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Gingival Crevicular Fluid Annexin-A1 Levels in Gingival Inflamation

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Abstract: This study aimed to examine the levels of annexin-1 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 annexin-1 levels were analyzed by ELISA. Gingivitis patients had similar GCF annexin-1 levels to healthy group ($p>0.05$). The present study demonstrated that annexin-1 in GCF of patients with gingival inflammation was similar to healthy ones.

Key words: GCF, annexin A1, ELISA, group, patient

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).

It is now known that lipocortin is the first characterized member of the annexin superfamily of proteins, so called since their main property is to bind (i.e., to annex) to cellular membranes in a Ca^{+2} dependent manner (Callard and Turner, 1990). Annexin A1 are ubiquitous proteins described in many organisms from mammals to moulds and even plants and implicated in several aspects of cell biology (Brownstein *et al*., 2004). A conserved core domain that contains either four or eight repeating units of approx. The 70 amino acids defines annexins structurally. The conserved repeats account for the shared abilities of the proteins to bind phospholipids whereas the specific functions of each annexin are probably related to their specific N-terminal regions (Swisher *et al*., 2007). As the first member of the family, lipocortin is now referred to as annexin 1. Scope of this review is to discuss the recent evidence on the mechanisms of the anti-inflammatory effects of annexin 1.

It is well established that one of the major anti-inflammatory properties of glucocorticoids is the ability to inhibit leukocyte migration. Annexin A1 may have profound effects on migration of neutrophils and monocyte/macrophages thereby mediating the inhibitory action of glucocorticoids. Annexin A1 an N-terminus fragment of the protein was also able to inhibit leukocyte migration annexin A1 role as a receptor for viruses and bacteria, to activate phagocytosis and to act as chemoattractant in the recruitment of key lymphocyte populations and in cell differentiation suggests its effective participation in microbiota clearance, inflammation and immune response (Armitage, 1999). The aim of the present study was to examine gingival crevicular fluid annexin a1 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). The purpose of the study was completely explained to each subject before entering the study and informed consent was obtained from each subject. 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

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disorders and pregnant females were excluded from the study. The selection of the patients was made according to the clinical and radiographic criteria (Armitage, 1999). The gingivitis group included 7 females and 8 males with varying degrees of gingival inflammation but no signs of attachment loss were observed. These patients ranged in age from 15-57 (mean age 34.4±10.2 years). The healthy group consisted of 8 females and 7 males who exhibited PPD <3 mm and no clinical attachment loss, clinical inflammation, sulcular bleeding and radiographic evidence of bone loss. These patients ranged in age from 21-62 (mean age 36.3±14.3 years) (Table 1). 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 (Crumpton and Debman, 1990).

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) and placed into a sterile eppendorff vials and kept at -40°C until being analyzed. The readings from the Periotron 8000 were converted to an actual volume (μL) by reference to the standard curve.

GCF processing: The GCF annexin a1 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 annexin a1 in each sample were calculated based on the dilutions and the results were expressed as total cytokines in the 30 sec GCF sample.

Table 1: Demographical variables between groups

Characteristics	Gingivitis	Healthy
Age	34.4±10.2	36.3±14.3
Gender	7F/8M	8F/7M

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 (Abacus Concept Inc., Berkely, CA).

RESULTS AND DISCUSSION

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

GCF annexin A1 levels: Gingivitis patients had elevated GCF annexin a1 levels compared to healthy (Table 2, $p>0.008$).

The role of annexin a1 as a mediator of anti-migratory effects of glucocorticoids was reinforced by data by Emingil *et al.* (2004) who showed using an intravital microscopy technique that the *in vivo* administration of an antiinflammatory dose of dexamethasone increased annexin A1 levels in circulating leukocytes. Most of the adherent leukocytes subsequently detached and returned to the blood stream whereas those that had entered into the diapedesis process exhibited a 3-4 fold longer latency than control cells before transmigration.

The analysis of proteine production levels has been also used as a tool for studying the local host response to a bacterial challenge. Several protein 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. However, little is known about the role and presence of the novel or newer cytokines in periodontal diseases and especially in GCF. Therefore, annexin A1 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 (Lamster *et al*., 1986). 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 (Ferlazzo *et al*., 2003). Furthermore,

Table 2: Correlations between annexin a1 levels between groups

Level	Gingivitis	Healthy
Annexin A1	13.08±7.4	10.64±6.8

$p<0.05$

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, annexin a1 could be detected in GCF samples collected from patients with gingival inflammation. Similar GCF annexin a1 levels were somewhat surprising, considering the proinflammatory effects of annexin A1.

It has been shown that pro and anti-iflammatory 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 (Young *et al*., 1999). Furthermore, researchers encounter redundant biological activities of different cytokines that we do not understand today. Local, site-specific, cellular immunoregulatory disarrangement may take place in periodontal disease (Cunha *et al*., 1992). The cytokine profiles are expected to show intra-individual and intra-site characteristics (Perretti *et al*., 2001). Annexin A1 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 annexin A1 in the healthy group's GCF.

CONCLUSION

Identification of novel proteins 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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