

Microbiological study of the subgingival biofilm in HIV+/HAART patients at a specialized dental service

Laura A. Gliosca^{1,4}, Luciana R. D'Eramo^{2,3,4}, Florencia L. Bozza^{1,4},
Luciana Soken^{1,4}, Lorena Abusamra⁵, Pablo A. Salgado^{1,2,4},
Aldo F. Squassi^{2,3,4}, Susana L. Molgatini^{1,4}

¹ Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Microbiología, Laboratorio de Diagnóstico Microbiológico, Buenos Aires, Argentina.

² Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Odontología Preventiva Comunitaria, Buenos Aires, Argentina.

³ Universidad de Buenos Aires, Facultad de Odontología, Clínica para La Atención de Pacientes de Alto Riesgo médico: (CLAPAR I), Buenos Aires, Argentina.

⁴ Universidad de Buenos Aires, Instituto de Investigaciones en Salud Pública, Buenos Aires, Argentina.

⁵ Servicio de Infectología, Hospital General de Agudos Dr. Juan A. Fernández, Buenos Aires, Argentina.

ABSTRACT

The aim of this study was to describe the microbiological profile of HIV patients under highly active antiretroviral treatment (HAART). This cross-sectional study comprised 32 HIV patients with periodontal disease (PD) who had been under HAART for more than 6 months. Information about the patients' medical history was obtained from clinical records. Clinical dental examination was performed by a calibrated researcher using standard dental instruments to determine probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP). A total 4,765 periodontal sites were evaluated, 125 of which were also studied microbiologically. Subgingival biofilm samples were obtained using sterile paper points; one set was used for microbiological culture studies and the other for end-point PCR. Statistical analysis was performed using Kruskal-Wallis and post-hoc Dunn-Bonferroni contrast tests. All participants were on HAART at the time of the study, and 90.6% had a viral load below 50 copies/mm³. Prevalence of periodontally active sites was low in the study

population. Microbiological studies: Black pigmented anaerobic bacteria and fusiform CFU counts were significantly higher in samples from sites with BOP and PD ≥ 4 mm (p 0.020 and p 0.005, respectively). Molecular Assays: Detection of *Porphyromonas gingivalis* (p 0.002), *Tannerella forsythia* (p 0.023) and *Treponema denticola* (p 0.015) was significantly more frequent at sites with BOP and PD ≥ 4 mm. Conclusions: The patients living with HIV/AIDS under HAART studied here had low prevalence of clinical periodontal disease signs. However, significant detection of *P. gingivalis*, *T. denticola*, and *T. forsythia* in periodontal active sites, and the involvement of these microorganisms as potential HIV reactivators, show the importance of creating awareness among dental health professionals of the need for close dental and periodontal monitoring in HIV patients.

Received: June 2019; Accepted: December 2019.

Keywords: HIV/AIDS, periodontal diseases, periodontitis, molecular biology, biofilm, risk factors.

Estudio microbiológico del biofilm subgingival de pacientes VIH+ bajo TARGA en un servicio dental especializado

RESUMEN

El objetivo de este estudio fue describir el perfil microbiológico del biofilm subgingival de los pacientes con VIH bajo tratamiento antirretroviral de alta actividad (TARGA). El estudio comprendió a 32 pacientes VIH seropositivos con enfermedad periodontal (EP) que se encontraron en tratamiento con TARGA por más de 6 meses. Los antecedentes médicos de los pacientes se obtuvieron de las historias clínicas. El examen clínico instrumental (profundidad de sondaje (PS), nivel de inserción clínico (NIC) y sangrado al sondaje (SS)) fue realizado con instrumental odontológico estándar por un investigador calibrado. De este modo, se evaluaron un total de 4.765 sitios periodontales de los cuales 125 fueron estudiados microbiológicamente. Las muestras de biopelícula subgingival se obtuvieron empleando conos de papel estéril. Las muestras se emplearon en estudios microbiológicos y moleculares por PCR de punto final. El análisis estadístico se realizó según Kruskal-Wallis y pruebas de contrastes post-hoc de Dunn-Bonferroni. El 90,6% de la población en estudio presentó carga viral inferior a 50 copias/mm³. La prevalencia de sitios

periodontales activos fue baja (1%). Los recuentos de bacterias anaerobias estrictas pigmentadas de negro y fusiformes fueron significativamente más altos en muestras de sitios periodontales con SS positivo y PS ≥ 4 mm (p 0.020 y p 0.005). La detección molecular de *Porphyromonas gingivalis* (p 0.002), *Tannerella forsythia* (p 0.023) y *Treponema denticola* (p 0.015) fue significativamente mayor en los sitios con SS y PS ≥ 4 mm. La prevalencia del 1% de enfermedad periodontal en el grupo de pacientes estudiados fue menor a la esperada, sin embargo; la detección significativa de *P. gingivalis*, *T. denticola* y *T. forsythia* en sitios periodontales activos y su potencial participación como agentes reactivadores del VIH, nos alerta de la importancia de crear conciencia en los profesionales de la salud (médicos y odontólogos) acerca de la necesidad de un monitoreo minucioso del estado periodontal de pacientes con características semejantes a las descriptas en la muestra poblacional estudiada.

Palabras clave: VIH/SIDA, enfermedad periodontal, periodontitis, biología molecular, biopelícula, factores de riesgo.

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is characterized by severe immunologic alterations mainly affecting CD4⁺ T cells, a subpopulation of T lymphocytes, and by subsequent development of potentially fatal opportunistic infections.

According to the World Health Organization, approximately 34 million people are infected with human immunodeficiency virus (HIV), and although there has been much progress in HIV research in recent years, including therapies such as highly active antiretroviral therapy (HAART), this disease still constitutes one of the most significant public health problems in the world¹. The National Ministry of Health estimates that in Argentina there are 120,000 people living with human immunodeficiency virus, 30% of whom are unaware of their HIV status².

Since the introduction of HAART (highly active antiretroviral therapy), the epidemiological profiles of the link between infectious disease and HIV have changed^{3,4}. The factors involved in these changes include partial restoration of the immune system, control of viral load and stability of the immune system over time⁵. HIV infection has turned into a chronic disease. Global mortality rates decreased from 97.4% in 1993 to 19.8% in 2001⁶. The current guidelines for antiretroviral therapy recommend initiating treatment earlier⁷.

However, the disease has not yet been completely eradicated, probably due to the ability of the virus to remain latent in host immune cells (T lymphocytes, macrophages and dendritic cells), as well as to its high mutation rate⁸.

Periodontitis, as a multifactorial ecological disease whose primary aetiology is bacterial biofilm, causes a chronic inflammatory response by the release of bacterial and host cell products⁹. Bacteria from subgingival periodontopathic biofilm have previously been shown to be a significant risk factor for many systemic diseases (heart disease, diabetes and low birth-weight, among others)¹⁰.

Epidemiological studies that used continuous measures of probing depth and clinical attachment loss have shown that advanced forms of periodontitis that resulting in severe loss of supporting structures and substantial tooth loss affect 10-15% of the population globally¹¹. This estimated prevalence range includes severe periodontitis, which primarily affects adolescents, young adults and adults, and whose prevalence increases with age in all populations¹²⁻¹⁴.

Longitudinal studies have shown that prolonged use of HAART in HIV-infected periodontal patients was associated with a decrease in the prevalence of putative periodontal pathogens as compared to seronegative patients. However, seropositive patients had species not usually associated with periodontitis, regardless of their periodontal status¹⁵. Establishing comparisons among studies conducted in the pre- and post HAART eras is complex given the need for appropriate and consistent characterization of HIV-associated periodontal disease to avoid underestimating the prevalence and severity of the infection¹⁶. The impact of HAART on periodontal disease in HIV patients in developing countries such as Argentina remains unclear, and reports on periodontal microbiota in this population are not conclusive.

Recent studies suggest that bacteria found in the oral cavity, including *P. gingivalis*, are able to reactivate the latent HIV virus within infected cells. In 2009, Huang et al. evaluated the capacity of bacteria found in the oral cavity to stimulate HIV promoter activation¹⁷.

Research has been conducted in which cellular T-lymphocyte, macrophage and fibroblast lines transfected with the HIV long terminal repeat promoter were stimulated with oral bacterial sonicates, and both the Gram-negative and Gram-positive species were able to elicit positive stimulatory activity¹⁸. Another assay suggests that *P. gingivalis* produces a fatty acid called butyric acid, which may induce reactivation of the latent HIV-1 virus⁹. González et al. looked specifically at the ability of supernatants (cytokines/chemokines) produced by human gingival fibroblasts and oral epithelial cells to modulate HIV promoter activation in macrophages when challenged with periodontal pathogens. Stimulation with *P. gingivalis* and extract of *F. nucleatum* showed a significant increase in cytokines/chemokines, especially Interleukin-6 and Interleukin-8, which have the ability to modulate the activation of HIV-1 promoters¹⁸.

The superposition of events such as these, with reactivation of the HIV virus due to the coexistence of active periodontal disease, increase the systemic inflammatory state of the host. This worsens the effector response and subsequent clinical outcome¹⁹. The aim of this study was to describe the microbiological profile of periodontal pathogens in a population of local patients living with HIV/AIDS on highly active antiretroviral therapy.

MATERIALS AND METHODS

The population study comprised male and female HIV-infected patients treated with HAART who received care the High-Risk Patients Dental Care Unit (CLAPAR I), School of Dentistry, University of Buenos Aires, Argentina. The patients were informed of the study objectives, risks and benefits. All participants agreed to participate voluntarily and signed an informed consent form approved by the Ethics Committee of the School of Dentistry, University of Buenos Aires. This study fulfils the requirements of STROBE rules for human observational studies.

This descriptive study focused on people between 21 and 65 years old living with HIV / AIDS who had been under treatment with HAART for at least six months prior to the dental examination. A non-probabilistic sequential sample of 50 subjects seeking care at the CLAPAR I was evaluated. Information on the patients' medical history was obtained from clinical records (antiretroviral treatment history, viral load and CD4⁺ levels). Viral load was detected by real-time quantitative polymerase chain reaction (qPCR) to quantify plasma HIV-1 RNA, using ABBOTT REAL TIME HIV-1 RNA VERSION 3.0 with a sensitivity of 40 HIV-1RNA copies/ml (a viral load below 50 copies / ml was considered undetectable). The missing data from the clinical histories were traced in each of the hospital's services.

Dental clinical examination was performed by a calibrated researcher (intra-examiner kappa >0.80) using standard dental instruments (Marquis periodontal probe) to determine probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP). Clinical parameters were evaluated at 4,765 periodontal sites, 8 sites per tooth, in order to provide more accuracy for the description of periodontal status.

A total 50 persons were given appointments for screening for eligibility. Exclusion criteria were: less than 6 teeth (n=3), antibiotic or antifungal treatment within the past 6 months (n=7), incomplete clinical histories, CD4⁺ lymphocyte level count lower than 250 cells / ml and absence of viral load data (n = 5), concomitant systemic or infectious diseases (n = 3). As a result of the selection, 32 patients were included in the study protocol. All evaluated patients received their dental treatment and follow-up in CLAPAR I (Figs.1 and 2).

Based on the most severe gingival inflammatory signs, one site per quadrant was selected to be studied microbiologically (n=128 sites). Samples of subgingival biofilm were collected by absorption using 4 sterile N°35 paper points. They were placed one at a time into the depth of the pocket and removed after 20 seconds. The 4 paper points from each site were placed in 1ml of refrigerated transport fluid (RTF)²⁰. Smears were prepared with

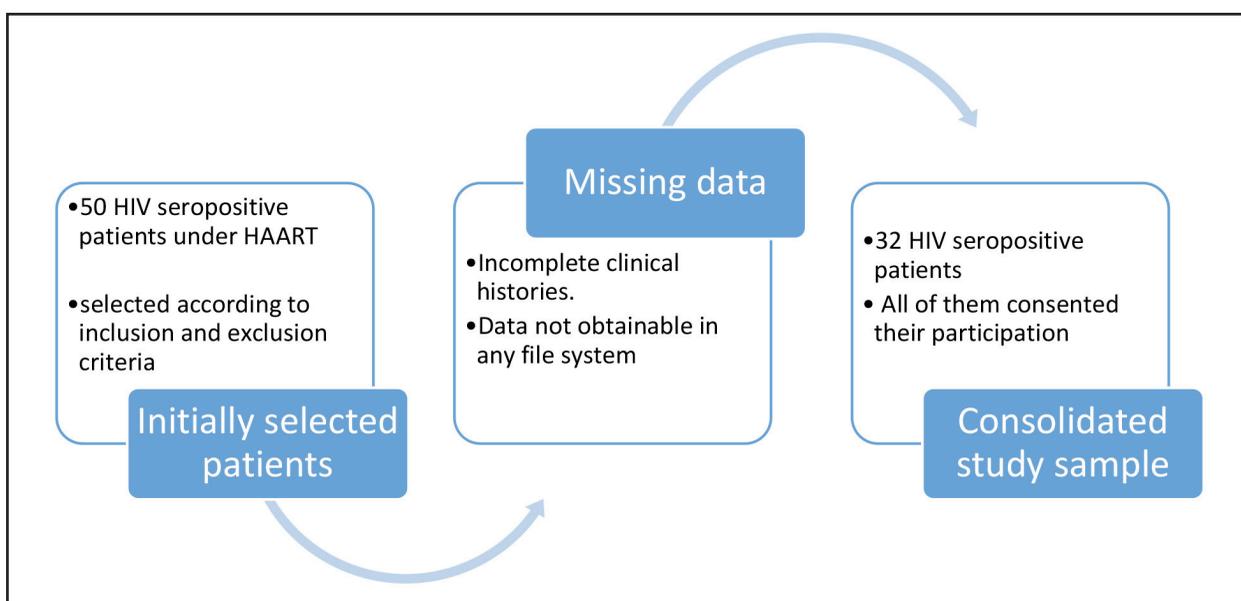


Fig. 1: Flowchart. Number of individuals at each stage of study.

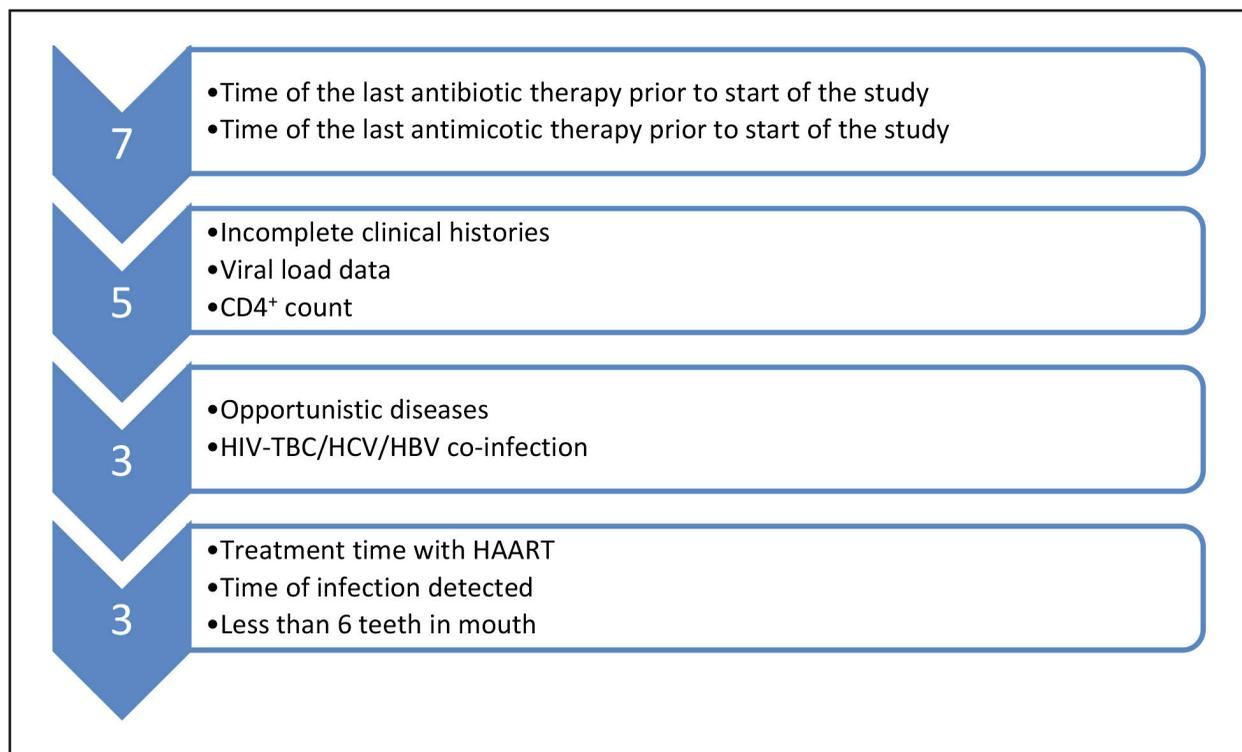


Fig. 2: Flowchart. Number of participants with missing data for each variable of interest.

the material obtained from the soft wall of each periodontal site.

For the microbiological assay, the periodontal pockets studied were assigned to four groups (Groups I to IV) according to their periodontal parameters such as bleeding on probing (BOP) and probing depth (PD). Group I: BOP/PD \geq 4mm n=21 (16.4 %); Group II: BOP/PD < 4mm n=30 (23.4%); Group III: NO BOP/PD \geq 4mm n=12 (9.4%); Group IV: NO BOP/PD < 4mm n=62 (49.6%). Details are provided in Table 3.

Microbiological Processing

To isolate strict anaerobic microorganisms, 100 μ l of the homogenized diluted (10^{-2} , 10^{-3}) samples were cultured in Anaerobic Blood Agar (ABA-BHI Agar) supplemented with 5% laked sheep blood (Laboratorio Gutiérrez, Argentina), 5 μ g/ml of hemin (Sigma-Aldrich[®], Argentina), and 1 μ g/ml of menadione (Sigma-Aldrich[®], Saint Louis, MO, USA). The plates were incubated under strict anaerobic conditions, and analysed at 7 and 15 days (GasPakTM, Mitsubishi, Medica-tec[®]). For detection of *Aggregatibacter actinomycetem comitans* (Aa), enteric bacteria, non-fermenting

gram negative bacilli (NFGNB), *Staphylococcus aureus* (Sa), coagulase-negative staphylococci (CoNS), and *Candida* spp, 100 μ l of the samples were cultured directly on selective and differential agar medium as follows: AASM²¹ for Aa, Levine (Oxoid[®], UK) and CLDE (Oxoid[®], UK) for enteric bacteria, bile esculin agar (Britania Laboratory, Argentina) supplemented with 0.04% potassium tellurite for enterococci, Chapman's mannitol salt agar (Oxoid[®], UK) for Sa, and CHROMagar[®] Candida for *Candida* spp. The plates were incubated under aerobic or capnophilic conditions according to bacterial atmospheric requirements, for 24h, 48h and 5 days. In all cases, the species were biotyped using conventional methods (Rosco[®] Diatabs) and a commercially available kit (Remel[®] enteric bacteria).

Molecular processing

For molecular processing, 200ul of each sample were stored at -20°C, and processed using a commercially available kit (Bioneer[®] Genbiotech) for extraction of genomic DNA. Seven "in house" end point polymerase chain reactions (PCR) were run for each sample (n=896), using specific primers

(Ashimoto et al. 1996) for detecting periodontopathic bacteria of the red complex (RCB) i.e. *Porphyromonas gingivalis* (*Pg*), *Treponema denticola* (*Td*), and *Tannerella forsythia* (*Tf*), and of the orange complex (OCB), i.e. *Fusobacterium nucleatum* (*Fn*), *Prevotella intermedia* (*Pi*) and *Aggregatibacter actinomycetem comitans* (*Aa*).

The amplification products were separated by electrophoresis in agarose gels (2%) in TAE buffer²², stained with Gold View (Montebio), and analysed using the Gel Doc™ XR+ Imaging System (Bio-rad). Negative and positive controls were used in all cases (*Porphyromonas gingivalis* ATCC 33277, *Prevotella intermedia* ATCC 25611, *Fusobacterium nucleatum* ATCC 10953, *Aggregatibacter actinomycetem comitans* ATCC 29522, *Tannerella forsythia* ATCC 43037), and DNA of wild-type strains, which were characterized, biotyped, and stored in the Collection of Microbial Cultures (Laboratory for Microbiological Diagnosis of the School of Dentistry, University of Buenos Aires, Argentina). Ubiquitous (Ubi) primers were also used to detect genomic bacterial DNA.

Statistical Analysis

Quantitative variables are expressed as average, standard deviation, median, and percentiles. Comparison of quantitative variables and factors or groups was performed using Student's t-test for independent samples. In all cases, homoscedasticity was evaluated using Levene's Test, and data normality was analysed using Kolmogorov Smirnov and Shapiro Wilks tests, as well as graphic normality tests. The data that did not meet parametric test assumptions were analysed using Kruskal Wallis test and post hoc Dunn-Bonferroni contrast test. Categorical variables were analysed

and compared using Chi-square test and Yates continuity corrections.

When distribution of data was binomial, percentages were compared using the proportions test (difference of percentages) and a normal approximation. Comparisons among more than two groups were performed applying a Bonferroni correction to the level of significance.

In all cases, the level of significance was set at 5% to rule out the null hypothesis. All statistical analyses were performed using statistical software SPSS (version 25), STATA 14, MS Excel 2016, Epidat 4.2.

RESULTS

The study comprised 32 patients (9 female and 23 male). All participants fulfilled the inclusion criteria, as shown in Flowcharts 1 and 2. Of the study patients, who were mostly Argentinians, 90.6% had less than 50 copies / mm³ viral load; data shown in Tables 1 and 2. The proportion of active periodontal sites and their distribution according to the analysis groups are shown in Table 3.

Isolates of conventional microorganisms were headed by *Candida* spp. (32.8%; *C. albicans*: 15.2%; *C. dubliniensis*: 16%) closely followed by Gram-negative bacilli (18.4%; NFGNB: 16%; enteric bacteria: 2.4%), *Staphylococcus* spp. 17.6% (*CoNS* 12.8%; *S. aureus*: 4.8%), and *Enterococcus faecalis* (0.8%) in the last position. Recovery rates of microorganisms using conventional cultures, and comparison of recovery rates among groups are shown in Table 4.

The mean anaerobic bacterial count (CFU) was 1.45 x 10⁶ CFU/ml, and there were significant differences in total counts of pigmented anaerobic and fusiform bacteria between Groups I and IV, as expected.

Table 1: Profile of HIV study population.

Population aspects		Frequency	Percentage
Sex	Female	9	28.1%
	Male	23	71.9%
Nationality	Argentine	30	93.7%
	Other	2	6.3%
Place of residence	Buenos Aires City	23	71.9%
	Buenos Aires Province	8	25.0%
	Other province in Argentina	1	3.1%

Conventional anaerobic culture and biotyping methods were unable to establish significant differences in the prevalence of *P.intermedia* and *P.gingivalis* in any of the study groups. Similarly,

different *Fusobacterium* species were observed but could not be identified at the species/subspecies level in any of the cases except as *F.nucleatum*.

Microbiological results for opportunistic bacteria such as *S.aureus* showed statistically significant differences in Group I versus the others ($p < 0.01$). Enteric bacteria were only isolated from Groups III and IV. No statistically significant difference was found for recovery rates for *E. faecalis*, NFGNB or total yeasts between the study groups.

Endpoint PCR results established statistically significant differences between groups according to periodontal clinical parameters (GI/GIV). *P.gingivalis*, *T.denticola* and *T.forsythia*, as perio-

Table 2: Relevant clinical data for study population health status.

Health status	Affected individuals (Total n=32)	Percentage
Initial CD4+ <350 cells/mm ³	12	37.5%
Initial CD4+ ≥350 cells/mm ³	20	62.5%
Initial VL < 50 copies/mm ³	29	90.6%

CD4+ (Lymphocytes cell cluster); VL (Virus load)

Table 3: Percentage of total study sites corresponding to each group.

Group	BOP	PD	Percentage of total sites corresponding to each group	
			Total sites included in periodontal study only (n=4765)	Total sites also studied microbiologically (n=125/4765)
I	Positive	≥ 4mm	3.7%	16.8%
II	Positive	< 4mm	3.3%	9.6%
III	Negative	≥ 4mm	14.1%	24.0%
IV	Negative	< 4mm	78.9%	49.6%

BOP: bleeding on probing; PD: probing depth.

Table 4: Microorganism recovery rates using conventional methods.

Microorganism	Group I %	Group II %	Group III %	Group IV %	p.
Total anaerobic bacteria	61.90	56.67	41.67	43.86	NS
Total pigmented bacteria	71.43*	53.33	25.00*	37.50	0.020
<i>Prevotella spp</i>	38.10	20.00	16.70	12.10	NS
<i>P. intermedia</i>	4.80	3.30	8.30	6.90	NS
<i>Porphyromonas spp</i>	19.00	13.30	0.00	5.20	NS
<i>P. gingivalis</i>	0.00	0.00	0.00	1.70	NS
<i>Fusobacterium spp</i>	71.40*	56.70	41.70	16.40*	0.001
<i>F. nucleatum</i>	9.50	16.70	25.00	10.90	NS
<i>Enterobacteria</i>	0.00	0.00	16.70*	1.60*	0.008
<i>E. faecalis</i>	0.00	3.30	0.00	0.00	NS
<i>S. aureus</i>	19.00*	0.00	0.00	3.20*	0.008
NFGNB	19.00	13.30	8.30	17.70	NS
Total yeasts	33.30	23.30	33.30	37.10	NS
<i>C. albicans</i>	31.60	11.50	8.30	15.00	NS
<i>C. dubliniensis</i>	5.30	11.50	16.70	23.30	NS

NS: No statistically significant difference

Kruskal Wallis test and post hoc Dunn-Bonferroni contrast test

Table 5: Percentage of microorganisms detected by PCR.

Microorganism	Group I %	Group II %	Group III %	Group IV %	p.
<i>A. actinomycetemcomitans</i>	4.80	6.70	0.00	1.60	NS
<i>P. intermedia</i>	0.00	3.30	0.00	4.80	NS
<i>P. gingivalis</i>	66.70*	30.00	41.70	21.00*	0.002
<i>T. denticola</i>	52.40*	40.00	33.30	19.40*	0.023
<i>T. forsythia</i>	66.70*	36.70	41.70	27.40*	0.015
<i>F. nucleatum</i>	52.40	63.30	50.00	33.90	NS

NS: No statistically significant difference
Kruskal Wallis test and post hoc Dunn-Bonferroni contrast test

odontopathic species, were the most prevalent (66.7% *Pg*, *Tf*; 52.4% *Td*) in the group of patients with active periodontal parameters (GI) (Table 5).

DISCUSSION

Studies reported in the literature have shown a similar composition of subgingival microbiota in seronegative and seropositive HIV patients^{23,24,15}. More recently, in 2019, a study on the subgingival periodontopathic microbiome showed that although *P.gingivalis* is the marker species of the “red complex”, *Treponema denticola* and *Tanerella forsythia* as well as *Filifactor alocis*, *Mitsuokella dentalis*, *Slackia exigua*, *Dialister pneumosintes*, *Selenomonas sputigena* and *Treponema lecithinolyticum* are among the ten best biomarkers for this pathology²⁵. However, other reports^{26,27} have found seropositive HIV patients to show a higher prevalence of putative periodontal pathogens such as *A. actinomycetemcomitans* spp., *P.intermedia* and *T.denticola*, as well as combinations of species. In addition, microbiological studies have reported isolation of species not usually associated with chronic periodontitis, including *Staphylococcus epidermidis*, *C.albicans*, *E.faecalis*, *Clostridium difficile*, *Clostridium clostridiforme*, *Klebsiella pneumoniae* and *Mycoplasma salivarius*, in subgingival biofilm samples from HIV-infected patients²⁸⁻³¹. Aas *et al.* studied the subgingival biofilm of seropositive patients with different immune status³². The authors found microorganisms such as *Saccharomyces cerevisiae* in subjects with higher viral load and low lymphocyte counts, lending support to the opportunistic nature of periodontal infections in these patients. Gonçalves *et al.* found higher prevalence of *Enterococcus*

faecalis and *Acinetobacter baumannii* in subgingival biofilm of HIV⁺ patients on HAART with CD4⁺ T-lymphocyte counts < 200 cells/mm³ and viral loads in the range of 105 copies/ml compared to seronegative patients, regardless of their periodontal status¹⁵. Conversely, recovery of opportunistic microorganisms was not significant in our study population, in which 90.6% of the participants showed undetectable viral loads, and 62.5% had CD4⁺ T lymphocyte counts above 350 cells / mm³. These discrepancies may be due to differences in the immune status of the study populations.

Gonçalves *et al.* observed a lower incidence of periodontal lesions in patients under HAART³³. Despite the lower frequency of clinical determinants of periodontal disease in our study population, more than 50% of the study sites in Group I were positive for periodontopathic bacteria belonging to Socransky’s red complex, which were detected using endpoint PCR³⁴. The primers designed by Ashimoto enable detection of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. denticola* and *T. forsythia* DNA in subgingival biofilm samples with high sensitivity and specificity (more than 99.9%). These primers have been used successfully by different authors³⁵ and therefore we consider them reliable for use in our study.

Brenchley *et al.* and Collman *et al.* posited that infections by Gram-negative bacteria and bacterial translocation associated with the mucosa in HIV-1-infected patients might directly or indirectly contribute to virus reactivation via pro-inflammatory cytokines released from stimulated cells^{36,37}. Periodontal disease, seen as a chronic polymicrobial, ecological and immune-inflammatory

disease, could play a role in viral reactivation in HIV seropositive patients³⁸.

Because HIV infection affects the host's immune response³⁹, gaining better understanding of the role of gingival-periodontal disease in immune activation in HIV seropositive patients would seem to be of the utmost importance for the development of therapeutic strategies aimed at controlling virus reactivation, patient immune status, and progression of AIDS.

Patients living with HIV / AIDS on HAART that were studied together in the High-Risk Patients Dental Care Unit (CLAPAR I) and the Microbiological Diagnostic Laboratory at School of Dentistry, University of Buenos Aires, Argentina showed low prevalence (1%) of periodontal lesions. Yeast and non-fermenting Gram-negative bacilli were distributed homogeneously, regardless of the depth of the periodontal pocket and in quantities that resemble a colonizing function.

Despite the low prevalence of periodontal disease and the moderate level of activity recorded in this

study, *P. gingivalis*, *T. denticola* and *T. forsythia* are within the framework of the microbiological profile of the subgingival biofilm of the eligible HIV-seropositive population. They were detected more frequently in periodontal active pockets (GI). As known, these pathogens and their products can contribute to systemic inflammation, so periodontal treatment could contribute to overcoming, and there by improving the general condition of seropositive HIV patients.

This is the first local study to record the prevalence of periodontopathic microorganisms in periodontal pockets of HIV patients. The results justify further in-depth longitudinal studies of the subgingival microbiome of local patients living with HIV / AIDS who are on HAART, which might clarify many of the interactions of these and other still unknown periodontal bacteria, describe new pathogenicity factors and contribute to understanding the particular dynamics of these subgingival microbiomes and HIV-1 virus homeostasis.

FUNDING

This work was partly supported by the Research Support Programme for Universidad de Buenos Aires UBACyT 20720120100008BA and UBACyT 20020120100324BA.

CORRESPONDENCE

Dra. Laura A. Gliosca
Cátedra de Microbiología, Facultad de Odontología, UBA.
Marcelo T de Alvear 2142 CABA, C1122AAH,
Buenos Aires, Argentina.
lgliosca@yahoo.com.ar

REFERENCES

1. WHO, UNICEF, UNAIDS. Progress report 2011: Global HIV/AIDS response. World Health Organization. 2011. URL: www.who.int/hiv/pub/progress_report2011/en/index.htm
2. National Ministry of Health, AIDS and STD Office. 2016. Bulletin on HIV-AIDS in Argentina. Year XIX. Issue 33. URL: http://www.msal.gob.ar/images/stories/bes/graficos/0000000918cnt-2016-11-30_boletin-vih-sida-2016.pdf
3. Holtzer CD, Jacobson MA, Hadley WK, Huang L, et al. Decline in the rate of specific opportunistic infections at San Francisco General Hospital, 1994-1997. *AIDS* 1998; 12: 1931-1933.
4. Dybul M, Fauci AS, Bartlett JG, Kaplan JE, et al. Guidelines for using antiretroviral agents among HIV-infected adults and adolescents: The Panel on Clinical Practices for Treatment of HIV. *Ann Intern Med* 2002; 137:381-433.
5. Lange C., Lederman M.. Immune reconstitution with antiretroviral therapies in chronic HIV-1 infection. *J Antimicrob Chemother* 2003; 51:1-4.
6. UNAIDS and World Health Organization. 2008. Report on the global HIV/AIDS epidemic 2008: executive summary, UNAIDS: Geneva. URL: https://www.unaids.org/sites/default/files/media_asset/jc1510_2008globalreport_en_0.pdf
7. WHO. 2013. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. Geneva. URL: <https://www.who.int/hiv/pub/guidelines/arv2013/en/>
8. Sungkanuparph S, Vibhagool A, Mootsikapun P, Chetchotisakd P. Opportunistic infections after the initiation of highly active antiretroviral therapy in advanced AIDS patients in an area with a high prevalence of tuberculosis. *AIDS* 2003; 17:2129-2131.
9. Imai K, Ochiai K, Okamoto T. Reactivation of latent HIV-1 infection by the periodontopathic bacterium *Porphyromonas gingivalis* involves histone modification. *J Immunol* 2009; 182:3688-3695.
10. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005; 366:1809-1820.
11. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. *Nat Rev Dis Primers* 2017; 22;3:17038. doi: 10.1038/nrdp.2017.38.
12. Jenkins WM, Papapanou PN.. Epidemiology of periodontal disease in children and adolescents. *Periodontol* 2000 2001; 26: 16-32.

13. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B et al. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. *J Dent Res* 2004; 93:1045-1053.
14. Holtfreter B, Albandar JM, Dietrich T, Dye BA et al. Standards for reporting chronic periodontitis prevalence and severity in epidemiologic studies: proposed standards from the Joint EU/USA Periodontal Epidemiology Working Group. *J. Clin. Periodontol* 2015; 42: 407-412.
15. Gonçalves LS, Soares Ferreira SM, Souza CO, Souto R et al. Clinical and microbiological profiles of human immunodeficiency virus (HIV) seropositive Brazilians undergoing highly active antiretroviral therapy and HIV seronegative Brazilians with chronic periodontitis. *J Periodontol* 2007; 78:87-96.
16. Vernon LT, Demko CA, Whalen CC, Lederman MM, et al. Characterizing traditionally defined periodontal disease in HIV⁺ adults. *Community Dent Oral Epidemiol* 2009; 37:427-437.
17. Huang CB, Emerson KA, Gonzalez OA, Ebersole JL. Oral bacteria induce a differential activation of human immunodeficiency virus-1 promoter in T cells, macrophages and dendritic cells. *Oral Microbiol Immunol* 2009; 24:401-407.
18. González OA, Ebersole JL, Huang CB.. Supernatants from oral epithelial cells and gingival fibroblasts modulate human immunodeficiency virus type 1 promoter activation induced by periodontopathogens in monocytes/macrophages. *Mol Oral Microbiol* 2010; 25:136-149.
19. Pólvara TLS, Nobre ÁVV, Tirapelli C, Taba M, et al. Relationship between human immunodeficiency virus (HIV-1) infection and chronic periodontitis. *Expert Rev Clin Immunol* 2018; 14:315-327
20. Syed SA and Loesche WJ. Survival of human dental plaque flora in various transport media. *Appl Microbiol* 1972;24: 638-644.
21. Tsuzukibashi O, Takada K, Saito M, Kimura C, et al A novel selective medium for isolation of *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*. *J Periodontal Res* 2008; 43:544-548.
22. Green MR, Sambrook J, Sambrook J. Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N. Y. 2012.
23. Brady LJ, Walker C, Oxford GE, Stewart C et al. Oral diseases, mycology and periodontal microbiology of HIV-1-infected women. *Oral Microbiol Immunol* 1996; 11: 371-380.
24. Tsang CS and Samaranayake LP. Predominant cultivable subgingival microbiota of healthy and HIV-infected ethnic Chinese. *APMIS* 2001; 109:117-126.
25. Schulz S, Porsch M, Grosse I, Hoffmann K, et al. Comparison of the oral microbiome of patients with generalized aggressive periodontitis and periodontitis-free subjects. *Arch Oral Biol* Mar 2019; 99:169-176.
26. Cross DL and Smith GL. Comparison of periodontal disease in HIV seropositive subjects and controls (II). Microbiology, immunology and predictors of disease progression. *J Clin Periodontol* 1995; 22:569-577.
27. Patel M, Coogan M, Galpin JS. Periodontal pathogens in subgingival plaque of HIV-positive subjects with chronic periodontitis. *Oral Microbiol Immunol* 2003; 18:199-201.
28. Zambon JJ, Reynolds HS, Genco RJ. Studies of the subgingival microflora in patients with acquired immunodeficiency syndrome. *J Periodontol* 1990; 61:699-704.
29. Moore LV, Moore WE, Riley C, Brooks CN, et al. Periodontal microflora of HIV positive subjects with gingivitis or adult periodontitis. *J Periodontol* 1993; 64:48-56.
30. Jabra-Rizk MA, Ferreira SM, Sabet M, Falkler WA, et al. Recovery of *Candida dubliniensis* and other yeasts from human immunodeficiency virus-associated periodontal lesions 2. *J Clin Microbiol* 2001; 39:4520-4522.
31. Gonçalves LS, Ferreira SM, Silva A Jr., Villoria GE, et al. Association of T CD4 lymphocyte levels and chronic periodontitis in HIV-infected Brazilian patients undergoing highly active anti-retroviral therapy: clinical results. *J Periodontol* 2005; 76:915-922.
32. Aas JA, Barbuto SM, Alpagot T, Olsen I et al. Subgingival plaque microbiota in HIV positive patients. *J Clin Periodontol* 2007; 34:189-195.
33. Gonçalves LS, Gonçalves BM, Fontes TV. Periodontal disease in HIV-infected adults in the HAART era: Clinical, immunological, and microbiological aspects. *Arch Oral Biol* 2013; 58:1385-1396.
34. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol* 1996; 11:266-273.
35. Herrera D, Contreras A, Gamonal J, Oteo A et al. Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain. *J Clin Periodontol* 2008; 35:106-113.
36. Brenchley JM, Price DA, Schacker TW, Asher TE, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006; 12: 1365-1371.
37. Collman RG, Perno CF, Crowe SM, Stevenson M et al. HIV and cells of macrophage/dendritic lineage and other non-T cell reservoirs: new answers yield new questions. *J Leukoc Biol* 2003; 74:631-634.
38. Huang CB, Alimova YV, Ebersole JL. HIV-1 reactivation in HIV-latently infected dendritic cells by oral microorganisms and LPS. *J Leukoc Biol* 2003; 74:631-634.
39. Cadogan M, Dalgleish AG. Pathogenesis of HIV: non-specific immune hyperactivity and its implications for vaccines. *Clin Med (Lond)* 2008; 8:267-271.