Cellular Profile and Expression of Immunologic Markers in Chronic Apical Periodontitis from HIV-infected Patients Undergoing Highly Active Antiretroviral Therapy

Túlio Gustavo Veiga Gama, DDS, MSc, PhD, Fabio Ramoa Pires, DDS, MSc, PhD, Luciana Armada, DDS, MSc, PhD, and Lucio Souza Gonçalves, DDS, MSc, PhD

Abstract

Introduction: This study tested the hypothesis that the inflammatory cell profile (CD3-, CD4-, CD8-, CD20-, and CD68-positive cells) and the expression of immunologic markers (tumor necrosis factor α, interferon-γ, interleukin-6, and interleukin-18) in chronic apical periodontitis are the same between non–HIV-infected patients and HIV-infected patients undergoing highly active antiretroviral therapy (HAART). **Methods:** Thirty-four surgically excised chronic apical periodontitis lesions were sampled from 34 patients (17 HIV-infected and 17 non–HIV-infected). The lesions were extracted from teeth with no previous endodontic treatment. All HIV-infected patients were undergoing HAART. The specimens were submitted to histopathologic and immunohistochemical analyses by using an optical microscope. Immunoexpression was graded into 2 levels, focal to weak and moderate to strong. The χ^2, Fisher exact, and Mann-Whitney tests were used to analyze all significant differences between groups. **Results:** Periapical cysts represented 70.6% and 52.9% of the lesions in the HIV-infected and non–HIV-infected groups, respectively; however, no statistically significant difference was observed ($P = .481$). There were no statistically significant differences between groups for the inflammatory cell profile and for any of the immunologic markers ($P > .05$). **Conclusions:** There are no statistically significant differences of the cellular profile and expression of immunologic markers in chronic apical periodontitis between non–HIV-infected patients and HIV-infected patients undergoing HAART. *(J Endod 2016;42:921–927)*

Key Words

Chronic apical periodontitis, HIV infection, immunohistochemistry, periapical cyst, periapical granuloma

Chronic apical periodontitis (CAP) is an inflammatory process in the periapical tissues that can develop after microbial invasion of the dental pulp. Intracanal microorganisms cause inflammatory responses that may trigger different forms of apical periodontitis (1), and the development of CAP represents a dynamic interaction between the immune system and infecting microorganisms (2). The persistence of the infectious stimulus within the root canal system leads to a chronic process that induces bone resorption and granuloma formation, which can subsequently give rise to a periapical cyst (3).

The role of bacteria and its products on the etiology and persistence of CAP is well-established (4). In addition, systemic conditions such as diabetes, herpes virus infection, and genetic polymorphisms as well as smoking, stress, and depression are also recognized as risk factors or disease modifiers because they can influence the host's immune response, impairing the balance between lesion progression and tissue repair (5). Because HIV infection may lead to an important immune deficiency, it is reasonable to believe that it may also act as a modifying factor on the development/repair of CAP (6).

However, since the establishment of highly active antiretroviral therapy (HAART) as the therapeutic protocol in HIV infection, there was a significant reduction in morbidity and mortality from HIV, an improvement in the patient's quality of life, restoration and preservation of the immunologic functions (7), and reduction of the viral load to undetectable levels (<50 copies per mL) (8). At the same time, untreated HIV infection is associated with persistently higher levels of proinflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor alfa (TNF-α) and coagulation biomarkers and acute phase proteins such as fibrinogen, D-dimer, and high-sensitivity C-reactive protein (9, 10).

The current study has hypothesized that the introduction of HAART has turned the protection against endodontic infection similar between HIV-infected and non–HIV-infected patients. Thus, the profile of the inflammatory cell infiltrate and the expression of immunologic markers in CAP were assessed in these 2 groups of patients and compared.

From the Post-graduation Program in Dentistry, Estácio de Sá University, Rio de Janeiro, Rio de Janeiro, Brazil.

Address requests for reprints to Dr Lucio Souza Gonçalves, Post-graduation Program in Dentistry, Estácio de Sá University, Av Alfredo Baltazar da Silveira, 580/cobertura, 22790-010 Recreio, Rio de Janeiro, RJ, Brazil. E-mail address: luciogoncalves@yahoo.com.br

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TABLE 1. Primary Antibodies Used in the Immunistochemical Analysis

Antibodies	Type	Manufacturer	Dilution	Positive control
Anti-IL-6	Mouse monoclonal	Santa Cruz Biotechnology	1:500	Lung
Anti-IL-18	Rabbit polyclonal	Santa Cruz Biotechnology	1:200	Spleen
Anti-CD3	Mouse monoclonal	Dako	1:200	Lymph node
Anti-CD4	Rabbit monoclonal	Spring Bioscience	1:100	Lichen planus
Anti-CD8	Mouse monoclonal	Dako	1:200	Lymph node
Anti-CD20	Mouse monoclonal	Dako	1:200	Lymph node
Anti-CD68	Mouse monoclonal	Dako	1:500	Mucocele
Anti-TNF-α	Mouse monoclonal	Santa Cruz Biotechnology	1:50	Lichen planus
Anti-IFN-γ	Rabbit polyclonal	Santa Cruz Biotechnology	1:200	Oral epithelium

Materials and Methods

Subject Population

Thirty-four patients (17 HIV-infected [group H] and 17 non–HIV-infected [group NH]) attending the Faculty of Dentistry at the Estácio de Sá University from 2011 to 2013 were selected. All patients were informed about the aims, risks, and benefits of the study and signed a consent form. Patients were >20 years old and presented at least 1 CAP lesion in a tooth indicated for extraction (loss of coronal structure by trauma or extensive dental caries, which made it impossible to restore the tooth), with clinical and radiographic confirmation. Exclusion criteria included the use of antibiotic prophylaxis for dental procedures, use of anti-inflammatory drugs, pregnancy, diabetes, and autoimmune diseases. Teeth with root fractures, endodontic-periodontal lesions, and/or previous endodontic treatment were also excluded. All HIV-infected patients were undergoing HAART for at least 18 months. The study protocol was approved by the Review Committee for Human Subjects of Estácio de Sá University (CAAE 26961014.0.0000.528).

Clinical and Radiographic Evaluation

Patients were submitted to an anamnesis questionnaire, and data about gender, age, ethnicity, tobacco use, and forms of HIV transmission were recorded. Information about CD4+ T-lymphocyte counts, plasma HIV viral load, and antiretroviral therapy was obtained from the patients' medical records. An oral examination included the visual inspection of the oral mucosa, periodontal evaluation, and pulp vitality tests. Full-mouth periapical and panoramic radiographs were taken. Teeth indicated for extraction were selected by clinical and radiographic evaluation.

TABLE 2. Sociodemographic and Health-related Behavior Characteristics of Both Groups

Characteristics	H group (n = 17)	NH group (n = 17)	P value
Sociodemographic parameters			
Age, y [median (minimum–maximum)]*	45 (31–72)	47 (29–64)	.783
Gender, n (%)†			.732
Male	7 (41.2)	9 (52.9)	
Female	10 (58.8)	8 (47.1)	
Ethnicity, n (%)‡			.560
White	11 (64.7)	10 (58.8)	
Black	7 (35.3)	7 (41.2)	
Health-related behavior			
Smoking, n (%)†			.398
Yes	2 (11.8)	5 (29.4)	
No	15 (88.2)	12 (70.6)	

*Mann-Whitney test.
†Fisher exact test.
‡χ^2 test.

Sample Collection

Thirty-four CAP lesions were sampled, 1 from each patient (17 HIV-infected and 17 non–HIV-infected). All lesions were obtained by curettage, placed in individual containers containing 10% buffered formalin for 48 hours, and sent to the Oral Pathology Laboratory (Faculty of Dentistry, Estácio de Sá University). All samples were routinely processed for histologic (hematoxylin-eosin staining in 5-μm sections) and immunohistochemical analyses. The final histologic diagnosis (periapical granuloma or periapical cyst) was rendered by 2 experienced observers.

Immunohistochemistry

Nine 3-μm sections from each CAP were obtained for the immunohistochemical analysis. These were used for the reactions with the selected primary antibodies (Table 1). Immunoreactions were performed according to previously published methods from the same laboratory and study group (11, 12). Positive controls (Table 1) and negative controls (omission of the primary antibodies) were included in all reactions.

Each immunoslide was analyzed by using an optical microscope with ×400 magnification (high-power field), and the expression was estimated on the basis of the amount of immunopositive cells/area in each high-power field. Each area observed was categorized according to the following scores: 1, focal to weak, if less than 30% of the cells were positively stained; and 2, moderate to strong, if more than 30% of the cells were positively stained. For each immunoslide, 5 different high-power fields were evaluated

TABLE 3. Characteristics Related to HIV-infected Group

Characteristics	Value
Plasma HIV viral load, n (%)*	
Detectable	3 (17.7)
Undetectable	14 (82.3)
CD4+ T lymphocytes, median (minimum–maximum)†	450 (355–1300)
Time of exposure to HIV, median (minimum–maximum)‡	5 (2–12)
Time of exposure to HAART, median (minimum–maximum)‡	4 (1.5–9)

*Copies/mL.
†Cells/mm^3 of blood.
‡Values in years.