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Serum Interleukin-6 as a Serologic Marker of Chronic Periapical Lesions: A Case-control Study

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Abstract

Background and aims. Chronic periapical disease with pulpal origin is an inflammatory condition caused by bacterial infection. Chronic infection could affect general health by increasing the production of cytokines such as interleukin-6 (IL-6) that probably play a role in pathogenesis of pulpal and periapical diseases. The aim of the present study was a comparative evaluation of the level of serum IL-6 in patients with periapical lesions and healthy controls.

Materials and methods. This analytical case-control study included 40 patients with chronic periapical lesions and 40 individuals without any oral diseases. All of the participants were in good general health. After obtaining an informed consent, clinical and radiographic examinations were carried out and blood samples were collected. Serum IL-6 was measured using ELISA. Data were analyzed using *t*-test by SPSS 14.0 computer software.

Results. Serum IL-6 concentration was significantly higher in test group compared to the controls ($P < 0.05$).

Conclusion. The results of the present study indicate that IL-6 produced in periapical lesions may serve as a marker of pathologic inflammatory activities in chronic periapical lesions.

Keywords: **Cytokine, interleukin-6, periapical lesions.**

Introduction

Periapical lesions are inflammatory diseases that develop as result of root canal bacterial infections[1] and may result in periapical bone destruction because of host defensive–microbial disturbances.[2] The host defensive reactions against root canal pathogens may elicit cellular and humoral immune responses as well as inflammatory processes.[3] Cytokines are low-weight messenger molecules between the host cells secreted by different immune cells and believed to have an important role in treatment and pathogenesis of many inflammatory diseases such as periradicular lesions.[4] The term interleukin (IL) is used to describe a cytokine that leukocytes use for intracellular communications.[5] Interleukin-6 (IL-6) has traditionally been considered to be a pro-inflammatory cytokine that may have a part in inflammatory process of periapical lesions.[6] However, emerging data suggest that IL-6 is a multifunctional cytokine which is produced by several types of immune cells including monocytes, macrophages, Th-2 lymphocytes, activated B cells,

and polymorphonuclear (PMN) cells.[7] IL-6 may also release locally in inflamed pulp and periradicular lesions, especially of chronic types.[8]

Since chronic infections could affect general health by increasing the production of cytokines, levels of these biologic markers can be used to improve the validity of predicting of threatening inflammatory pulpal diseases. In fact, the amount of these molecules in healthy and diseased pulpal tissues is of great importance.[5] Elevated levels of IL-6 and IL-8 in fibroblast subpopulation have been demonstrated in adult periodontitis.[9] Moreover, serum IL-6 amounts were reported significantly higher in more-extensive periodontal lesions compared to normal periodontal tissue.[10]

On the other hand, an increased local production of IL-6 in periapical lesions was observed in several studies;[8,7,11] there was a clear relationship between levels of IL-6 and the extent of tissue damage.[11] However, to the best of our knowledge, little data exist on the correlation of these lesions and changes of IL-6 in serum of affected individuals. Therefore, the purpose of this study was to determine the serum concentrations of IL-6 in patients with chronic periapical lesions and compare the results with healthy controls.

Materials and Methods

The study population consisted individuals referring to the Department of Oral Medicine, Hamadan University of Medical Sciences, from January 2006 to June 2007 to receive dental treatments. Selection criteria included patients with good general health who had a recent panoramic radiograph. Clinical examination was performed according to the standard clinical criteria. Exclusion criteria included (1) known systemic diseases such as diabetes, hepatitis, HIV infection, immunosuppressive chemotherapy, bleeding disorder, severely compromised immune function, and inflammatory or autoimmune diseases like Behçet's syndrome, arthritis, Reiter syndrome, and AIDS; [12] (2) history and/or presence of other infections; (3) specific physiological condition (pregnancy or menstruation); (4) periodontal diseases; (5) any type of oral ulcers; (6) current or previous smoking; (7) treatment with any medication known to affect the serum level of inflammatory markers in the preceding three months; and (8) any clinical signs including spontaneous pain, swelling, and tenderness around the periapical overlying mucosa, pain during percussion, presence of pus or sinus tract as well as positive dental vitality signs/symptoms.

A written informed consent was obtained from each participant after the procedure had been explained to them in detail. For careful evaluation, a periapical radiographic examination was performed after detection of periapical lesion in panoramic radiographic view of involved tooth. Two examiners (an oral medicine specialist and an endodontist) evaluated the radiographs and determined the size of periapical radiolucent area by a digital caliper (Mitutoyo, Illinois, USA). Subjects were divided into two groups according to the radiographic findings: 40 patients with 2–5 mm radiolucent asymptomatic periapical lesions (case group), and 40 patients without any radiolucent periapical lesion (age and gender matched control group). All of the subjects in the case group were asymptomatic at the time of sample collection without any history of previous exacerbation of periapical lesions. The participants of both groups were from the same geographic area.

Fasting venous blood samples were collected all within the time frame between 8 am to 10 am. Serum was extracted from the blood samples by centrifuge and then stored at -70°C for subsequent analysis.

The concentrations of IL-6 were analyzed using a commercial enzyme-linked immunosorbent assay kit (BMS213/2, Bender Med System, Austria). The test was performed according to the manufacture's instructions. Concentration of IL-6 present in serum of participants was calculated with reference to a standard curve that was constructed using 450 nm wave length spectro-photometer (ELISA reader, Averneas, USA) and reported on the scale of pictogram per milliliter (pg/ml).

Statistical differences of serum IL-6 concentrations between case and control groups were determined by student's *t*-test. Values of $P < 0.05$ were considered significant.

Results

This study quantified the levels of IL-6 in serum of 80 participants, with an age range of 17 to 46 years old (Table 1). No significant differences were found between groups regarding age and gender ($P > 0.05$). Table 2 demonstrates the average levels of IL-6 in each group. Serum concentrations of IL-6

Table 1. Demographic data of the study participants

Study groups (number)	Gender (number, %)	Age (mean ± SD)
Case (40)	Female (14, 35%) Male (26, 65%)	27.8 ± 3.1
Control (40)	Female (18, 45%) Male (22, 55%)	23.8 ± 8.2

Table 2. Serum IL-6 concentrations

Study groups (number)	Serum IL-6 level (Mean ± SD)	MD*	SE †	CI‡	t	P-value
Case (40)	1.92±1.55	1.31	0.27	0.59 ≈ 2.03	4.81	0.000
Control (40)	0.61±0.73					

* Mean Difference
† Standard Error
‡ Confidence Interval

were significantly correlated with presence of periapical lesions ($P < 0.05$).

Discussion

The present study was undertaken to establish the association between periapical lesions and increased IL-6 in serum of affected patients. A wealth of information has shown that cytokines have become one of the most important aspects of clinical research because they are new markers for diagnosis or treatment and have been implicated in the pathogenesis of several diseases including peritoneal sepsis, inflammatory bowel disease, systemic sclerosis, cardiovascular diseases and periapical lesions.[13-16] Various cytokines and prostaglandins are produced locally in periapical lesions and serve as important communication factors for cross-regulation of immune responses.[16] Furthermore, these substances may become diagnostic markers of periapical periodontitis.[16] T-cell–derived cytokines are categorized to substances produced by Th-1 such as IFN-γ, IL-2, and IL-1, and those produced by Th-2 including IL-4, IL-10, and IL-13.[11] Elevated levels of prostaglandins and cytokines such as IL-8,[16-18] $IL\text{-}1_{ra}$,[4] IL-6,[4,8,12,19] TGFβ-1,[4] IL-2,[5] IFN-γ,[20] IL-4,[20] IL-1β,[12,19] TNF-α,[19,21] IL-10,[12] and IL-1α[21] were found to be highly associated with periapical lesions in several human and animal studies. IL-6 was also detected in neutrophils from peripheral blood and inflammatory periradicular tissues.[22] It was shown that IL-6 plays a central and complex role in regulation of the immune response.[2] An animal study has demonstrated that IL-6 is a pro-inflammatory mediator because it is produced locally and immediately, following stimulation by IL-1 and TNF-α in the inflammatory cascade.[6] Finally, IL-6 increases the number of osteoclasts derived from colony-forming granulocyte macrophages[6] and has been demonstrated to have the capacity of bone resorption.[11] On the other hand, IL-6 interferes with apoptosis in neutrophils.[22] Kawashima et al[11] observed the production of IL-6 in normal murine periapical tissues and found that it is further induced specially on day 14 after pulp exposure; however, they failed to detect IL-6 mRNA. Redics et al[7] suggested that higher local IL-6 concentrations existed in symptomatic lesions containing epithelial cells, representing an active stage of apical periodontitis, compared to asymptomatic, poor PMN cell, non-epithelialized chronic and healing lesions. In a recent study about periradicular lesions, the effect of a new antibiotic (linezolide) was evaluated on the levels of some cytokines such as IL-6. Concentrations of this cytokine were reported between 0.1 to 2.9 pg/ml in periapical tissue extracts.[4] This slight discrepancy could be attributed to the differences in the type of samples, methods and units used for measuring IL-6. Although cytokines were produced locally in the inflammation area, there was the possibility of an increased cytokine level in the blood as capillaries are located close to the endodontic microbial flora, an assumption confirmed by the present study.

Some in-vitro studies have shown that significant blood levels of IL-6 were produced

by neutrophils stimulated with microbial lipopolisaccharides compared with unstimulated cells. Their findings indicated that there was a dose-dependent increase in IL-6 levels when cells were stimulated with *A. actinomycetecomitans* and *P. gingivalis* extract, the response in the latter being to a lesser extent.[22] These results are compatible with the present study regarding the elevated levels of IL-6. The higher level of IL-6 in the mentioned study compared to our results could be explained by the fact that IL-6 can be produced by a variety of cells such as neutrophils. In other words, direct stimulation of neutrophils might have contributed to a higher level of IL-6 in the blood.

Conclusion

The results of the present study showed significant amounts of IL-6 produced in serum of patients with periapical lesions at a 3.14 fold higher level than healthy controls. This highlights the potential for improving our understanding of IL-6 as a sensitive indicator of inflammation and may be useful for evaluation of periapical periodontitis. These data might provide preliminary information toward establishing a clear relationship between elevated levels of serum IL-6 and chronic periapical lesions.

Since interleukins are of major importance in the pathogenesis of human chronic periradicular lesions, a more comprehensive analysis of different inflammatory mediators may provide better diagnostic indicators of pathogenesis of periradicular lesions as well as prognostic indicators of response to the medical treatment.

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