

# Detection of IL-4, IL-6 and IL-12 Serum Levels in Generalized Aggressive Periodontitis

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## ABSTRACT

**Background:** Periodontitis is a multifactorial chronic inflammatory disease characterized by destruction of tooth-supporting tissues. Environmental and genetic factors as well as the immune system participate in this process. Recent studies have attempted to elucidate the role of cytokine networks involved in periodontal diseases. **Objective:** To assess and compare the levels of IL-4, IL-6 and IL-12 in serum samples of patients with generalized aggressive periodontitis (GAgP) and control individuals. **Methods:** A total of 50 subjects were included in the study of which 25 patients had generalized aggressive periodontitis and 25 were healthy unrelated age and gender matched patients undergoing extraction and surgical crown lengthening (control group). Local blood samples of patients were collected from surgical sites of pocket reduction and from healthy individuals before tooth extraction or crown lengthening from non inflamed sites. The levels of IL-4, IL-6 and IL-12 were determined by an ELISA assay using serum samples separated from the whole blood of both groups. **Results:** The level of IL-4 increased significantly in control group in comparison with the test group ( $p=0.002$ ). The amount of IL-6 in GAgP patients increased strongly compared with control group ( $p<0.0001$ ). There was no significant difference between the two groups concerning the level of IL-12. **Conclusion:** There is an association between generalized aggressive periodontitis and low level of IL-4 as an anti-inflammatory cytokine, and high level of IL-6 as a proinflammatory cytokine. No correlation between IL-12 and generalized aggressive periodontitis was found.

**Keywords:** Aggressive Periodontitis, IL-4, IL-6, IL-12, Serum

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## INTRODUCTION

Chronic periodontitis is characterized by an interaction between dental plaque antigen and components of the host defense system (1); therefore, periodontitis is considered to be an immunological disease with several unique immunological features, including elevated cellular and humoral immune responses (2). The antigenic persistence contributes to the amplification of the immune inflammatory response that determines the progressive destruction of the periodontium, the detachment of the periodontal ligaments, the bone resorption and the teeth mobilization (3). T cells which are the key players of immune modulation, have been extensively investigated in the pathogenesis of periodontitis. T helper (Th) cells can be classified into at least three distinct subsets according to their cytokine production and functional properties: Th1, Th2 and Th17 cells (4). Cytokines are the mean of communication between immune and non-immune cells (5). Interleukin 12 (IL-12) is involved in the differentiation of naive T cells into Th1 cells. IL-12 is known as a T cell stimulating factor, which can stimulate the growth and function of T cells. It has been suggested that susceptibility to periodontitis may involve a Th2 response to specific types of periodontal bacteria (6). The major type of cytokine produced by Th2 cells are IL-4, IL-5, IL-6 and IL-10 which also assist in antibody production IL-4 is considered as B cell stimulatory factor and promotes immunoglobulin (Ig) class switching to IgE (7). The interleukin 6 (IL-6) is an important parameter in periodontal research because of its role in inflammation and bone resorption by stimulating activity of the osteoclasts (8,9).

During the past decade numerous investigators have shown altered cytokine production in periodontitis and attempted to elucidate their role in periodontal diseases. For example, several studies have demonstrated that localized absence of IL-4 in diseased periodontal tissues is associated with periodontal disease activity and progression (10,11). This led to the hypothesis of Shapira et al. (11) that the absence of IL-4 triggers periodontal disease.

It has been found that IL-1 $\beta$  increased significantly in the periodontal tissues and gingival fluid from diseased sites, compared with healthy sites (12,13,14).

The aim of the present study was to further investigate the role of IL-4, IL-6 and IL-12 in patients with generalized aggressive periodontitis (GAgP) in comparison with control group.

## MATERIALS AND METHODS

**Sample Collection.** Twenty-five Iranian patients aged 20 to 36 years, male and female, with GAgP referred to the Periodontal Clinic of the Ahvaz Jundishapur University of Medical Sciences participated in the study. Informed consent was obtained from all participants with the signed form that was previously reviewed and approved by the Ethical Committee for the use of human subjects in research.

The control group included 25 healthy unrelated patients age matched undergoing surgical and extraction crown lengthening, who were examined by an experienced clinician before inclusion in the study.

Subjects completed personal and familial medical and dental history questionnaires; the exclusion criteria of this study a history of allergy, current pregnancy or lactation,

radiotherapy in the past year, a history of hepatitis or HIV infection and on any medication that could affect cytokine profiles and smoking during 3 month later.

Inclusion criteria were generalized loss of proximal attachment, affecting at least 3 teeth other than the incisors and first molars. At least 5 or 6 teeth in each patient had sites with PD  $\geq$ 5 mm, and all selected patients showed extensive associated bone loss on radiographs. At the time of examination, none of the GAgP patients had been treated previously.

**Surgery Technique.** Pocket reduction included both apical displacement of the flaps and osseous recontouring (elimination of bony defects) to obtain proper pocket elimination, crown lengthening include procedures may be required to solve problems such as (1) inadequate amount of tooth structure for proper restorative therapy, (2) subgingival location of fracture lines, and (3) subgingival location of carious lesions that remove gingiva and bone to solve the problems.

**Blood Sample Collection.** Whole blood from patients was collected during routine periodontal surgery with the use of syringe from surgical site of pocket reduction. During blood collection we put sterile gauze in surgical site to prevent contamination with saliva. Collection of blood from healthy individuals was performed before tooth extraction or crown lengthening from noninflamed sites. Serum samples were separated from the whole blood, aliquated and stored at  $-70^{\circ}\text{C}$  until used.

**Cytokine Assay.** The level of IL-4, IL-6 and IL-12 were determined by enzyme-linked immunosorbent assay (ELISA) kit (Bender Medsystem, S: Germany). Cytokine concentrations were determined with a standard curve derived from known amounts of the relevant cytokine using absorbance readings at 450 nm on a spectrophotometer (TECAN). The minimum detection level for the cytokines was  $< 2$  pg/ml.

**Statistical Analysis.** Comparison between two groups was performed using student's t-test. Data was presented as mean  $\pm$  SEM and  $p < 0.05$  was considered statistically significant

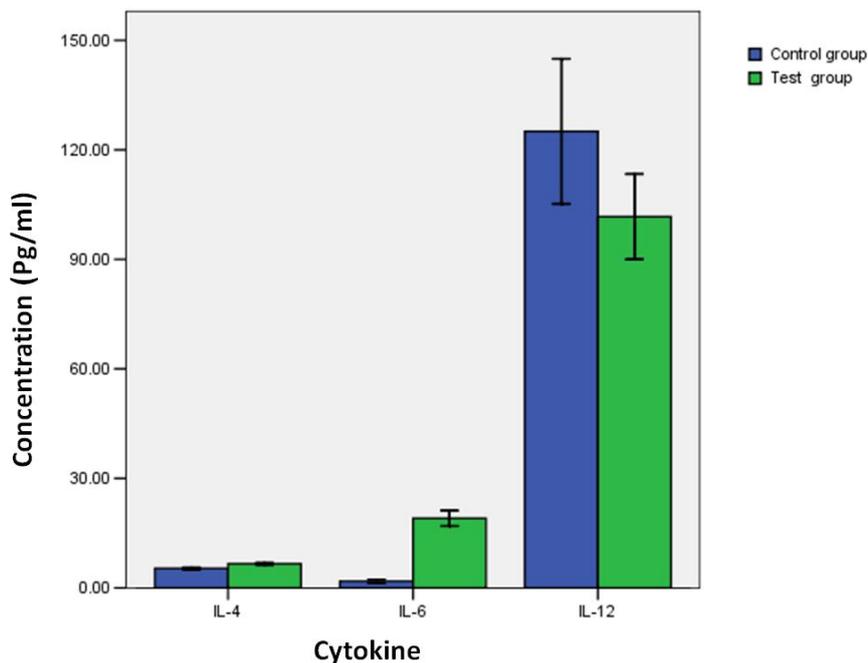
## RESULTS

The results indicated that the cytokines are present in sera of all GAgP patients and control group with different amounts (Figure 1). The level of IL-4 decreased significantly in test group in comparison with control group ( $p=0.002$ ).

However, the level of IL-6 increased significantly in test group in comparison with control group ( $p < 0.0001$ ). Although the level of IL-12 decreased in test group compared with control group, the difference was found to be non-significant ( $p=0.316$ ). Table 1 provides a summary of the statistical analysis in case and control groups.

**Table 1. The level of different cytokines (mean  $\pm$  SEM) in sera of test and control groups.**

Cytokines (pg/ml)	test group (n=25)	control group (n=25)	P value
IL-4	5.33 $\pm$ 0.29	6.60 $\pm$ 0.27	0.002
IL-6	19.07 $\pm$ 2.11	1.81 $\pm$ 0.51	$< 0.0001$
IL-12	101.67 $\pm$ 11.70	125.08 $\pm$ 19.85	0.316



**Figure 1.** The concentration of different cytokines among tests and control groups.

## DISCUSSION

The host defense system and the cells of the periodontium are all linked by complex processes in which soluble mediators and cytokines coordinate tissue turnover, inflammatory processes and immune response. In this study, we analyzed the expression of key cytokines (IL-4, IL-6 and IL-12) that seem to play an important role in the initiation and progression of periodontitis. The study was performed on collected serum samples from 25 patients with documented GAgP and 25 periodontally healthy subjects. The findings of the present study indicate that the concentration of IL-4 in sera of test group was lower compared to that of healthy subjects. In agreement with our study Pradeep et al. (15) reported that the mean concentration of IL-4 decreased from periodontal health to disease in gingival crevicular fluid (GCF). Giannopoulou et al. (16) have also shown IL-4 was higher in the periodontally healthy group, but very low in the periodontal disease group. This is in agreement with Kabashima et al. (11) who reported lack of IL-4 in GCF from severe inflammatory sites. High levels of TNF- $\alpha$  and low levels of IL-4 were observed in diseased sites of generalized aggressive periodontitis individuals (17). Changes in cytokine levels including IL-1 $\beta$ , IL-2, IL-4, IL-8 and IFN- $\gamma$  were also reported in inflamed shallow and deep periodontal sites from patients with generalized chronic periodontitis (GCP) and GAgP, in comparison to shallow sites from subjects with gingivitis using multiplexed bead immunoassay (18). All these findings suggest that localized absence of IL-4 might lead to the development of gingivitis into periodontitis. IL-4 is a very efficient cytokine in inhibition of

production of proinflammatory cytokine, TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and also IL-8 chemokine(19,20)

Contractly, Michel et al. (21) reported 27.8% of their investigated patients were positive for the IL-4 promoter-and intron polymorphisms. But, levels of IL-4 in the sera of patients were below the limit of detection and could not be quantified by ELISA, which corresponds well with the hypothesis of Shapira et al. (10). It has been shown by polyclonal monospecific antibodies that Th2 cell is more abundant than Th1 in periodontal disease, in other words IL-4 increases in comparison to IL-2 in periodontitis granulation tissue (22). This result is consistent with the findings of Manhart et al. (23), Aoyagi et al. (24), and Gemmell et al. (25).

The results of the present study also showed that the level of IL-6 increased in the sera of the patients compared with control group. Our results concerning IL-6 are in agreement with those of other studies, showing higher amounts of IL-6 in patients with periodontitis (18,21,24,26,27,28) as well as in sites undergoing orthodontic movement, emphasizing the role of IL-6 in the bone remodeling process (29). Also, a significantly higher expression of IL-6 and IFN- $\alpha$  mRNA in diseased tissues compared to healthy tissues in adult periodontitis patients is reported (30). Elevated levels of IL-17, TGF- $\beta$ , IL-1 $\beta$ , IL-6, and IL-23 messenger RNA and protein in diseased tissues as well as the presence of Th17 cells in gingiva from patients with periodontitis has also been reported (28).

This study also indicated that the level of IL-12 decreased in test group compared with control group, but there was no significant difference between two groups. According to Tsai et al. IL-12 could be related to the pathogenesis of inflammatory periodontal disease (31). They showed that there is an elevated level of IL-12 in GCF in chronic periodontitis compared to healthy individuals. Very little IL-12 has been detected with levels decreasing with increasing inflammation in GCF samples of periodontitis patients (32). Another study demonstrated no significant difference in IL-12 concentration (33) which is in agreement with our study. However, in order to clarify the role of cellular immunity in pathogenesis of GAgP, other factors should be investigated.

Considering the available data, it seems that the main difference in the studies is related to sample collection, test protocols and genetically different populations. Thus, cytokine assay focusing on local tissue sample or GCF using more advanced methods is needed to establish these findings.

The results of this study indicate that the concentration of IL-4 in sera of GAgP subjects is lower compared with healthy subjects. This data likely highlights the protective role of IL-4 in the disease. Lack of IL-4 may results in a breakdown of the regulation of immune function and enhanced macrophage survival in the inflammatory lesion. The increase level of IL-6 is expression of inflammatory response in GAgP which maybe involved in destruction of the periodontiu in patients. With regard to non-significant enhancement of IL-12, it seems that there is no relationship between cellular immunity and GAgP which requires more investigations.

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