

## Analysis of *IL-10* in HIV-1 patients with chronic periodontitis in northern Brazil

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**Aim:** The objective of this study was to investigate the levels of IL-10 in the gingival crevicular fluid in HIV-1 positive patients with chronic periodontitis and to compare with HIV-1 negative patients with chronic periodontitis, also to correlate clinical periodontal parameters, viral load and count of CD<sub>4</sub><sup>+</sup> and CD<sub>8</sub><sup>+</sup> lymphocytes (LTCD<sub>4</sub><sup>+</sup> and LTCD<sub>8</sub><sup>+</sup>). **Methods:** 33 patients were selected and splitted into two groups: 16 HIV-1 positive patients and 17 HIV-1 negative patients and all with chronic periodontitis. The clinical periodontal parameters recorded were: Probing Depth (PD) and Clinical Attachment Level (CAL); the sistematical parameters LTCD<sub>4</sub><sup>+</sup>, LTCD<sub>8</sub><sup>+</sup> and viral load were analyzed by the gingival crevicular fluid collected from all patients. Enzyme-linked immunosorbent assay (ELISA) was used to determine the concentrations of Interleukin (IL)-10. For the statistical analysis the Student t, Mann-Whitney and Spearman tests were performed. IL-10 levels were significantly lower in both patients groups. **Results:** There was statistical difference between groups for probing depth (p=0.015) and clinical attachment level (p=0.011), no significant correlation was found among the analyzed variables. **Conclusion:** The IL-10 levels in HIV-1 positive patients had no influence in periodontal and medical parameters.

**Keywords:** Interleukin-10. HIV and Chronic Periodontitis.

## Introduction

Acquired Immuno Deficiency Syndrome (AIDS) is characterized by an advanced state of immunodepression, is still considered a serious global public health problem, estimated at around 34 million people infected with HIV worldwide<sup>1</sup>. Despite the reduction in the morbidity and mortality of patients who use antiretroviral therapy (ART), there are still a large number of individuals carrying the Human Immunodeficiency Virus 1 (HIV-1)<sup>2,3</sup>.

The HIV-1 infects  $\text{TCD}_4^+$  lymphocytes ( $\text{LTCD}_4^+$ ), also known as lymphocyte T helper (LTh) and the decrease in the number of these cells may contribute to the appearance of several opportunistic infections and several pathologies, such as periodontal disease<sup>4</sup>. According to Chin<sup>5</sup> (2017), immunodeficiency caused by HIV-1 may have a direct influence on the pathology of periodontal disease.

The periodontal manifestations are recognized as an important characteristic and are widely associated with the immunity caused by the virus, and can be considered one of the first clinical signs of HIV infection, which can be mitigated by the use of ART<sup>6</sup>. According to Elizondo et al.<sup>7</sup> (2017) the presence of gingivitis and the severity of periodontitis, is indicated by the bone loss and increased PD, is directly related to HIV infection.

Periodontitis, when installed, has in its gingival crevicular fluid (GFC) several proteins linked to the inflammatory process, such as cytokines, which may be beneficial to diagnose the current status of the periodontium, as well as the effects of periodontal therapy<sup>8</sup>.

Among the cytokines present in the GFC, there is the presence of Interleukin (IL) 10. *IL-10* is an important cytokine suppressor of inflammatory activity, modulating the production and secretion of other cytokines. The LTh 2 activated secretes *IL-10* that act inhibiting the cytotoxic  $\text{TCD}_8^+$  lymphocyte ( $\text{LTCD}_8^+$ ), causing immunosuppression of the response. It also inhibits the production of interferon (IFN) by T lymphocytes, co-stimulates the proliferation and differentiation of B lymphocytes and suppresses the production and secretion of pro-inflammatory cytokines, constituting an important suppressor of cellular immunity<sup>8,9</sup>.

The susceptibility and extension of tissue destruction seem to be determined by the complex cytokine balance produced by the presence of numerous associations between periodontal microorganisms. When the host's response is exacerbated, it can lead to tissue damage, causing loss of periodontal support. The study of inflammatory mediators associated with periodontal disease, by immunological or biochemical methods, allows the evaluation of the host's response to this disease<sup>10,11</sup>.

So, the knowledge of the cytokines involved in the progression or not of periodontal disease, especially in HIV-infected individuals, is of fundamental importance for a better therapeutic behavior and, consequently, the quality of life of these patients<sup>11</sup>. In order to contribute to the characterization of the cytokine profile presented by patients with HIV-1 with chronic periodontitis, the objective of this study was to investigate the levels of IL-10 in the gingival crevicular fluid in HIV-1 positive patients with chronic periodontitis and to compare with HIV-1 negative patients with chronic periodontitis, also to correlate clinical periodontal parameters, viral load and count of  $\text{CD}_4^+$  and  $\text{CD}_8^+$  lymphocytes.

## Material and Methods

### Study population

The sample size was calculated according to the PD (mean and standard deviation) of both groups by Student's Sample T-test. The level of significance was 5%, with an effect of 0.80. Considering a statistical power of 95%, the sample size was fixed in 16 patients per group. Thirty-three individuals with chronic periodontitis, aged between 34 and 60 years were selected. Of these, 16 patients were HIV-1 positive and used ART regularly, all the patients selected used the same drug at the same frequency. The other 17 patients have no medical conditions. All subjects presented at least 20 teeth and were without periodontal therapy for about 1 year. The sites had a PD greater than or equal to 5 mm and radiographically must presented a great bone destruction. The teeth selected for the collection of GFC were all natural, intact, without prosthesis and did not present any dysfunction in relation to the occlusion<sup>12</sup>.

The periodontitis was diagnosed based on the study of Armitage<sup>13</sup> (1996). The samples were collected by a single Calibrated Researcher (C.R), who had previously experience 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 parameters were assessed: CAL and PD. The exams were carried out randomly only in upper posterior molars belonging to two different quadrants, the Williams periodontal probe was introduced gently in each of the sites. The collection sites were chosen by C.R based on analysis of periodontal parameters and presence of periodontal pocket  $\geq 5$  mm.

To confirm that the individuals in the control group were HIV-1 negative, blood samples were collected and they were sent to the clinical analysis laboratory for diagnostic purposes. An ELISA type immunoenzyme assay were performed (*DiaSorin, anti-HIV tetra Elisa, Biotest, Germany*), which includes a recombinant antigens, one of the envelope and two antigens of the viral capsid<sup>7</sup>.

In the probability of a HIV-1 positive sample, the blood samples would be submitted to serological screening for anti-HIV antibodies with the microparticle immunoenzymatic method (*Axsym-System-Abbott, Germany*), followed by indirect immunofluorescence confirmation.

Were excluded from this study: pregnant, lactating women, diabetic, smokers, patients on systemic or local antimicrobial therapy, hormone therapy or any other analgesic and anti-inflammatory drug with at least a 30-day interval until sample collection<sup>12</sup>.

### Collect of samples

#### *Clinical measurement*

Clinical parameters were evaluated in all teeth, excluding third molars, and included the following: PD and CAL. Six sites were examined for each tooth: mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual. One calibrated examiner 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.

### ***Collection of Gingival Crevicular Fluid (GCF)***

GCF samples were obtained from 2 sites in the periodontally affected sites at the mesio-buccal gingival sulci at 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. GCF 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 30 seconds. Strips contaminated by saliva or blood were excluded from the sampled group. 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<sup>12</sup>.

### ***ELISA test for quantification of IL-10***

The concentration of the immunoinflammatory mediator present in the GFC was evaluated by the enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions (eBioscience, 10240 Science Center, San Diego, CA, USA). All experiments were performed in duplicates for each biological triplicate.

### ***Lymphocyte count and viral load***

The grade of impairment of the immune system was assessed through CD<sub>4</sub><sup>+</sup> and CD<sub>8</sub><sup>+</sup> levels, as well as the viral load present in the medical records of HIV-1 patients involved in the research. The exams were mandatory, at least 30 days before the samples were collected.

The counts were recorded in patient medical records, individually, to relate with the IL-10 found in GFC, as well as the level of clinical impairment of the periodontium.

### ***Quantification of TCD<sub>4</sub><sup>+</sup> and TCD<sub>8</sub><sup>+</sup> lymphocytes***

The blood samples from HIV-1 subjects were quantified by T-lymphocyte count using the flow cytometry technique (*FacsCalibur*, Becton & Dickinson, USA), using the BD Trucount TMTubes and BD multitest kit according the standard protocol recommended by the manufacturer (*Becton Dickinson*, USA) and used in the National Network for Quantification of TCD<sub>4</sub><sup>+</sup> and TCD<sub>8</sub><sup>+</sup> lymphocytes<sup>12</sup>.

### ***Quantification of HIV-1 plasmatic viral load***

The plasma viral load in HIV-1 patients was determined by the branched DNA (*bDNA*) method using the *Versant® HIV-1 RNA 3.0 Assay bDNA* kit (*Bayer Corporation*, Massachusetts, USA), using the *System 340 bDNA Analyzer* (*Siemens*, Deerfield, USA).

### ***Statistical analysis***

The data on IL-10 level, serological indicators and clinical parameters of chronic periodontitis were submitted to descriptive and inferential analyzes. To determine the proportion between patients of the different gender that composed the two groups, HIV-1 positive and negative, Chi-square test was used. In order to verify any age difference between the patients of both groups, the T Student test was used. To compare the clinical parameters of chronic periodontitis (CAL and PD) and the level of IL-10,

between patients HIV-1 positive and negative, T Student or Mann-Whitney tests were applied. Spearman's tests were used to verify correlations between clinical parameters of periodontitis and levels of interleukin. These tests were also used to assess the correlation between viral load and serologic indicators (LTCD<sub>4</sub><sup>+</sup> and LTCD<sub>8</sub><sup>+</sup>) with IL-10 levels. The significance level adopted was 5%, and statistical calculations were conducted in the SPSS 20 program (SPSS Inc., Chicago, IL, USA).

## Results

Of the 33 patients, the number of HIV-1 carriers were 9 males (58.3%) and 7 females (41.6%), with the average of 47.1 ( $\pm$  6.7) years. The HIV-1 negative patients, 9 belonged to the male gender (52.9%) and 8 to the female (47.1%), with the average of 47.0 ( $\pm$  5.4) years. Chi-square test showed no significant difference between the groups regarding the proportion of male and female participants ( $p = 1,000$ ). The T Student test indicated that there was no significant difference in age between the patients belonging to the groups of HIV-1 carriers and HIV-1 non-carriers ( $p = 0.971$ ).

T Student test showed that the CAL ( $p=0.011$ ) and PD ( $p=0.015$ ) were significantly different and higher in patients with HIV-1 positive. Mann-Whitney test revealed that the level of IL-10 did not present significant difference between the two studied groups, as demonstrated in table 1. No significant correlation was found between IL-10 and CAL in HIV-1 patients group and control group. IL-10 levels also did not correlate with PD in both groups. The amount of LTCD<sub>4</sub><sup>+</sup> showed no significant correlation with the level of IL-10. As for LTCD<sub>8</sub><sup>+</sup>, its quantity had no correlation with the cytokine concentration studied. Viral load did not correlate with IL-10 level. These results are demonstrated in table 2.

**Table 1.** Mean values and standard deviation of cytokine and clinical parameters of chronic periodontitis among HIV-1 seropositive and seronegative patients.

Parameters			p value
	HIV <sup>+</sup>	HIV <sup>-</sup>	
Clinical level of insertion (mm)	7.1 (0.7)	6.4 (0.8)	$p = 0.011^*$
Probing depth (mm)	6.6 (0.7)	5.9 (0.7)	$p = 0.015^*$
IL-10 (pg/mL)	1.23 (1.97)	0.60 (0.07)	$p = 0.452^{**}$

\* Values of p based on T-Student test.

\*\* Values of p based on Mann-Whitney test

\*\*\* Significant at the 5% level.

**Table 2.** Results of the Spearman correlation test between IL-10 and the clinical parameters of chronic periodontitis, serology and viral load in HIV-1 and non-HIV-1 carriers.

	Parameters				
	Clinical level of insertion	Probing depth	LTCD <sub>4</sub> <sup>+</sup>	LTCD <sub>8</sub> <sup>+</sup>	Viral load
HIV <sup>+</sup>	$p = 0.794$	$p = 0.514$	$p = 0.569$	$p = 0.629$	$p = 0.894$
HIV <sup>-</sup>	$p = 1.000$	$p = 1.000$	-	-	-

\* Significant at the 5% level.

## Discussion

Usually seropositive patients presents a more severe periodontitis. This sistemical condition enhance the CAL, the PD, gingival recession leading to a tooth loss. Due to patient's immunosupression, a increased viral load and a diffused invasion into the gingival tissue of opportunistic bacteria, fungi and viruses, present in the oral cavity cause a greater inflammatory response in tissues and a considerable bone destruction<sup>13</sup>.

In seronegative patients when periodontitis is installed, in the GCF several cytokines linked to the inflammatory process, such as IL-10, these cytokines act in immunological activities and in pro-inflammatory and anti-inflammatory processes in periodontal tissues to protect against microbial activity<sup>14</sup>.

However, in seropositive individuals, these immunological reactions are weakened leading to a greater severity and progression of periodontitis, as the progression of the disease is determined by factors linked to the host's immune response and the virulence of the bacteria. For this reason, biological characteristics such as cytokine profile have been studied in an attempt to better assess the behavior of the immune system in seropositive patients<sup>15</sup>.

Interleukin-10 is an anti-inflammatory cytokine that inhibits the production of proinflammatory cytokines<sup>15</sup>. The *IL-10* can attenuate the inflammatory response and exert an immunosuppressive and immunostimulatory effect, operating on a great variety of cellular types<sup>16</sup>. As a regulator of the cell-mediated immune response, *IL-10* can generally suppress the production of proinflammatory cytokines and chemokines. *IL-10* induce the proliferation of B lymphocytes and the proliferation and activation of natural killer cells<sup>16,17</sup>.

Based on its biological activities, it is evident that the reduction of *IL-10* production is associated, with higher susceptibility, to any type of infectious disease<sup>15</sup>. For this reason, some studies have analyzed the participation of this cytokine in infectious processes, such as periodontal diseases and peri-implants<sup>16,17</sup>. Periodontitis is probably the most common chronic disease in adults. Several studies have shown that the profile of locally produced cytokines may be relevant for periodontal destruction<sup>18</sup>.

In the study, the CAL as well as the PD were significantly higher in HIV-1 patients than HIV-1 seronegative patients, which may demonstrate the interference of HIV infection in the natural history of periodontal disease<sup>10,18</sup>. However, this relationship was not always found<sup>19</sup>.

The present study used traditional periodontal clinical parameters to determine the level of *IL-10* in seronegative HIV-1 patients with chronic periodontitis. The clinical parameters chosen for the analysis consider the reality of public or private clinics, where simple and rapid methods are used for clinical follow-up. The *IL-10* evaluated in the present study, did not present significant differences within the studied groups in relation to the clinical parameters<sup>15-20</sup>.

*IL-10* suppresses the immune response and, thus, modulates the production of other cytokines and, according to Ujiie et al.<sup>21</sup> (2016) and Jaradat et al.<sup>22</sup> (2012), there is an increase in the levels of *IL-10* in the presence of HIV infection. In contrast, Teles et al.<sup>23</sup> (2009) did not obtain relevant levels of *IL-10*, which agrees with the present research that obtained low levels of IL-10 in both HIV-1 and non-HIV-1 carriers.

In this study, *IL-10* showed low levels in both HIV-1 and non-carrier patients. These lower levels of *IL-10* are characteristic of the decline in Th2 response, were also observed by Silveira et al.<sup>17</sup> (2016), Cullinan et al.<sup>24</sup> (2008) and Lappin et al.<sup>25</sup> (2001), which may suggest a deficiency in the control of the immune response directed against pathogens, resulting in an exacerbated inflammation.

According to Silveira et al.<sup>17</sup> (2016), the chemoattraction characteristics of *IL-10* producing cells could control the destructive potential of the disease. As *IL-10* is associated with suppression of bone resorption, the low expression in patients with chronic periodontitis would be related to the more severe form of the disease.

The *IL-10* low levels were found in both groups. The studied cytokine is involved in the regulation of inflammatory responses, it is suggested that the low levels of this molecule, observed among patients with chronic periodontitis, contribute to the development of the disease. Also as a result, *IL-10* showed no significant difference with viral load<sup>23,24</sup>.

The analysis of the cytokine profile in chronic periodontitis does not aim to replace the routinely used clinical exams, but could complement the clinical evaluation, however would assist the diagnosis and possible therapeutic interventions. Thus, clinical data related to cytokine levels in the gingival crevicular fluid may elucidate the pattern of local immune response, supporting a better understanding of the pathogenesis of periodontitis, especially in individuals with other chronic infections, such as that caused by HIV-1<sup>24-26</sup>.

The periodontal disease being proven to be more severe in seropositive patients with periodontitis, the *IL-10* levels were expected to be lower because it is a cytokine with innate and immune antiinflammatory activity. However, the results in the present manuscript conclude that this cytokine does not interfere in the severity of periodontal disease in these immunodepressed patients. The *IL-10* levels in the gingival crevicular fluid had no influence on the periodontal clinical parameters of seropositive patients, according to our results we could not find correlations between lymphocyte serum levels, viral load, and this Interleukin.

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