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Communication

Cytokines and MMPs levels in gingival crevicular fluid from patients with chronic periodontitis before and after non-surgical periodontal therapy.

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Abstract: The study of host response in periodontal disease may provide a mechanism to monitor disease progression. The purpose of the present research was to determine the levels of IL-1α, IL-1β, TNF-α, IL-6, IL-6sR, IL-8, IL-10, MMP-3 and MMP-8 in gingival crevicular fluid (GCF), before and after non-surgical periodontal treatment (NSPT) in order to evaluate therapy response. Methodology: Eleven patients diagnosed with chronic periodontitis and eleven healthy subjects were selected for this study. Clinical measurements, including probing depth (PD) and clinical attachment loss (CAL) were carried out in patients diagnosed with chronic periodontitis and periodontal healthy controls. The clinical indexes evaluated were: gingival index (GI) and plaque index (PI). Samples of GCF were taken from one tooth per quadrant before and 45 days after NSPT. The levels of inflammatory mediators were measured by ELISA. Results: The values of all clinical parameters decreased significantly after treatment. The concentration levels of all cytokines and MMP-3 and MMP-8 in the GCF sample were higher in patients diagnosed with chronic periodontitis compared to the healthy group. All inflammatory mediators decreased after therapy, but did not reach control values; IL-6, Il-6sR, IL-10 and TNF-α, attained the highest reduction (70% - 54%); the vales of MMP3, IL-1α, IL-1β and IL-8 were reduced between 50% - 34%; and MMP-8 showed the lowest decrease (28%). Conclusion: All clinical parameters and cytokines levels decreased after NSPT. The mediators TNF-α IL-6, IL-6sR, and IL-10 showed the largest variation between before and after NSPT and could thus be used to evaluate therapy response.

Keywords: cytokine; metalloproteinases; periodontitis; gingival crevicular fluid.

INTRODUCTION.

Periodontitis is characterized by chronic infection, inflammation, and destruction of supporting periodontal tissues.[1] Disease manifestation results from the interaction between host defense mechanisms, microbial agents, and environmental and genetic factors.[2] The host response to pathogens results in the production of inflammatory mediators by cells like neutrophils, macrophages, T-cells, mast cells and fibroblast.

Pro-inflammatory cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor (TNF-α) and prostaglandin E2 (PGE2) can promote extracellular matrix destruction by matrix metalloproteinases (MMPs) in the periodontium and stimulate bone resorption.[3]

Increased levels of several cytokines, including interleukin IL-1, IL-2, IL-6, IL-8 and TNF-α have been observed in GCF from patients with periodontal disease. Considerable interest has been focused on the potential of these mediators as diagnostic or prognostic markers for

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periodontal disease activity and wound healing.[1]

The pro-inflammatory cytokine IL-1 plays a central role in immune regulation and in a variety of inflammatory responses as modulator of extracellular matrix and bone.[4] The multifunctional cytokine IL-6 has many biological activities, including B-lymphocyte differentiation, T-lymphocyte proliferation and stimulation of immunoglobulin (Ig) secretion by B-lymphocytes. IL-6 induces bone resorption by itself and in conjunction with other bone-resorbing agents and acts synergistically with IL-1α. IL-8, formerly known as neutrophil-activating peptide-1 (NAP-1), is important for the initiation and development of inflammatory processes through its capacity to attract and activate neutrophils.[5] TNF-α, secreted predominantly by monocytes and macrophages, is a potent inflammatory cytokine that up-regulates the production of collagenases, PGE_2, chemokines and cytokines, cell adhesion molecules, bone resorption-related factors[5] and induces/up-regulates MMP-8 expression via gingival fibroblasts.[1]

The pro-inflammatory cytokines stimulate host cells to produce several matrix metalloproteinases (MMPs), facilitate leucocyte recruitment, cytokine and chemokine processing, and matrix remodeling. MMP-8, a collagenase, and MMP-3 or stromelysin-1, have been associated with periodontal disease. MMP-3 has the capacity to activate pro-MMP-1, pro-MMP-8, and pro-MMP-9 in activation cascades. Several studies have shown the potential utility of MMP-8 as a marker for periodontal treatment effectiveness and to identify patients at risk of progressing attachment loss.[1]

Chronic periodontal disease can be successfully treated by non-surgical or surgical mechanical therapy to reduce tissue inflammation; the evaluation of cytokines levels before and after periodontal treatment may provide a mechanism to monitor host response in periodontal disease and evaluate treatment outcome.

The aim of this study is to determine the levels of IL-1α, IL-1β, TNF-α, IL-6, IL-6sR, IL-8, IL-10, MMP-3, MMP-8 in GCF, and clinical parameters before and after NSPT, to evaluate therapy response and to compare results with a group of healthy subjects.

MATERIALS AND METHODS.

Design and subjects

A prospective cohort study was carried out. Eleven patients diagnosed with chronic periodontitis and eleven healthy subjects, with a mean age of 41±15 and 40±11 respectively, were selected in this study.

Patients attended the Periodontics Postgraduate Clinic at the School of Dentistry, Universidad Central de Venezuela, from January to December 2014. Periodontal diagnosis was established following the clinical and radiographic criteria defined by the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions[6] under the following inclusion criteria: diagnosis of chronic periodontitis from moderate to severe, with depths to probing ≥4mm and insertion loss ≥5mm in more than 8 teeth, regardless of age or gender; no history of systemic diseases or intake of drugs 6 months prior to the study. Pregnant or lactating women were excluded. Individuals in the control group were systemically and periodontally healthy.

Bioethical considerations

All patients signed an informed consent form. The study was approved by the Bioethics Committee of Universidad Central de Venezuela (UCV) no. 006-2007, which was supported by the Council of Scientific and Humanistic Development of the Central University of Venezuela, Grant no. 10-00-7070-2007.

Data collection

Clinical measurements, including probing depth (PD), clinical attachment loss (CAL), gingival index (GI) and plaque index (PI), were carried out in patients diagnosed with chronic periodontitis (9 females and 2 males) and periodontally healthy controls (8 females and 3 males). The clinical indexes were evaluated by one calibrated examiner. The measurements were taken at six sites per tooth, excluding third molars in each subject.

Samples of GCF were taken from one tooth per quadrant before and 45 days after the NSPT. The levels of inflammatory mediators were measured by ELISA. The teeth were gently washed with water, and the sites under study were isolated with cotton rolls and dried out with an air syringe. Four paper points were carefully inserted 1mm into the gingival crevice of each tooth selected for sampling and allowed to remain there for 30 seconds. Precautions were taken to avoid mechanical injury. Paper points from individual sites were stored at –30ºC until further processing.

The GCF absorbed by each paper point was eluted with 100µL of buffer A (12mM Tris-Hcl pH 7.2, 0.1 M NaCl and 0.05% Tween 20), extracted by vortexing for 30 seconds,

repeating the procedure three times. The paper points were then removed, and the eluted sample was centrifuged for 5 min at 3000*g*; the supernatants were collected and stored at -30ºC for use in the subsequent assay.

The presence and levels of inflammatory mediators in GCF were measured using a commercial ELISA kit (Quantikine, R&D Systems Inc., Minneapolis, Minnesota, USA) following the manufacturer's specifications.

Statistical analysis

Mean and standard deviation were calculated. Data normality was confirmed by Shapiro-Wilks test.

Data was analyzed using t-student test to compare study and control groups; Pearson's correlation coefficient was used to establish relationships among clinical parameters and mediator levels in GCF. Statistical significance was set at $p<0.05$.

Table 1. Clinical parameters in patients with chronic periodontitis, before and after undergoing non-surgical periodontal treatment, compared to healthy control subjects.

Clinical parameters	CPG-BT±SD (pg/ml)	CPG-AT±SD (pg/ml)	*p-value	CG±SD (pg/ml)	**p-value	***p-value
PI	1.72 ± 0.5	0.57 ± 0.2	0.00	0.63 ± 0.5	0.00	0.47
GI	2.14 ± 0.5	0.47 ± 0.3	0.00	0.46 ± 0.4	0.00	0.84
PD (mm)	4.39 ± 0.8	3.19 ± 0.5	0.00	1.89 ± 0.4	0.00	0.00
CAL (mm)	5.18 ± 1.2	4.47 ± 1.6	0.02	1.9 ± 0.5	0.00	0.00
BOP	0.65 ± 0.4	0.43 ± 0.3	0.00	0.03 ± 0.1	0.00	0.00

Data are presented as the **mean±standard deviation**=SD. **n**=11.
CPG=Chronic Periodontitis Group. **CG**= Control Group. **BT**= Before Treatment. **AT**= After Treatment; PI= Plaque index. **GI**= Gingival Index. **PD**= Pocket depth; **CAL**= Clinical Attachment Loss. **BOP**=Bleeding on probing. *Comparison between CPG before and after treatment. ** Comparison between CPG-BT and CG. ***Comparison between CPG-AT and CG.

Table 2. Levels of cytokines, MMP-3 and MMP-8 in gingival crevicular fluid in patients with chronic periodontitis before and after non-surgical treatment compared to healthy control subjects.

	CPG-BT ± SD (pg/ml)	CPG-AT±SD (pg/ml)	*p-value	CG±SD (pg/ml)	**p-value	***p-value
IL-α	208.3 ± 58.3	116.2 ± 23.1	0.001	97.8 ± 15.8	0.001	0.041
IL-β	173.7 ± 37.1	89.7 ± 26.2	0.002	63.4 ± 19	0.003	0.014
TNF-α	11.2 ± 1.5	5.2 ± 0.9	0.003	1.15 ± 0.8	0.001	0.000
IL-6	4.34 ± 1.8	1.3 ± 0.4	0.002	0.4 ± 0.2	0.003	0.088
IL-6sR	605.2 ± 107.8	236.5 ± 46.9	0.003	73.3 ± 23.2	0.001	0.000
IL-8	474.7 ± 169.3	299.1 ± 64.4	0.003	206.1 ± 46.6	0.002	0.000
IL-10	11.3 ± 3.6	4.7 ± 1.59	0.001	2.4 ± 0.6	0.001	0.000
MMP-3	1835.7 ± 1747.6	919.8 ± 508.1	0.009	724.8 ± 230.1	0.008	0.001
MMP-8	4913.3 ± 3549.8	3549.8 ± 2268.2	0.001	1534.6 ± 567	0.001	0.000

Data are presented as the **mean ± standard deviation**=SD. **n**=11.
CPG= Chronic Periodontitis Group. **CG**= Control Group. **BT**= Before Treatment. **AT**= After Treatment.
*Comparison for CPG before and after treatment. ** Comparison between CPG-BT and CG. ***Comparison between CPG-AT and CG

RESULTS.

Table 1 shows the comparison between clinical parameters before and after treatment, and with the healthy group. Table 2 displays the levels of inflammatory mediator before and after NSPT, and in healthy subjects. Moderate positive correlation was found between CAL and TNF-α (r=0.635; $p\leq0.04$) before treatment. Strong negative correlation was found between PI with IL-10 (r=-0.847; $p<0.001$) and moderate negative correlation between bleeding on probing (BOP) and IL-6 (r=-0.656; $p<0.02$), in the control group.

DISCUSSION.

Gingival crevicular fluid has shown potential value to evaluate periodontal therapy efficacy, displaying the greatest degree of sensitivity and specificity compared with saliva biomarkers in the identification of periodontal disease progression.[7]

Our results showed that concentration levels of all inflammatory mediators evaluated in the GCF sample were higher in the patients diagnosed with chronic periodontitis compared with the healthy group. These

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results were consistent with other authors who found high levels of IL-1-α, IL-6, IL-8, IL1-β and TNF-α in patients with chronic periodontitis.[8] The average cytokines concentration values showed large standard deviations indicating great variability among the samples, especially within the periodontitis group. The evaluated cytokines can be grouped into three levels according to their measured concentration values: low expression (TNF-α, IL-6, and IL-10), intermediate expression (IL-1β, IL1α, IL-6sR and IL-8), and high expression levels (MMP-3, MMP-8). These results are agreement with Offenbacher *et al.,*[9] who evaluated 33 cytokines levels in patients with chronic gingivitis, reporting three different groups according to their concentration: a group composed by cytokines with constitutively *"low"* expression levels (IL-6, IL-10, TNF-α); an intermediate cluster (IL-1α), and the group with the highest basal secretion levels (IL-1α, IL-8 and IL-1α).

Levels of all inflammatory mediators decreased after therapy, without reaching the values found in the control group; IL-6, IL-6sR, IL-10 and TNF-α, showed the highest reduction (70% to 54%); MMP3, IL-1α, IL-1β and IL-8 showed moderate reductions (50% - 34%); and levels of MMP-8 decrease the least (28%). The clinical parameters measurements decreased significantly after treatment, however, pocket depth, clinical attachment level and bleeding on probing, did not decrease down to the values shown by the control group. The degree of reduction of these cytokines may be due to their activation induced by the presence of plaque and gingival inflammation, although no statistically significant correlation was observed.

The smallest decrease observed for MMP8 may be related to the degradation of the organic matrix, and not to the presence of plaque.

Before treatment, only TNF-α showed moderate correlation with CAL. These results confirm those reported by Min Ki Noh *et al.,*[5] as they associated TNF-α, as a potent inflammatory cytokine, with factors connected to bone resorption.

No significant correlation between the clinical parameters and the expression of inflammatory cytokines in GCF after therapy was found. In the healthy periodontal group, the anti-inflammatory IL-10 showed the highest correlation with plaque level, followed by IL-6 with BOP, and these mediators showed the highest reduction after NSPT.

IL-10 is a multifunctional anti-inflammatory cytokine with regulatory effects in periodontal inflammation. It has been reported that lower levels of IL-10 may contribute to the progression of periodontal disease and that larger amounts have a protecting effect on tissue destruction, by suppressing the secretion of pro-inflammatory cytokines such as IL-1 and IL-8.[10]

CONCLUSION.

All clinical parameters and cytokines levels decreased after NSPT. TNF-α, IL-6, IL-6sR, and IL-10 are the cytokines whose levels varied the most before and after NSPT, and could be used to evaluate therapy response.

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