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Evaluation of gingival crevicular fluid cyclophilin a and extracellular matrix metalloproteinase inducer levels in different periodontal diseases

Gülnihal Eren[a,*], Oya Türkoğlu[a], Harika Atmaca[b], Gül Atilla[a]

[a] Ege University, School of Dentistry, Department of Periodontology, İzmir, Turkey
[b] Celal Bayar University, Faculty of Science, Department of Biology, Manisa, Turkey

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ABSTRACT

Objective: Cyclophilin A (CypA) is able to regulate inflammatory responses and matrix metalloproteinase production via its interaction with extracellular matrix metalloproteinase inducer (EMMPRIN). EMMPRIN is the cell surface receptor of CypA. The aim of the present study was to evaluate the gingival crevicular fluid (GCF) CypA and EMMPRIN levels in patients with chronic periodontitis (CP), generalized aggressive periodontitis (G-AgP) and periodontally healthy controls.
Methods: Twenty CP patients, 19 G-AgP patients and 20 healthy control subjects were included in the present study. All study participants were non-smokers. Full mouth clinical periodontal parameters including probing depth, clinical attachment level, plaque index, and papilla bleeding index were recorded. GCF CypA and EMMPRIN levels were analyzed by enzyme-linked immunosorbent assay. Data were analyzed statistically with parametric and non-parametric tests.
Results: GCF CypA total amount was higher in the G-AgP group compared to healthy controls ($p < 0.05$), whereas CypA total amounts were similar in CP and healthy controls ($p > 0.05$). No significant difference in GCF CypA total amount between CP and G-AgP was observed ($p > 0.05$). Also, there was no significant difference in GCF EMMPRIN total amounts among the study groups ($p > 0.05$).
Conclusion: Higher levels of GCF CypA in patients with G-AgP might demonstrate that CypA is associated with the inflammatory infiltrate and alveolar bone destruction of G-AgP. However, GCF CypA level does not seem to be affected by CP. Similar GCF EMMPRIN levels in diseased and healthy groups might suggest that EMMPRIN has role in the turn over of connective tissues in physiological conditions as well as pathological state.

1. Introduction

Periodontitis is an inflammatory disease initiated by oral microbial biofilm and resulting in the destruction of periodontal tissues. The inflammatory response in periodontitis includes leukocyte recruitment and releasing inflammatory mediators and cytokines from the general circulation into gingival connective tissue (Ishikawa, 2007). Infiltrating neutrophils, lymphocytes and monocyte/macrophages secrete various mediators. These mediators and cytokines appear as a key step in the pathogenesis of periodontal diseases and initiate the synthesis of matrix metalloproteinases (MMPs), which contribute to periodontal tissue destruction during periodontitis (Kinane & Attstrom, 2005; Potempa, Banbula, & Travis, 2000). Several members of the MMP family have been proven to be involved in connective tissue breakdown and expression of MMPs, including complex interactions between cell surface receptors, extracellular matrix and cytokines (Birkedal-Hansen et al., 1993).

MMP activity is regulated by several inducers and inhibitors (Gabison, Hoang-Xuan, Mauviel, & Menashi, 2005; Tang et al., 2005). Extracellular matrix metalloproteinase inducer (EMMPRIN) is a transmembrane glycoprotein belonging to immunoglobulin superfamily. It was initially isolated from the surface of tumor cells (Biswas et al., 1995). It exhibits several molecular and cellular characteristics, yet one major function of EMMPRIN is contribution to both release and upregulation of several MMPs (Biswas et al., 1995). Immunohistochemical studies reported expression of

* Corresponding author at: Ege University, School of Dentistry, Department of Periodontology, 35100 Bornova, İzmir, Turkey.
E-mail address: gulnihal_karasu@hotmail.com (G. Eren).

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EMMPRIN was found in both normal and periodontitis-affected human gingival tissues (Dong, Xiang, Li, Cao, & Huang, 2009; Xiang, Cao, Dong, & Li, 2009). Also, increased EMMPRIN levels were detected in the gingival crevicular fluid (GCF) of patients with periodontitis by Western blot technique (Emingil et al., 2006).

Cyclophilin A (CypA) has been first originally identified as an intracellular ligand for the cyclosporine A (Yurchenko et al., 2002) and also can be released extracellularly by macrophages in response to inflammatory stimuli (Nabeshima et al., 2006; Sherry, Yarlett, Strupp, & Cerami, 1992). CypA is able to stimulate the production of MMPs in some diseases, such as rheumatoid arthritis and atherosclerosis (Wang et al., 2010; Seizer et al., 2010). Significant levels of CypA have also been measured in synovial fluid and plasma in patients with rheumatoid arthritis and coronary heart disease (Satoh et al., 2013; Billich, Winkler, Aschauer, Rot, & Peichl, 1997). It has been shown that CypA is associated with alveolar destruction in periodontitis in rats (Liu et al., 2010) and it is able to regulate inflammatory responses via interaction with its cell surface receptor EMMPRIN (Yurchenko et al., 2002). Liu, Li, Xiang, Dong, and Cao, (2013) showed increased CypA immunostaining cells in the extracellular matrix of inflamed human gingival tissues. Also, they stated that CypA might participate in human periodontitis through inducing the chemotaxis of mononuclear cells and neutrophils (Liu et al., 2013).

CypA and EMMPRIN expression were demonstrated in gingival tissues of periodontitis patients by immunohistochemical analyses and CypA–EMMPRIN interaction was shown in the gingival tissues with periodontitis (Dong et al., 2009; Xiang et al., 2009; Liu et al., 2010, 2013). However, there are few studies investigating GCF EMMPRIN levels in periodontitis patients, and Western blot technique has been used in these studies (Emingil et al., 2006; Emingil, Atilla, Sorsa, & Tervahartiala, 2008). To the best of our knowledge, no study has investigated the levels of CypA and EMMPRIN in GCF by the enzyme-linked immunosorbent assay (ELISA) from patients with periodontitis. CypA–EMMPRIN interaction is related to chemotaxis, production of matrix degrading enzymes and expression of cytokines (Kim et al., 2005; Yang et al., 2008). CypA and EMMPRIN have several roles in inflammation and GCF is considered to reflect ongoing events in the periodontal tissues. In the present study, it was hypothesized that GCF CypA and EMMPRIN levels might be increased by the presence of periodontal inflammation. Therefore, we aimed to evaluate the CypA and EMMPRIN levels of patients with periodontitis and subjects with healthy periodontium in GCF with ELISA technique.

2. Materials and methods

2.1. Study population

A total of 59 subjects, 20 patients with CP, 19 patients with G-AgP and 20 subjects with healthy periodontium were recruited from Ege University, School of Dentistry, Department of Periodontology during the period between October 2014 and August 2015. The purpose and procedures were fully explained to all participants prior to participation and written informed consent was obtained from in accordance with Helsinki Declaration. This study protocol was approved by the Ethical Committee of Ege University School of Medicine (No: 15-1/2). The subjects in study groups were selected according to criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions (Armitage, 1999). In order to confirm the clinical periodontal diagnosis, radiographs were also taken to evaluate alveolar bone destruction. All study participants had a minimum of 16 teeth in their mouth. Smokers and former smokers, patients with systemic diseases, immunological disorders, hepatitis, and HIV infections were excluded from the study. Patients who were pregnant or breastfeeding and those taking oral contraceptive drugs were also excluded from the study. Subjects who had received antibiotics within the last 3 months or treatment for periodontal disease within the last 6 months were not included into the study.

2.2. Clinical examination

The clinical periodontal parameters including probing depth (PD) (mm) and clinical attachment level (CAL) (mm) were assessed at six sites per tooth excluding third molars with Williams periodontal probe (Hu Friedy, Chicago, IL, USA). Plaque index (PI) (Quigley & Hein, 1962), papilla bleeding index (PBI) (Muhlemann & Son, 1971) and bleeding on probing (BOP) (Ainamo & Bay, 1975) were also determined. All clinical measurements were performed by a calibrated examiner (G.E.) 1 day before GCF sampling. The intra-examiner reliability was revealed by intraclass correlation coefficient and it was 0.86 and 0.87 for PD and CAL measurements, respectively.

2.3. Study groups

2.3.1. Chronic periodontitis (CP) group

Twenty CP patients aged between 31 and 60 years (8 females and 12 males, mean age 47.2 ± 7.3 years) were included. They had at least four non-adjacent teeth with sites with CAL ≥ 5 mm and PD ≥ 6 mm.

2.3.2. Generalized aggressive periodontitis (G-AgP) group

A total of nineteen G-AgP patients aged between 23 and 39 years (12 females and 7 males, mean age 30.8 ± 5.6) were included. These patients demonstrated severe destruction of periodontal tissues and CAL ≥ 5 mm and PD ≥ 6 mm on eight or more teeth, at least three of these were other than central incisors of first molars.

2.3.3. Healthy control group

Twenty subjects with healthy periodontium aged between 21 and 60 (10 females and 10 males, mean age 38.9 ± 9.4 years) were included in healthy control group. Subjects had no sites with >2 mm CAL and >3 mm PD. Also, they had BOP score $<15\%$ at examination and no alveolar bone loss present in radiographs.

2.4. GCF sampling

GCF samples were obtained from buccal aspects of the mesial or distal interproximal sites of a single rooted tooth. In the CP and G-AgP groups, GCF samples were collected teeth that exhibited PD ≥ 6 mm and CAL ≥ 5 mm. In the healthy group, GCF samples were taken from one site of a tooth exhibiting PD ≤ 3 mm and no BOP. Before sampling, supragingival plaque was removed from the interproximal surfaces with a sterile curette and the surface was gently dried by an air syringe and the sampling sites were isolated by cotton rolls before GCF sampling. Paper strips (Periopaper, ProFlow Inc., Amityville, NY, USA) were gently placed 1 mm into the crevice and remain for 30 s (Lamster, Oshrain, & Gordon, 1986). Attempts were made to avoid the insertion of the strips to the full depth of the pocket, to minimize the risk of contaminating the GCF with blood. Strips contaminated with blood were discarded and an alternative site was sampled. The absorbed GCF volume of each strip was calculated by an electronic device (Periotron 8000; Oraflow Inc., Plainview, NY, USA). Then each strip was placed into a dry sterile polypropylene tube, frozen immediately at chair side and kept at $-40\,^{\circ}C$ until being analyzed. A standard curve was taken as a reference to convert the readings from the electronic device to an actual volume (μl).