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Gingival Crevicular Fluid IL-33 Levels in Gingival Inflamation

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Abstract: This study aimed to examine the levels of interleukin-33 in Gingival Crevicular Fluid (GCF) in gingival inflammation. A total of 20 subjects, 10 patients with gingivitis and 10 healthy subjects were included in this study. Periodontal status was evaluated GCF interleukin-33 levels were analyzed by ELISA. Gingivitis patients had similar GCF interleukin-33 levels to healthy group ($p>0.05$). The present study demonstrated that interleukin-33 in GCF of patients with gingival inflammation was similar to healthy ones.

Key words: GCF, IL-33, ELISA, patients, inflammation

INTRODUCTION

Dental plaque-induced gingivitis is the most prevalent disease that affects the periodontium. Microbial dental plaque is considered to be the key etiological factor associated with the development of gingivitis (Kornman *et al*., 1997).

Cytokines, small polypeptides with a wide spectrum of inflammatory, hemopoietic, metabolic and immunomodulatory properties are produced by a variety of cells including the macrophage/monocyte system, dendritic cells, lymphocytes, neutrophils, endothelial cells and fibroblasts (Callard and Turner, 1990). As a consequence, cytokines, together with their receptors form a network of high complexity that is under tight but complex biological control including positive and negative feedback by the cytokines themselves. It is well known that immunity depends on two major types of specific immune responses, termed the cellular and humoral responses. Cytokines play an important role in controlling inflammatory processes and tissue homeostasis. Their pleiotropic action includes numerous effects on the cells of the immune system and modulation of inflammatory responses. The analysis of cytokine production levels has been also used as a tool for studying the local host response to a bacterial challenge. Interleukin (IL)-33 is an important member of the IL-1 family that has pleiotropic activities in innate and adaptive immune responses in host defense and disease. The aim of the present study was to examine gingival crevicular fluid IL-33 levels in gingival inflammation.

MATERIALS AND METHODS

Study population: A total of 20 subjects were included in this study. All consecutive subjects were recruited from the Department of Periodontology, School of Dentistry, Ege University, Izmir, Turkey. The use of human participants satisfied the requirements of Ege University Institutional Review Board. The purpose of the study was completely explained to each subject before entering the study and informed consent was obtained from each subject in accordance with the Helsinki Declaration of 1975 (revised in 2000). Complete medical and dental histories were taken from all subjects. All of the patients were non-smokers had at least 20 teeth in the mouth. None of the subjects had a history of systemic disease and had received antibiotics or other medications or periodontal treatment within the past 4 months. Patients with severe medical disorders including diabetes mellitus, immunological disorders and pregnant females were excluded from the study. The study of the patients was made according to the clinical and radiographic criteria (Armitage, 1999). The gingivitis group included 4 females and 6 males with varying degrees of gingival inflammation but no signs of attachment loss were observed. These patients ranged in age from 15-53 (mean age 32.6±11.1 years) (Table 1). The healthy group consisted of 5 females and 5 males who exhibited

Table 1: Demographical variables between groups

Characteristics	Gingivitis	Healthy
Age	32.6±11.1	35.9±13.6
Gender	4F/6M	5F/5M

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PPD <3 mm and no clinical attachment loss, clinical inflammation, sulcular bleeding and radiographic evidence of bone loss (mean age 35.9±13.6 years; range 22-62 years). These individuals were healthy volunteers from the Department of Periodontology. At the screening stage to determine the clinical periodontal status. Dichotomous measurement of supragingival plaque accumulation and bleeding on probing were also recorded.

Collection of GCF samples: After being selected for the study, GCF sampling was done from one approximal site of a tooth with bleeding and ≥2 mm probing depth in the gingivitis group. In the healthy group, GCF samples were collected from one approximal site of a tooth with ≤2 mm probing depth. Prior to GCF sampling, the supragingival plaque was removed from the interproximal surfaces with a sterile curette; these surfaces were dried gently by an air syringe and were isolated by cotton rolls. GCF was sampled with filter paper. Paper strips were carefully inserted into the crevice until mild resistance was felt and left there for 30 sec (Lamster *et al*., 1985). Care was taken to avoid mechanical injury. Strips contaminated with blood were discarded (Cimasoni, 1983). The absorbed GCF volume of each strip was determined by electronic impedance (Periotron 8000, ProFlow, Inc., Amityville, NY. USA) and placed into a sterile eppendorff vials and kept at -40°C until being analyzed. The readings from the Periotron 8000 (Periotron 8000, ProFlow, Inc., Amityville, NY, USA) were converted to an actual volume (μL) by reference to the standard curve.

GCF processing: The GCF IL-33 were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) for quantification of this protein in the GCF samples. Manufacturers' guidelines were followed for each assay and 96 well plates precoated with appropriate antibodies were used. The amounts of IL-33 in each sample were calculated based on the dilutions and the results were expressed as total cytokines in the 30 sec GCF sample.

Statistical analysis: Statistical analysis was performed using non-parametrical techniques. Comparisons between the study groups were performed using the Mann-Whitney U-test. The $p<0.05$ were considered to be statistically significant. All data analysis was performed using a statistical package.

RESULTS

Gingivitis patients had significantly higher percentage of sites with bleeding on probing and plaque compared to the healthy group ($p<0.008$).

Table 2: Correlations between IL-33 levels between groups

Characteristics	Gingivitis	Healthy
Interleukin-33	14.67±8.2	19.03±7.1

‡p<0.05

GCF IL-33 levels: Gingivitis patients had elevated GCF IL-33 levels compared to healthy (Table 2, $p>0.008$).

DISCUSSION

The analysis of cytokine production levels has been also used as a tool for studying the local host response to a bacterial challenge. The Interleukin-1 (IL-1) family of cytokines are important mediators of destructive inflammatory disorders such as rheumatoid arthritis and periodontitis. Several biomarkers can be detected in the Gingival Crevicular Fluid (GCF) which provides a convenient diagnostic fluid to assess the levels of inflammatory mediators released during periodontal disease progression. Increased levels of several proinflammatory cytokines in GCF have been associated with different periodontal disease such as Interleukin (IL)-1β, IL-2, Interferon (IFN)-γ and IL-8. However, little is known about the role and presence of the novel or newer cytokines in periodontal diseases and especially in GCF. Therefore, IL-33 in GCF from patients with different periodontal diseases were analyzed by ELISA in the present study.

It has been generally accepted that analysis of GCF constituents can provide information of association between specific metabolic change and disease status (Emingil *et al*., 2004). One important consideration in the analysis of host mediators in GCF is the method of presenting the data. Some researchers have stated that expression of GCF data as total amount per standardized sampling time is a more sensitive way than reporting them as concentration (Lamster *et al*., 1986). Furthermore, biochemical GCF analysis involving the noninvasive sampling technique when combined with the clinical assessments of the disease could provide information about the specific metabolic changes in periodontium reflecting the clinical disease status. In the present study, IL-33 could be detected in GCF samples collected from patients with gingival inflammation. Similar GCF IL-33 levels were somewhat surprising, considering the proinflammatory effects of IL-33.

It has been shown that pro and antiniflammatory mediators are associated with periodontal disease as evidenced by the presence of these molecules in the GCF. Conflicting results concerning nodes in the cytokine network obtained in different studies may be related to differences in the selection of patients whose disease activities and clinical stages vary considerably

(Hanioka *et al*., 2000). Furthermore, researchers encounter redundant biological activities of different cytokines that researchers do not understand today. Local, site-specific, cellular immunoregulatory disarrangement may take place in periodontal disease (Gemmell *et al*., 2000). The cytokine profiles are expected to show intra-individual and intra-site characteristics (Golub *et al*., 1997). IL-33 might regulate leukocyte migration to the inflammatory sites and could mediate the destruction of infected host tissues extracellular matrix and basement membrane proteins. In the present study, researchers could find interleukin-33 in the healthy group's GCF.

CONCLUSION

Identification of novel cytokines as molecular markers which correlated with periodontal disease amend and further extend the understanding of the pathogenesis of periodontal diseases. Additional studies are necessary to clear the role, regulation and function of these cytokines in the pathogenesis of periodontal disease.

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