stages of the disease such as necrotizing ulcerative periodontitis or gingivitis[8].

In the Amazon region, CMV is one of the most prevalent herpesviruses in the HIV positive population due to the environmental and socioeconomic conditions of the northern Brazilian region[9]. It is known that CMV activity in the periodontal pocket leads to a more rapid progression of CP. In addition, studies indicate that CMV is the most prevalent herpesvirus in the gingival tissue in HIV-positive patients. With the advent of ART therapy, seropositive patients achieve a reduction in HIV viral load and elevated lymphocyte levels,

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inducing CMV to enter tissue and remain in the latent stage[10]. The aim of the present study was to determine the prevalence of CMV in the saliva and at subgingival sites of HIV patients with gingivitis, chronic periodontitis and without chronic periodontitis, in Belem, State of Pará, Brazil.

This cross-sectional study was conducted in Pará State, northern Brazil between January 2014 and December 2016. The study evaluated patients with HIV and periodontal disease (CP or gingivitis). One hundred and thirty HIV positive individuals, aged between 20 and 60 years, all of whom were ART users were selected. There were 50 patients with HIV-1 and without periodontal disease placed in the control group G1, and 30 HIV-1 positive patients with gingivitis were placed in group G2; 50 other patients with HIV-1 and CP were placed in group G3.

Periodontitis was diagnosed based on the study of Armitage[11] (1996). The samples were collected by a single Calibrated Researcher (CR), who was experienced in clinical studies. The assessment was made using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA), a mouth mirror, and clinical tweezers, all of which were sterile, consisted of disposable materials, and were used under natural lighting. The following clinical parameters were assessed: CAL, bleeding on probing (BoP) and probing depth (PD)[12].

The inclusion criteria were individuals with at least 20 teeth and who had not undergone periodontal treatment for 1 year. Patients with CP should have a PD ≥ 5 mm and must radiographically present bone destruction in two different sextants and BoP. All individuals with PD less than 5 mm, without gingival inflammation, without BoP, and without CAL were considered to be periodontally healthy. Patients with gingivitis should have inflamed and edematous gingiva with swelling of the interdental papilla, easily induced bleeding, and no CAL. Patients younger than 20 years, pregnant women, lactating women, diabetics, smokers, and patients on systemic or local antimicrobial therapy, on hormone replacement therapy, or on any analgesic and anti-inflammatory drugs less than 30 days prior to sample collection were excluded from the study[12].

To confirm that all individuals were HIV-1 positive, blood samples were collected and sent to the clinical laboratory for diagnostic purposes. ELISA type immunoenzyme assay was performed (DiaSorin, anti-HIV tetra Elisa, Biotest, Germany), which includes a recombinant antigen, one of the envelope and two antigens of the viral capsid[8,12].

Clinical parameters were evaluated in all teeth, excluding third molars, and included the following: BoP, PD, and CAL. Six sites were examined for each tooth: mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual. One CR monitored the patients and collected the clinical reports. Data were collected and averaged between the sites collected divided by the number of teeth examined per patient[12,13].

Gingival crevicular fluid samples were obtained from 2 sites in the periodontally affected sites at the mesiobuccal gingival sulci of teeth 16 and 26. After isolating the tooth with a cotton roll, supragingival plaque was removed with curettes (Hu Friedy, Gracey, IL, USA), without touching the marginal gingiva. The crevicular site was then dried gently with an air syringe. Gingival crevicular fluid was collected with paper strips (ProFlow, Amityville, NY, USA). Strips were placed into the sulci/pocket until mild resistance was sensed and left in place for 1 minute. Strips contaminated by saliva or blood were excluded from the sampled group. Besides the collections from the pockets, saliva was also sampled by placing the *Endo points* in the sublingual region for 1 minute. Each tip was placed in a sterile polystyrene tube (Eppendorf, Sigma, CA, USA) which was sealed and identified with patient data and the site where the sample was collected and was immediately sent, under refrigeration in a cooler with ice packs, to the Laboratory of Virology of the Federal University of Pará and stored at -70ºC until analysis[8,12,13].

Peripheral blood samples (10 mL) were collected by venipuncture in two vacuum EDTA tubes for the detection of possible CMV infection and for CD_4^+ T lymphocyte count and determination of HIV viral load. After collection, the samples were transported to the Laboratory of Virology, at the Institute of Biological Sciences of the Federal University of Pará (UFPA). The samples were centrifuged at 3000 rpm for 15 minutes for separation of plasma from the cellular components and stored at -70ºC[12,13]. Plasma was tested for anti-CMV IgM and IgG antibodies using the commercially available ELISA (DiaSorin, Saluggia, Italy).

The HIV viral load was measured by real-time PCR whereas CD_4^+ T lymphocyte count was determined by flow cytometry (FACSCount, Becton & Dickinson, San Jose, CA, USA) using the FacsCount™ Reagents immunomonitoring kit, following the protocol recommended by the manufacturer (Becton Dickinson, San Jose, CA, USA). Both procedures were routinely conducted at the Laboratory of Virology, which is one of the Brazilian reference laboratories for these measurements[12,13].

Genomic DNA was extracted from the blood samples, subgingival sites, and saliva using the *DNA BioPur Mini Spin* kit (Biopur, Reinach, Switzerland), according to the manufacturer's instructions. CMV was detected by real-time PCR (qPCR) for amplification of *IE2* (UL122 assay) and *pp65* (UL83 assay) genes as described previously. Thirty positive and negative controls were obtained using Acrometrix™ CMV (Thermo Fisher Scientific, Waltham, USA) and to guarantee the presence of DNA and absence of PCR inhibitors in the samples, a segment of the *GAPDH* gene was amplified according to the previously described protocol[12,13].

Statistical analyses were conducted. Means and standard deviations were calculated for age, periodontal parameters, and duration of HIV infection in years. The presence of CMV, duration of ART use, and HIV viral load are described as absolute and relative frequencies. The student *t*-test was used to assess the difference between groups regarding the parameters evaluated. The significance level was set at 0.05.

Three groups of HIV-1 patients were assessed: control patients – without periodontal disease (G1), patients with gingivitis (G2), and patients with periodontitis (G3). G2 patients were predominantly male (53.3%), with a mean age of 33 years, and had BOP and average PD of 2.9 mm; most G2 patients had an undetectable HIV-1 viral load (53.3%), but CMV was

detected in one sample (0.83%) of an individual who had just recently initiated ART.

G3 predominantly comprised women (56%) with a mean age of 36 years; in this group, individuals had an average PD of 5.6 mm, and despite the high prevalence of undetectable HIV-1 viral load (66%), CMV was detected in two subgingival samples (1.66%). This could be due to the short time since HIV-1 infection (average of 2.5 years) and because those patients had just initiated ART. In G1, as expected, the epidemiological characteristics and periodontal parameters revealed lower values than in the other groups.

Epidemiological characteristics, periodontal parameters, CMV and HIV viral loads, and use of ART are displayed in **Table 1**.

When activated in the periodontium, the mechanism of CMV infection consists of the herpesviral-bacterial interactive model, in which dental plaque composed by bacteria and herpesviruses induce inflammation of the gingiva. Once the inflammation process is established, the junctional epithelial immune cells and the gingival connective tissue respond to bacterial aggression by releasing interleukin-1b, monocytes, macrophages, neutrophils, and $LTCD_4^{+}$[1-7].

According to Contreras, Botero and Slots[6] (2014) the CMV infection triggers in macrophages and T lymphocytes a major release of interleukin-1b and tumor necrosis factor-α. These proinflammatory mediators induce osteoclast differentiation, the release of matrix metalloproteinases and the impairment of antibacterial immune mechanisms, causing an upgrowth of periodontopathic bacteria[6]. In HIV-positive patients, CMV infection occurs with the decrease in cellular immunity, herpesvirus activation, and inhibition of phagocytic activity. Knowing these immunological factors, the increase in periodontopathogenic bacteria levels of the red complex in the periodontal pockets, favors a new mechanism of periodontal disease activity, leading to more severe periodontitis[7,8].

ART has improved the prognosis, quality and life expectancy of HIV patients. It allows the reduction of morbidity and mortality rates by increasing the count of $LTCD_4^{+}$ cells, reducing viral load and restoring the immune system[13–15]. In our study, all 130 HIV-positive patients were under ART at the Specialized Center in Pará State; the patients were on a cocktail of 3 drugs, including valganciclovir[14]. This drug is a pro-drug used for prophylaxis and treatment of CMV in HIV-positive individuals; this drug decreases CMV levels in saliva and blood, minimizes viral replication, and contributes to reactivation of T lymphocyte cells in HIV-positive patients[14].

When analyzing the results of this manuscript, we verified whether the use of valganciclovir in the ART therapy of seropositive patients might have directly influenced the low prevalence of CMV in the analyzed samples. In the group of patients with gingivitis, the CMV-positive patient had an undetectable HIV viral load, but he stated that he began to use ART during the study, which may induce a positive result in this group. In group G3 CMV-positive patients had been diagnosed with HIV contemporaneously with the study, so they began to use ART at the moment of sample collection[7–10].

TABLE 1: Epidemiological characteristics, periodontal parameters, CMV and HIV viral loads, and use of ART in HIV-1 patients without periodontal disease (G1), with gingivitis (G2), and with periodontitis (G3)

	G1 (n=50)	G2 (n=30)	G3 (n=50)
Age (years)	36 (SD 8.5)	33.4 (SD 8.8)	36 (SD 9.4)
Sex			
male	31 (62%)	16 (53.3%)	22 (44%)
female	19 (38%)	14 (46.7%)	28 (56%)
Periodontal parameters			
probing depth (mm)	2.5 (SD 0.6)	2.9 (SD 0.4)	5.6 (SD 0.7)
bleeding on probing (sites)	1.0 (SD 2.0)	8.9 (SD 3.0)	12.2 (SD 7)
Presence of CMV			
blood	0	0	0
subgingival sites	0	1 (0.83%)	2 (1.66%)
saliva	0	0	0
HIV viral load (% of individuals)			
undetectable (<50)	24 (48%)	16 (53.3%)	33 (66%)
low (<10,000)	20 (40%)	8 (26.7%)	10 (20%)
moderate/high (>10,000)	6 (12%)	6 (20%)	7 (14%)
HIV infection (years)	5.9 (SD 6.0)	4.1 (SD 3.6)	2.5 (SD 2.3)
Use of ART (% of individuals)			
yes	42 (84%)	19 (63.3%)	34 (68%)
no	8 (16%)	11 (36.7%)	16 (32%)

SD: Standard deviation; **CMV:** cytomegalovirus; **HIV:** Human immunodeficiency virus; **ART:** antiretroviral therapy.

Nibali et al.[13] (2004) and Stein *et al.*[15] (2013) revealed a low prevalence of CMV in the studied samples corroborating the findings of this study. According to the authors, the type of periodontal disease assessed, social, ethnic, geographical factors and sample size influenced the prevalence of CMV. The present study aimed to evaluate the prevalence of CMV in a specific and differentiated population from the other studies, in the region amazon where CMV has a high prevalence and a considerable sample size to determine if the presence of CMV was statistically relevant as it is clinically. However, ART proved to have more influence in our study than in the other studies due to the type of medication used by the patients.

In conclusion, the data from our study did not indicate any association between herpesviruses and periodontopathogenic bacteria due to the very low prevalence of cytomegalovirus in the analyzed samples of seropositive patients. This result was not expected, and in order to better clarify the co-infection model, further studies with different methodologies regarding the type of samples collected should be used to improve the methodological limitations.

Conflict of Interest

The authors declare that there is no conflict of interest.

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