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ORIGINAL ARTICLE

IL-12 and IL-18 levels in serum and gingival tissue in aggressive and chronic periodontitis

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OBJECTIVE: The aim of this study was to compare the levels of interleukin-12 (IL-12) and IL-18 in gingival tissue and serum between patients with chronic (*n* = 18) or aggressive periodontitis (*n* = 12) and healthy subjects (HS) (*n* = 9).

METHODS: Gingival tissue biopsies and serum were obtained from all study subjects. The tissue was homogenized and cytokines IL-12 and IL-18 were quantified by enzyme-linked immunosorbent assay.

RESULTS: Interleukin-12 levels in gingival tissue were significantly higher in aggressive periodontitis patients than in HS; serum IL-12 was significantly elevated in aggressive periodontitis relative to both chronic periodontitis (CP) and HS. IL-18 levels in gingival tissue showed no significant differences between the groups. Patients with CP showed significantly elevated levels of serum IL-18 compared with HS; however, the aggressive periodontitis group showed no significant differences with either the CP group or the HS.

CONCLUSIONS: Our results showed higher levels of IL-12 in gingival tissue and serum of patients with aggressive periodontitis, and IL-18 was elevated in the serum of CP patients. The patterns of IL-12 and IL-18 are different in chronic and aggressive periodontitis; this finding suggests distinctive mechanisms of immunopathogenesis between these forms of periodontitis.

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Introduction

Periodontal disease is one of the two major dental diseases that affect human populations worldwide (Papapanou, 1999; Petersen and Ogawa, 2005). This pathology includes the inflammatory disorders of gingivitis and periodontitis, which are caused by specific pathogens, such as *Porphyromonas gingivalis, Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans*, in the bacterial biofilm that forms adjacent to teeth on a daily basis. These pathogens have been implicated as etiological agents and are associated with the progressive form of the disease (Seymour, 1991; Pihlstrom *et al*, 2005; Van Dyke and Sheilesh, 2005).

Periodontal diseases are a group of disorders with different etiologies and clinical manifestations. They include periodontitis, which is distinguished by a strong inflammatory response. Two main forms of periodontitis are identified; chronic (CP) and aggressive (AP), and they are characterized by attachment loss, bone destruction, periodontal pockets and gingival inflammation. However, attachment loss and tissue destruction is faster in AP than CP (Lang *et al*, 1999; Lindhe *et al*, 1999).

Recently, some studies have highlighted the importance of the immuno-inflammatory response to bacterial infection in the pathogenesis of periodontitis and tissue damage (Armitage and Robertson, 2009). The immuno-inflammatory response is initiated by the release of matrix metalloproteinases (MMPs) 8 and 9, prostaglandin E_2 and high levels of proinflammatory cytokines, including interleukin 1 (IL-1), tumor necrosis factor-α (TNF-α), IL-12 and IL-18 (Page and Kornman, 1997; Okada and Murakami, 1998; Graves and Cochran, 2003; Rai *et al*, 2008). This inflammatory process can gradually lead to the destruction of connective tissue and alveolar bone (Cochran, 2008; Graves, 2008).

Bioactive IL-12 is a 70-kDa heterodimer (p70) consisting of a 35-kDa light chain (p35) and a 40-kDa heavy chain (p40) (Gately *et al*, 1998). This cytokine is involved in both innate and adaptive immune responses; its most important functions are to stimulate T and NK cells to produce interferon-γ (IFN-γ) and to promote the Th1 response (Gately *et al*, 1998; O'Garra, 1998; Murphy *et al*, 2000; Opal and DePalo, 2000).

IL-18 is the major inducer of IFN-γ, and it is produced by a wide range of cells involved in regulating the innate and adaptive immune responses (Okamura *et al*, 1995; Dinarello and Fantuzzi, 2003). IL-18 acts synergistically with IL-12 on NK and T cells to induce IFN-γ production and the Th1 response (Nakahira *et al*, 2002).

Both IL-12 and IL-18 have been studied in patients with periodontal disease. IL-12 is reported to occur as IL-12p70 and IL-12p40 in the gingival crevicular fluid (GCF) of patients with periodontitis at lower levels than those observed in patients with gingivitis (Orozco *et al*, 2006). In another study, the total levels, but not the concentration, of IL-12 were higher in the GCF of patients with CP than in healthy subjects (HS) (Yucel *et al*, 2008). By contrast, IL-12p70 levels were found to be reduced in gingival biopsies of patients with periodontitis compared with those of HS (Johnson and Serio, 2005). IL-12p40 levels were found to be higher than those of the active form (IL-12p70) in serum of patients with periodontitis and gingivitis (Orozco *et al*, 2006); in another study, IL-12 levels were higher in serum of CP than AP patients (Cairo *et al*, 2010).

It has been reported than IL-18 levels were found to be locally higher in both gingival tissue biopsies and GCF of patients with periodontitis compared with patients with gingivitis and HS (Orozco *et al*, 2006; Figueredo *et al*, 2008; Pradeep *et al*, 2009). Similar results have been observed in serum, however, were not significant (Orozco *et al*, 2006). Moreover, it has been observed an increase of IL-18 in gingival biopsy tissues correlates directly with the pocket deep (Johnson and Serio, 2005) Several studies, mainly of GCF, have shown that levels of IL-12 and IL-18 in patients with periodontitis differ from those in HS and patients with gingivitis. However, this difference has not been addressed with respect to the different clinical characteristics of the two forms of periodontitis, AP and CP (Table 1). To assess if there are differences in the levels of IL-12 and IL-18 in these forms of periodontitis, we measured the levels of these cytokines in gingival tissue biopsies and serum of HS and patients with CP or AP.

Materials and methods

Study subjects

Thirty-nine subjects who had attended the Periodontal Clinic of the Dentistry School of University of Guadalajara for routine dental and radiographic examinations were recruited to participate in this study. Patients

Table 1 Comparative analysis between the levels of IL-12 and IL-18 in periodontal disease

Study group	*Sample studied*	*Method used*	*Status of cytokine studied*	*Reference*
IL-12				
HS (n = 6, 34 sites), G (n = 19, 32 sites) and CP (n = 19, 34 sites)	GCF	ELISA	IL-12 levels were higher in CP than those in HS and G.	Tsai *et al* (2005)
G (n = 10) and P (n = 10)	GCF	ELISA	IL-12p70 levels were lower in CP than those in G	Orozco *et al* (2006)
HS (n = 14), G (n = 14) and CP (n = 12)	GCF	ELISA	IL-12 levels were higher in CP than those in HS	Yucel *et al* (2008)
Grouped according with pocket depth: ≤3 mm (n = 42); 3 mm (n = 36); 4–6 mm (n = 39) and >6 mm (15)	Gingival biopsies	ELISA	There was an inverse correlation between IL-12 level and pocket depth	Johnson *et al* (2005)
G (n = 23) and CP (n = 25)	Gingival biopsies	Real-time PCR	IL-12p40 expression was higher in CP than that in G	Honda *et al* (2006)
G (n = 22) and CP (n = 24)	Gingival biopsies	Real-time PCR	IL-12p35 expression was higher in CP than G	Honda *et al* (2008)
Healthy sites (n = 11) and CP lesions (n = 15)	Gingival biopsies	Real-time PCR IHC	IL-12 expression was higher in CP lesions than in healthy sites	Ohyama *et al* (2009)
IL-18				
Grouped according with pocket depth: ≤3 mm (n = 42); 3 mm (n = 36); 4 to 6 mm (n = 39) and >6 mm (15)	Gingival Biopsies	ELISA	There was an direct correlation between IL-18 level and pocket depth	Johnson *et al* (2005)
G (n = 15) and CP (n = 18)	GCF	ELISA	IL-18 levels were higher in CP than those in G	Figueredo *et al* (2008)
HS (n = 20), G (n = 20) and CP (n = 20)	GCF	ELISA	IL-18 levels were higher in CP than those in G and HS	Pradeep *et al* (2009)
G (n = 10) and P (n = 10)	GCF and serum	ELISA	IL-18 levels were higher in P than those in G in GCF; in serum was not significantly	Orozco *et al* (2006)
JIA (n = 38) and HS (n = 29)	Serum	ELISA	IL-18 levels were higher in JIA than those in HS	Miranda *et al* (2005)

G, gingivitis; P, periodontitis; HS, healthy subjects; CP, chronic periodontitis; JIA, juvenile idiopathic arthritis; GCF, gingival crevicular fluid.

who had clinical features typical of chronic or aggressive periodontitis (as described in detail later) were enrolled consecutively.

All the subjects were in good general health and had not received previous periodontal therapy or taken antibiotic, immunomodulatory, or anti-inflammatory drugs in the 6 months prior to the study. Pregnant women, smokers, patients that were undergoing antibiotic prophylaxis for dental treatment, patients with any systemic disease, or those who were on long-term medication that could affect the expression of gingivitis or periodontitis were excluded from the study.

The purpose of this study was explained to each subject before he/she agreed to participate in the study, and their informed consent was obtained according to the Declaration of Helsinki. The study was approved by the medical ethical review committee at University of Guadalajara, and all subjects gave their written approval before participating based on the General Health Law and the NOM-008-SSA2-1993 norm.

Clinical examination

Clinical examinations were performed on all existing teeth of the participants and periodontal conditions were assessed based on the following parameters: sites with plaque (SP), bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL). The examination was performed using a periodontal probe (Hu-friedy, Chicago, IL, USA) by a single investigator, and the mean sulcular depth was calculated.

Clinical parameters were obtained on six sites per tooth with the exception of CAL, for which four sites were examined, and the results were expressed as percent of sites (for plaque and BOP) and as mean values accompanied by standard deviation for PD and CAL).

Study groups

Medical and dental records were obtained for all participants and were diagnosed according to the classification of the American Academy of Periodontology (Armitage, 1999; Duarte *et al*, 2010).

HS group. The healthy group consisted of nine HS (six women and three men; mean age: 38.1, age range: 16–65 years) who attended the clinic for esthetic surgery with no evidence of clinical attachment loss, clinical inflammation, sulcular bleeding, or radiographic evidence of bone loss. The gingival tissue of this group was defined as clinically healthy when the mean sulcular depth was ≤3 mm and there was no evidence of BOP any surface (Loe, 1967).

CP group. The CP group included 18 adult patients (13 women and five men; mean age: 47.9; age range: 33–62 years). They presented localized damage at various sites in the oral cavity, including ≥5 mm of CAL and a PD of ≥6 mm.

AP group. The aggressive periodontitis group included 12 patients (11 women and one men; mean age: 33.5; age range: 16–44 years) with a pattern of severe damage and CAL ≥5 mm on eight or more teeth, at least three of which were not central incisors or first molars.

In all periodontitis patients, gingiva with redness or marked redness, edema, glazing, BOP or spontaneous bleeding was considered inflamed (Loe, 1967).

Sample collection

Gingival tissue samples. Inflamed gingival tissue (only from active periodontal sites) from patients was collected by a flap operation during routine periodontal surgery using a scalpel, after which the wound was secured with sutures. Prior to surgery, all individuals underwent an initial periodontal treatment phase. Collection of gingival tissue from HS was performed before tooth extraction for orthodontic/prosthetic indications. Tissues samples were frozen at −70°C until analysis.

Tissue preparation

The gingival tissue was solubilized according to the techniques described by (Gorska *et al*, 2003; Johnson *et al*, 2005) Briefly, the tissue was blotted, weighed on a microbalance, cut into small pieces (1–2 mm^3) with a scalpel and then placed in a 1.5-ml microtube with a sufficient volume of phosphate-buffered saline (PBS) to ensure the following dilution: 10 mg tissue/100 μl PBS plus protease inhibitor (Sigma Chemicals, St Louis, MO, USA). Subsequently, the tissue was macerated with a polypropylene pestle and a vortexer. Next, it was centrifuged at 600 *g*, and the supernatant was frozen at −70°C until analysis by cytokine ELISA. The procedure was carried out at 4°C.

Serum collection

Blood obtained by venipuncture was centrifuged to isolate serum, which was immediately stored at −70°C until the cytokine ELISA for IL-12 and IL-18 was carried out.

Protein assay

A standard Bradford micromethod was used to assess the protein concentration in each gingival sample (Bradford, 1976). The absorbance was read at 570 nm in a microplate spectrophotometer. Protein concentrations were calculated from a bovine serum albumin standard curve (Sigma Chemical) and were expressed as $\mu g/\mu l$. The Bradford reagent used consisted of 100 mg of Coomassie brilliant blue G-250 (Research Organics, Cleveland, OH, USA), 50 ml of 95% ethanol (Caledon Laboratories Ltd, Georgetown, ON, Canada), 100 ml of concentrated phosphoric acid (Caledon Laboratories Ltd) and 200 ml of distilled water.

Enzyme-linked immunosorbent assay (ELISA)

Aliquots from either tissue homogenates or cytokine standards were added in triplicate to the wells of microtiter plates to determine the concentrations of human IL-12 and IL-18 using an ultrasensitive IL-12 p70 ELISA and IL-18 ELISA kit (BioSource, Camarillo, CA, USA), respectively. The absorbance of each well was read at 450 nm in a microplate spectrophotometer, and the

concentration of each biomarker was calculated from the standard curve included with each assay kit. Method and antiserum-specificity controls were included in each assay. Controls for plate-to-plate variation were also used when appropriate. The concentrations of IL-12 and IL-18 were expressed as pg/mg of gingival tissue and as pg/ml of serum.

Statistical analysis

The results are expressed as mean ± s.d. or number of cases (percent). The chi-square test was used to compare gender, SP and BOP of the study groups. The data were tested for normal distribution using the Shapiro–Wilk test for small sample size. One-way ANOVA followed by *post hoc* DMS was used to compare age and PD; *post hoc* Tamhane was used to CAL.

Cytokine data were log-transformed because of their non-normal distribution. The cytokine level values were corrected by adding 1 to obtain log values for statistical analysis, and the results were expressed as mean ± s.e.m. One-way ANOVA was carried out followed by *post hoc* DMS for defining differences within groups.

Pearson correlation was used to study the correlation between the cytokine levels and clinical findings. All the statistical analyses were performed considering $P < 0.05$ to be significant using SPSS 15.0 software (SPSS, Chicago, IL, USA), and graphs were plotted using GraphPad Prism 5.1 software (San Diego, CA, USA).

Results

The demographic and clinical data of the participants are shown in Table 2. The mean age (years) was statistically significantly higher in the CP group (47.9 ± 9.3) than in the HS or AP group (38.1 ± 14.6 and 33.6 ± 8.3 respectively). The percentage of women was higher in all three groups, with no significant differences between them. All CP and AP patients showed high percentages of SP and BOP, while in the HS these were not observed. PD was significantly different between the HS and CP ($P < 0.0001$), HS and AP ($P < 0.0001$) and CP and AP ($P = 0.0132$) groups; CAL only showed significant differences between the HS and CP ($P < 0.0001$) and HS and AP groups ($P < 0.0001$).

The levels of IL-12 in tissue were higher in patients with AP (0.56 ± 0.17 pg/mg of tissue) than those in the HS (0.22 ± 0.03 pg/mg of tissue) or CP (0.39 ± 0.05 pg/mg of tissue) group, but significant differences were only found between the AP and HS groups ($P = 0.021$) (Figure 1a). In serum, IL-12 was significantly higher in the AP group (63.5 ± 8.6 pg/ml) than that in the CP (44.9 ± 4.8 pg/ml; $P = 0.036$) or HS (39.4 ± 5; $P = 0.024$) group (Figure 1b).

Interleukin-18 levels in gingival tissues were higher in the HS (14.6 ± 5.6 pg/mg of tissue) and CP (14.5 ± 2.7 pg/mg of tissue) groups than those in the AP group (10.5 ± 2.1 pg/mg of tissue), but no statistically significant differences were observed (Figure 2a). We found that serum IL-18 levels were higher in the CP group (427.3 ± 56.9 pg/ml) than those in the HS (278.2 ± 41.6 pg/ml) or AP (348.5 ± 39.2 pg/ml) groups, but significant differences were only observed between the CP and HS groups ($P = 0.027$) (Figure 2b).

Correlation analysis comparing tissue and serum IL-12 and IL-18 levels with PD and CAL found only a significant correlation between levels of serum IL-12 and PD (Pearson correlation, $r = 0.411$; $P = 0.037$).

Discussion

Periodontal disease is characterized by an immunoinflammatory response in gingiva to periodontopathic bacteria (mainly Gram negative) that is regulated by proinflammatory cytokines such as IL-1β, TNF-α, IL-12, IL-18 and others present in the local environment. IL-12 and IL-18 are released by monocytes-macrophages in response to antigenic stimuli such as lipopolysaccharide of Gram-negative bacteria (Nau *et al*, 2003; Foster *et al*, 2007). IL-12 is involved in the promotion of the Th1 response, whereas IL-18 acts synergistically with IL-12 to promote this response and induces IFN-γ production by T and NK cells (Altare *et al*, 1998; Nakahira *et al*, 2002; Lopez Roa *et al*, 2008). However, in the absence of IL-12, IL-18 induces

Table 2 Mean ± s.d. clinical and demographic characteristics of HS, CP, and AP groups

				P-*value**		
	HS (n = *9*)	*CP* (n = *18*)	*AP* (n = *12*)	*HS vs* CP	*HS vs* AP	*CP vs* AP
Mean age (years)	38.1 ± 14.6	47.9 ± 9.3	33.6 ± 8.3	0.0297	0.3357	0.0009
Female/male %	6/3 66.7/33.3	13/5 77.8/22.2	11/1 91.7/8.3	NS	NS	NS
SP (%)	0	100	100	<0.0001	<0.0001	NS
BOP (%)	0	100	100	<0.0001	<0.0001	NS
PD (mm)	1.6 ± 0.4	3.5 ± 0.7	4.5 ± 0.9	<0.0001	<0.0001	0.0132
CAL (mm)	0.7 ± 0.3	5.6 ± 1.3	4.8 ± 1.7	<0.0001	<0.0001	0.5465

*Significance level $P < 0.05$. Chi-square test was used to compare gender, SP and BOP. One-way ANOVA followed by *post hoc* DMS was used to compare age and PD; *post hoc* Tamhane was used to CAL.
AP, aggressive periodontitis; BOP, bleeding on probing; CAL, clinical attachment level; CP, chronic periodontitis patients; HS, healthy subjects; PD, probing depth; s.d., standard deviation; SP, sites with plaque.

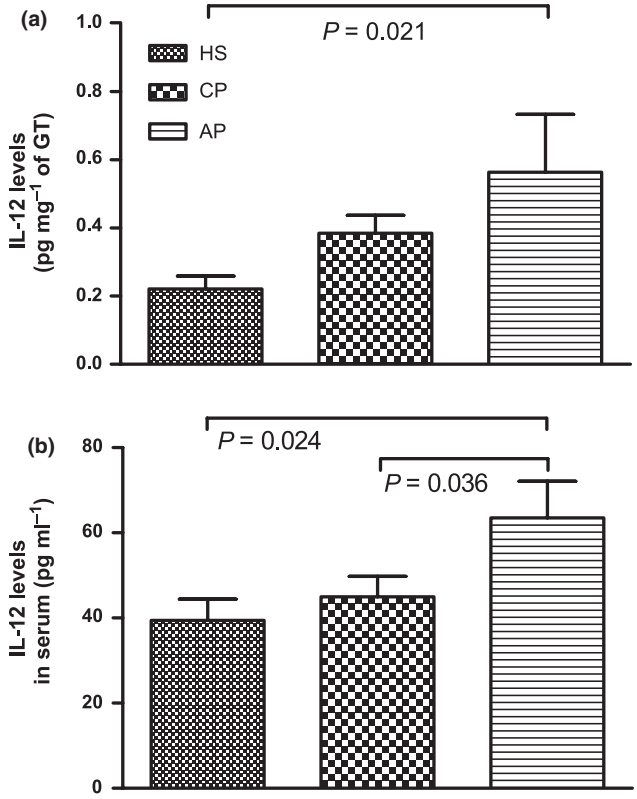

Figure 1 IL-12 levels in healthy subjects (HS), chronic periodontitis (CP) and aggressive periodontitis (AP) patients. The levels of IL-12 were detected using ELISA in gingival tissue (GT) biopsies (**a**) and serum (**b**) of HS, CP, and AP patients. The results are showed as mean ± s.e.m. ANOVA test and *post hoc* DMS was used; $*P < 0.05$ was considered as significant

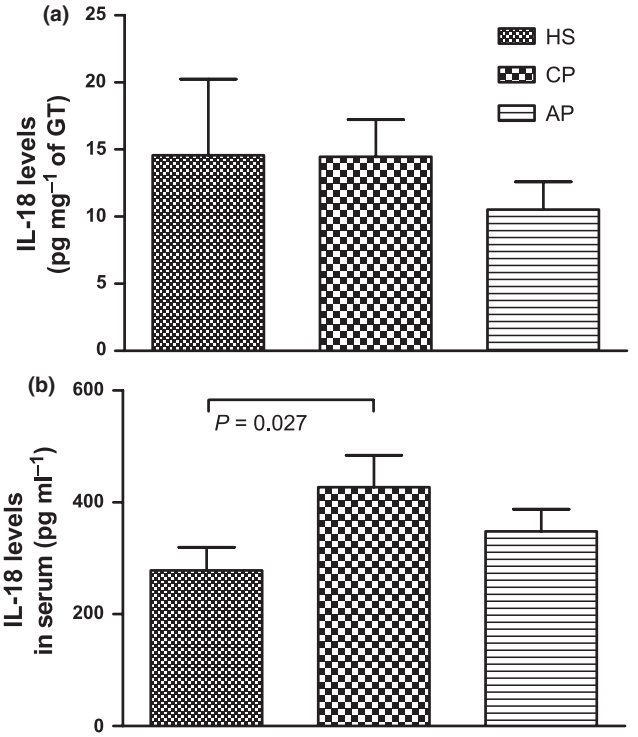

Figure 2 IL-18 levels in healthy subjects (HS), chronic periodontitis (CP), and aggressive periodontitis (AP) patients. The levels of IL-18 were detected using ELISA in gingival tissue (GT) biopsies (**a**) and serum (**b**) of HS, CP, and AP. The results are showed as mean ± s.e.m. ANOVA test and *post hoc* DMS was used; $*P < 0.05$ was considered as significant

the Th2 response, releasing IL-4 and IL-13 (Nakanishi *et al*, 2001). Studies elucidating the role of IL-12 and IL-18 in periodontal diseases by measuring protein levels or gene expression in different sample types are summarized in Table 1. However, the levels of these cytokines have not been assessed with respect to the two forms of periodontitis, chronic and aggressive. Thus, in this study, we report for the first time the levels of IL-12 and IL-18 in both forms of periodontitis.

We found that IL-12 levels in gingival tissues and serum are higher in patients with AP than in those with CP or HS; and CP than HS, which may be involved in the immuno-inflammatory response observed in this most destructive form of this disease. In this regard, it has been reported that GCF IL-12 levels were higher in CP patients than those in HS (Tsai *et al*, 2005; Yucel *et al*, 2008). Moreover, an elevated IL-12p35 gene expression in CP lesions compared with those in gingivitis lesions or healthy control sites was also reported (Honda *et al*, 2008; Ohyama *et al*, 2009). However, other studies show inconsistent results regarding IL-12 levels, it has been reported that IL-12p40 levels in serum, were higher in patients with periodontitis than in patients with gingivitis, whereas IL-12p70 in GCF and in serum were lower in patients with periodontitis than in patients with gingivitis, (Orozco *et al*, 2006), in addition, other findings describe an inverse correlation between IL-12p70 levels and PD (Johnson and Serio, 2005).

The increased levels of IL-12 in tissue in AP described in this research suggest a local immune response to bacteria that are present at the site of the infection. Multiple studies have demonstrated that *P. gingivalis* and *A. actinomycetemcomitans* induce IL-12 expression in dendritic cells (Sugano *et al*, 2004; Vernal *et al*, 2008, 2009). In addition, it has been reported that in a murine model, the Th1 response promotes bone loss in mice immunized with *P. gingivalis* (Stashenko *et al*, 2007). In this context, IL-12 plays an important role in promoting the Th1 response and IFN-γ production (Trinchieri, 2003). IFN-γ mRNA levels in gingival tissue samples were described to be higher in patients with AP than in those with CP or HS (Garlet *et al*, 2003, 2004; Suarez *et al*, 2004; Honda *et al*, 2006) and elevated levels of IFN-γ protein in gingival tissues have been reported only in CP relative to HS (Dutzan *et al*, 2009). IFN-γ acts on monocytes/macrophages to positively regulate IL-12 production in a feedback loop and to induce IL-1β and TNF-α production (Watford *et al*, 2003; Schroder *et al*, 2004). These proinflammatory cytokines induce bone resorption indirectly by stimulating osteoblastic cells to produce RANK-L, which binds to RANK (receptor activator of NF-κB) expressed on osteoclast precursors to promote osteoclast differentiation and subsequent activation (Taubman and Kawai, 2001).

In this study, patients with AP showed higher levels of IL-12 in gingival tissue compared with patients with CP or HS, which suggests that the increased levels of IFN-γ mRNA present in AP (Garlet *et al*, 2003, 2004) could be attributable to an elevated level of IL-12, possibly explaining the bone destruction in this form of periodontitis. However, we did not measure the levels of IFN-γ, which makes it necessary to assess the levels of IFN-γ in AP to determine its relation with bone destruction.

In this study, we observed elevated levels of serum IL-12 in patients with AP. In this regard, we found a significant correlation between IL-12 in serum and PD, suggesting that this cytokine is associated with gingival tissue damage and it is reflected at systemic level, as has been reported for others molecules. For example, Shi *et al* reported increases in globulin and leukocyte levels and reduced albumin/globulin ratios in AP, which might be associated with severity of periodontal destruction (Shi *et al*, 2008). Loos *et al* reported elevated levels of C–reactive protein and IL-6 in periodontitis, which suggests that these markers of inflammation may be associated with cardiovascular events (Loos *et al*, 2000). It is unknown whether the increase in IL-12 levels observed in AP might be associated with increased risk of cardiovascular events. Therefore, additional research is needed in this regard.

On the other hand, we found that the levels of IL-18 in gingival tissue biopsies showed a tendency to be higher in HS and in patients with CP than in those with AP, but this trend did not reach significance. Our findings are consistent with the results of other studies in which the levels of IL-18 in GCF were higher in patients with periodontitis than in gingivitis patients or HS (Orozco *et al*, 2006; Figueredo *et al*, 2008; Pradeep *et al*, 2009). It has been reported that the increased IL-18 in gingival biopsy tissues correlates with the severity of chronic inflammation (Johnson and Serio, 2005).

Moreover, our results showed levels of IL-18 in serum statistically higher in CP than in HS ($P = 0.027$). Miranda *et al* reported high levels of IL-18 in serum of patients with juvenile idiopathic arthritis, which is associated with periodontal attachment loss, suggesting that this may be attributed to an alteration in the systemic inflammatory response that makes tissue more prone to destruction in the presence of periodontal bacteria (Miranda *et al*, 2005). In this context, the high levels of IL-18 that we observed in CP patients are probably responsible for disease's persistence and the bone loss. Moreover, it is possible that high levels of IL-18 is associated with the presence of *P. gingivalis*, which has been observed to induce IL-18 production in human umbilical vein endothelial cells and monocytes and is one of the factors involved in periodontal disease (Foster *et al*, 2007; Wang *et al*, 2008).

Therefore, it is possible that IL-18 participates in the neutrophil response observed in periodontal disease. Neutrophils have been suggested to have a role in the pathogenesis of periodontitis, and they have been reported to have a hyperreactive phenotype. The neutrophils of patients with CP show increased expression of IFN-γ-stimulated genes compared with those from HS (Wright *et al*, 2008). IL-18 is also capable of activating neutrophils to produce cytokines such as IL-8 and IL-1β and to release granules, and it increases the respiratory burst, thereby promoting inflammation (Buchmann *et al*, 2002). This finding suggests that this cytokine is involved in chronic inflammation and tissue destruction in patients with periodontitis.

Our results could be explained in the context of Th1/Th2 responses by comparing the AP and CP groups with HS. Thus, in AP the only difference was observed in IL-12 in both tissue and serum, and these conditions would favor a Th1 response. By contrast, in CP a difference was observed in IL-18 only in serum, and these conditions would induce a Th2 response. The role of Th1/Th2 in periodontitis is still under debate: several lines of evidence suggest that Th1 contributes to bone loss by inducing osteoclastogenesis, whereas Th2 cells plays a protective role in bone destruction (Taubman and Kawai, 2001; Teng, 2003). However, controversy still exists because Th17 cells, a recently described population of T cells, have been implicated in various autoimmune and inflammatory diseases, including periodontal disease. Several authors have described the presence of Th17 cells and the expression of IL-17 in gingival tissue and GCF of CP patients (Takahashi *et al*, 2005; Honda *et al*, 2008; Cardoso *et al*, 2009; Ohyama *et al*, 2009; Pradeep *et al*, 2009). Recently, high levels of IL-17 have been reported in serum of patients with AP (Duarte *et al*, 2010; Schenkein *et al*, 2010). This evidence suggests that Th17 cells may play an important role in the pathogenesis of bone destruction in periodontitis. These cells are known to produce large amounts of IL-17, which induces the expression of RANK-L on osteoblasts, fibroblasts, and Th17 cells themselves. In turn, IL-17 also promotes inflammation and the production of cytokines such as TNF-α and IL-6, which also promote the expression of RANK-L. Recently, Sato suggested that the Th1/Th2 response inhibits osteoclastogenesis and proposed that Th17 cells are an osteoclastogenic Th cell subpopulation in rheumatoid arthritis (Sato *et al*, 2006). This model of osteoclastogenesis by Th17 could be addressed in future research.

In summary, our results provide data about IL-12 and IL-18 levels in the spectrum of periodontal diseases that have not been reported until now. The higher levels of IL-12 in gingival tissue and serum of patients with AP, and increased level of IL-18 in the serum of CP patients suggest distinctive mechanisms of immunopathogenesis between these forms of periodontitis. In addition, it would be interesting to investigate the Th1, Th2, and Th17 profile cytokines in both chronic and aggressive periodontitis to understand their pathogenic mechanisms and to identify potential therapeutic targets.

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Author contributions

C. Guerrero-Velázquez and P. E. Sánchez-Hernández, designed the study, performed the experimental work described in the study; searched and updated scientific literature and contributed scientific ideas and wrote manuscript. A. L. Zamora-Perez, participated in the design of the study and reviewed the manuscript. M. Fuentes-Lerma, orthodontic specialist, was responsible for the selection of controls (healthy subjects) and sampling. C. Robles-Gómez, specialist in periodontics, determined the diagnosis, classification, clinical parameters, patient selection and sample collection. R. P. Mariaud-Schmidt, contributed with scientific ideas and technical support, got the project grant.

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