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# Correlation of chronic periodontitis in tropical area and IFN-γ, IL-10, IL-17 levels

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ABSTRACT

**Objective:** To evaluate the correlation of chronic periodontitis in tropical area and IFN-γ, IL-10 and IL-17 levels. **Methods:** One hundred and forty-eight patients and one hundred and thirty-two healthy control subjects were included in the study. Clinical parameters (PI, GI and PD) and GCF levels of IFN-γ, IL-10 and IL-17 were measured at baseline, week 8, week 16 and week 24 after mechanical removal of dental plaque. IFN-γ and IL-10 were determined with ELISA methods and IL-17 was determined with the cytometric bead array. **Results:** Removal of dental plaque resulted in improvement in all clinical parameters. Meanwhile, GCF IL-17 declined to control levels, while GCF IFN-γ and IL-10 levels remained unchanged. **Conclusions:** The decline of GCF IL-17 levels in patients with resolution of periodontitis suggests that IL-17 is involved in the periodontal inflammatory process.

## 1. Introduction

Periodontitis is a chronic inflammatory disorder affecting the supporting tissues of teeth. The pathogenesis of periodontitis involves the interaction between periodontal pathogens and host inflammatory and immune responses. Cytokines play a critical role in mediating inflammatory processes and tissue homeostasis underlying periodontitis. Extensive research has been conducted to demonstrate the expression and changes of various kinds of cytokines in normal periodontal tissues and pathological conditions[1,2].

Of the cytokines that are implicated in the development of periodontitis, it is unclear how exactly IFN-γ participates in periodontal tissue destruction. IFN-γ is secreted by Th1 cells and induces IL-1β, TNF-γ and prostaglandin E2 (PGE2) production by macrophages. Therefore, it is proinflammatory in nature. However, IFN-γ also inhibits osteoclastogenesis by interfering with the RANKL-RANK signaling pathway[3], suggesting that it may serve a dual function. In contrast, IL-10 is thought to be an anti-inflammatory cytokine and suppresses immune and inflammatory responses[4]. The expression profile of IL-10 in healthy and diseased periodontal conditions has yet to be elucidated. IL-17 has recently received broad attention for its role in periodontal tissue destruction. Considered a proinflammatory cytokine and secreted by CD4 Th17 cells, IL-17 has been found to be involved in a number of systemic conditions, including rheumatoid arthritis and several autoimmune disorders. However, studies have reported varying results concerning levels of IL-17 in

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Gingival crevicular fluid (GCF) and in periodontal tissues[5,6]. The present study was carried out to investigate GCF levels of IFN-γ, IL-10 and IL-17 in both healthy and diseased periodontal states in a tropical population.

## 2. Materials and methods

### 2.1. Patient recruitment and study protocol

All 148 patients with chronic periodontitis admitted from November 2010 to May 2012 were selected. In addition, 132 periodontally healthy individuals were assigned into the control group. The vast majority of the patients and healthy controls were natives of Hainan, a tropical island in southern China. Informed consent was acquired from each participant prior to recruitment. Patients meeting the following selection criteria were randomly admitted into the research project: (1) the subject was a nonsmoker between 35-60 years of age; (2) there was no systemic infection, diabetes, immunodeficiency disorder and autoimmune disease; (3) no periodontal therapy of any form had been provided and no antibiotics, nonsteroid antiinflammatory drugs and glucocorticoids had been taken in the preceding six months; (4) the selected tooth had a periodontal pocket measuring between 4-6 mm.

For each patient or control subject, four periodontal sites from four different quadrants were selected. Baseline measurements, consisting of clinical parameters and GCF samples, were taken before mechanical removal of dental plaque was initiated. Clinical parameters included plaque index (PI) (Silness and Löe, 1964)[7], gingival index (GI) (Löe and Silness, 1963)[8] and probing depth (PD), and GCF samples were determined for GCF volume, IFN-γ, IL-10 and IL-17 levels. After baseline measurements, each patient received oral hygiene instructions, supragingival scaling and root planing, while control subjects received only oral hygiene instructions. The same measurements were taken at week 8, week 16 and week 24, respectively.

### 2.2. GCF collection

Before GCF collection, the teeth were isolated with cotton rolls and supragingival plaque was removed with a scaler, which was kept clear of gingiva. For each tooth, GCF was obtained at the junction of the buccal and mesial surfaces. A Periopaper® (Oraflow Inc., NY, USA) strip was gently inserted into the gingival sulcus until slight resistance was encountered. After 30 seconds, the strip was withdrawn and GCF volume was measured using Periotron (Model 8000, Oraflow, NY, USA) calibrated with a 0.01 M sodium phosphate buffer. The sample was then placed in a 1.5 mL micro centrifuge tube containing 150 μL PBS-Tween 20 (0.1M PBS, 0.05% Tween 20, pH 7.4) and stored at -70 ℃ until cytokine quantification. Strips contaminated with blood were discarded and samples were re-collected at a later time.

### 2.3. Quantification of GCF IFN-γ, IL-10 and IL-17 levels

IFN-γ levels in the GCF samples were determined with an IFN-γ ELISA kit by Millipore (Millipore Corporation, MA, USA); the levels of IL-10 were determined with a cytometric bead array kit (BD Bioscience-Pharmingen, CA, USA); and the levels of IL-17 were measured with a Human IL-17 ELISA (Quantikine R&D Systems, MN, USA). All the assays were conducted according to manufacturers' instructions.

### 2.4. Statistical analysis

Data analysis was performed using the statistical package SPSS version 14.0. Values are expressed as the mean±SD. Data from the chronic periodontitis groups and the control groups were compared at each time point. Student *t*-tests were used where appropriate. $P < 0.05$ was considered statistically significant.

## 3. Results

The chronic periodontitis group included 76 male and 72 female patients, with a mean age of 43.8±5.51, while the control group had 68 male and 64 female subjects, with a mean age of 41.3±5.06. GCF volumes were much higher at chronic periodontitis sites than at periodontally healthy control sites ($P < 0.01$). After mechanical removal of dental plaque, there was a sharp decline in GCF volume. However, the GCF volumes remained higher at week 8, week 16 and week 24 when compared with those at healthy sites ($P < 0.05$) (Table 1).

Mechanical plaque removal led to marked improvement in all clinical parameters. At week 8, mean PI, GI and PD scores all showed significant decrease. From week 8 to week 24, the chronic periodontitis group's PI scores were on longer significantly different from those of the control group. In contrast, the patients' GI scores remained slightly higher than the healthy controls' scores ($P < 0.05$) (Table 2). Both before and after dental plaque removal, GCF IFN-γ levels were not statistically different between the two groups, although mean values for the chronic periodontitis group seemed slightly higher at all time points (Table 3). A similar pattern could be seen for IL-10 (Table 3). GCF IL-17 levels in the chronic periodontitis group was higher than in the control group at baseline, but at subsequent time points, there was no difference between the two groups (Table 3).

**Table 1**

Influence of periodontal basic therapy on GCF volume (mean±SD) (μ/L).

| Group | Baseline | Week 8 | Week 16 | Week 24 |
|---|---|---|---|---|
| Periodontitis | 0.153±0.068** | 0.063±0.016* | 0.076±0.015* | 0.067±0.018* |
| Control | 0.036±0.009 | 0.023±0.007 | 0.032±0.006 | 0.031±0.008 |

*$P < 0.05$, **$P < 0.01$ compared with controls.

**Table 2**

Effect of periodontal basic therapy on clinical parameters (mean±SD) (μ/L).

| Group | | Baseline | Week 8 | Week 16 | Week 24 |
|---|---|---|---|---|---|
| Periodontitis | PL | 2.12±0.52** | 1.09±0.34 | 0.91±0.27 | 0.93±0.25 |
| | GI | 2.33±0.57** | 0.63±0.16* | 0.68±0.19* | 0.64±0.18* |
| | PD | 4.88±0.62** | 3.59±0.43** | 3.36±0.40** | 3.39±0.38** |
| Control | PL | 1.08±0.35 | 0.87±0.23 | 0.78±0.29 | 0.84±0.19 |
| | GI | 0.40±0.15 | 0.36±0.11 | 0.39±0.13 | 0.42±0.16 |
| | PD | 0.77±0.21 | 0.76±0.20 | 0.77±0.19 | 0.75±0.18 |

*$P < 0.05$, **$P < 0.01$ compared with controls.

**Table 3**

Effect of periodontal basic therapy on IFN-γ, IL-10 and IL-17 (mean±SD) (pg/mL).

| Group | | Baseline | Week 8 | Week 16 | Week 24 |
|---|---|---|---|---|---|
| Periodontitis | IFN-γ | 0.26±0.13 | 0.20±0.15 | 0.24±0.16 | 0.22±0.19 |
| | IL-10 | 16.10±5.40 | 13.20±4.70 | 14.80±5.00 | 12.90±4.60 |
| | IL-17 | 4.60±1.39** | 2.69±1.10 | 2.65±1.40 | 2.58±1.30 |
| Control | IFN-γ | 0.19±0.14 | 0.16±0.11 | 0.18±0.13 | 0.17±0.10 |
| | IL-10 | 12.60±4.90 | 11.80±4.30 | 13.50±4.40 | 12.10±4.20 |
| | IL-17 | 2.10±0.87 | 2.34±1.00 | 2.46±1.20 | 2.32±1.10 |

**$P < 0.01$ compared with controls.

## 4. Discussion

In this study, we analyzed GCF IFN-γ, IL-10 and IL-17 levels in patients with chronic periodontitis both before and following mechanical dental plaque removal. Accompanying overall improvement in clinical measurements, IL-17 levels showed decline after plaque removal. However, no change seems to have occurred in GCF IFN-γ and IL-10 levels. Previous studies on these cytokines have generated conflicting and sometimes confusing data. Our results are largely in agreement with those from a study by Zhao *et al*, in which GCF IL-17 and IL-21 levels were decreased and IFN-γ levels were unchanged in a group of Chinese periodontitis patients after receiving periodontal treatment[9]. It needs to be pointed out that our subjects represent a tropical population in southern China. One study on an Indian population failed to detect IL-17 in GCF[10]. It is not clear whether regional alone can adequately account for the discrepancy. Most studies have also been unable to find any difference in GCF IFN-γ levels after resolution of periodontal inflammation[11]. Experimental studies have demonstrated that IFN-γ positive cells such as Th1 cells play a prominent role in mediating periodontal tissue destruction, although detailed processes still need to be revealed[12]. In addition to the three cytokines, a number of other proinflammatory cytokines, such as IL-1α, IL-1β, IL-6, IL-8 and TNF-α, have been shown to be closely involved in the pathogenesis of periodontal disease. There is a positive correlation between periodontal disease activity and tissue cytokine levels[13,14]. Host responses mediated by cytokines are believed to be essential in the breakdown of connective tissue and bone, which is the key feature of the disease process.

For a long time, our understanding of T helper cell-mediated immunity was based on the Th1/Th2 model. According to this model, Th1 cells, which secret IL-2 and IFN-γ, are responsible for cell-mediated responses, such as inflammatory processes, while Th2 cells, producing IL-4, IL-5 and IL-13, lead to humoral immune responses, including allergic reactions[15]. The identification of the Th17 subset has brought about a new perspective on immune mechanisms involved in inflammatory disorders. Th17 cells produce IL-17, IL-22, IL-26 and IL-21. IL-17 is capable of stimulating a variety of cell types to produce inflammatory mediators such as IL-1, IL-6, TNF-α, metalloproteinases, and chemokines. Th17 cells are crucial in immune responses against extracellular bacteria[16]. They also participate in the pathogenesis of several autoimmune and inflammatory disorders. It has been shown that IL-17A produced by Th17 cells promotes the development of osteoclasts when osteoblasts are present[17]. In addition, IL-17 is expressed at gingival sites in patients with periodontitis[18]. As our data show, GCF IL-17 levels in patients with periodontitis are much higher than in healthy subjects. Furthermore, IL-17 expression has been observed in the alveolar bone of patients with chronic periodontitis[18]. It has been recently demonstrated that levels of IL-21 in gingival tissues are increased in patients with chronic periodontitis[19]. Therefore, all lines of evidence point to the involvement Th17 cells and their cytokines in the pathogenesis of periodontal disease.

In the present study, resolution of periodontal inflammation was achieved exclusively with mechanical dental plaque removal. The significant improvement in patients' periodontal conditions had been expected. In the past three decades, a large number of studies have clearly shown that, in many cases, nonsurgical therapy can be a valid alternative to surgical therapy[20,21]. It seems that outcomes of treatment modalities are to a large extent dependent on the probing depth. In shallow periodontal pockets (1-3 mm), nonsurgical therapy results in 0.3 mm less clinical attachment loss than surgical therapy. There is also less probing depth reduction (about 0.1 mm). In pockets ranging from 4-6 mm, nonsurgical therapy results in 0.3 mm more clinical attachment gain but 0.3 mm less probing depth reduction than surgical therapy. For pockets deeper than 6 mm, surgical therapy shows an advantage in probing depth reduction[22,23]. There is no statistically significant difference in clinical attachment level gain between treatment modalities. Where long-term treatment outcomes are concerned, no significant difference between treatment modalities has been found for shallow pockets. However, surgery provides a greater reduction in probing depth for pockets exceeding 4 mm[24,25]. Since our primary objective was to examine the relationship between clinical periodontal health status and GCF cytokine levels, no attempt was made to compare the effectiveness of different treatment modalities.

In conclusion, after resolution of periodontal inflammation, clinical parameters improved significantly and GCF IL-17 levels in patients with chronic periodontitis declined accordingly. In contrast, GCF IFN-γ and IL-10 levels remained largely unchanged. The results suggest that IL-17 is involved in the periodontal inflammatory process.

## Conflict of interest statement

We declare that we have no conflict of interest.

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