Cytokine measurements in gingival crevicular fluid and periodontal ligament: Are they correlated?

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Introduction: Mechanical stress can induce molecular changes in gingival crevicular fluid (GCF) and the periodontal ligament (PDL). It is still not clear whether changes in the PDL and GCF are linked. In this study, we aimed to analyze the expression of cytokines in GCF and PDL after mechanical stress. **Methods:** Twenty-three healthy patients were included. The experimental group consisted of premolars subjected to a force of 0.980 N for 1, 3, 7, 14, 21, or 28 days. The contralateral teeth were the controls. GCF and PDL samples were collected at the same time points for analysis of cytokines using the cytometric bead array. **Results:** Interleukin (IL)-6 (IL-6) production was significantly elevated in the PDL on day 1 after force application. Significantly strong positive correlations between GCF and PDL in experimental group were seen on days 3 (interferon-gamma), 7 (IL-10), 14 (IL-17A), and 28 (IL-17A, tumor necrosis factor-alpha), and significantly strong negative correlation were seen on days 14 (interferon-gamma) and 21 (IL-2, IL-10). **Conclusions:** Different patterns of IL-6 expression were seen in the PDL and GCF after mechanical stress. Despite occasional correlations between GCF and PDL, the molecular contributions of the PDL to the GCF changes could not be clearly defined by our model. (Am J Orthod Dentofacial Orthop 2015;148:293-301)

Gingival crevicular fluid (GCF) is a transudate of interstitial tissues produced by an osmotic gradient.[1,2] This initial preinflammatory fluid, on stimulation, becomes an inflammatory exudate, approaching serum concentrations.[3] It contains a mixture of molecules from blood, host tissue, and plaque biofilms, such as electrolytes, proteins, small molecules, cytokines, antibodies, enzymes, and bacterial antigens.[3,4] Under conditions that induce bone and connective tissue remodeling, such as periodontitis and orthodontic tooth movement, there is a release of inflammatory mediators in GCF and the periodontal ligament (PDL). In these circumstances, augmented levels of different cytokines have been reported.[1,2,4-7] GCF provides a unique window for the analysis of periodontal conditions. However, there is no practical and accurate periodontal indicator based on GCF because of extremely complex issues such as periodontal diseases, their microbial causes, cell regulations, and tissue reactions during inflammation and healing.[3,8] In addition, the information regarding the release of molecules in GCF after orthodontic tooth movement is still limited.[9]

Although GCF is considered to be associated with changes in the PDL,[10] no studies are available that have concomitantly evaluated cytokine expression in GCF and PDL. It is hypothesized that mechanical stimuli might trigger similar molecular changes in the PDL and GCF. It is still not clear whether these changes are, in part, linked. The aim of this study was to estimate

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the contributions of the PDL to the GCF changes, by concomitantly evaluating the molecular profiles in these 2 areas with mechanical stimuli.

MATERIAL AND METHODS

Twenty-three patients (11 male, 12 female), aged 10 to 24 years (mean, 13.43 ± 2,64 years), seen in the Department of Pediatric Dentistry and Orthodontics, Faculty of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, from August 2011 to July 2013, were selected to participate in this study. All patients required extraction of their premolars for orthodontic reasons, such as crowding, dental biprotrusion, or Class II malocclusion, before orthodontic treatment. The inclusion criteria were as follows: (1) healthy patients with no evidence of type 1 or type 2 diabetes mellitus or osteoporosis; (2) patients who had not taken systemic antibiotics, or anti-inflammatory or hormonal drugs for 6 months before the study; (3) patients who required tooth extractions before treatment with fixed appliances; and (4) patients with good periodontal health and no radiographic evidence of periodontal bone loss.

This study was approved by our institutional ethics committee (protocol number 175.320/2012) and conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from each participant and his or her guardian if the patient was less than 18 years of age. At the first appointment (baseline), the Silness and Löe plaque index[11] and the gingival bleeding index[12] were recorded to ensure that the patients had no periodontal alteration at the start of the study. The mandibular or maxillary premolars without orthodontic appliances were used as the controls. The experimental group consisted of the contralateral teeth from the same arch that previously received orthodontic mechanical loading. The orthodontic appliance consisted of 0.022 × 0.028-in light Roth tubes (Eurodonto, Curitiba, Paraná, Brazil) and brackets (Aditek do Brasil, Cravinhos, São Paulo, Brazil) bonded with Transbond XT (3M Unitek, Monrovia, Calif). A 0.017 × 0.025-in beta-titanium alloy cantilever and a 0.010-in metallic ligature (Morelli Orthodontics, Sorocaba, São Paulo, Brazil) were placed between the premolar and the first molar on the same side by an orthodontist (D.F.M.) (Fig 1, *A*). A force in the apical direction was applied to the premolar. The force magnitude was 0.980 N, measured with a digital tensiometer (model FGV-1X; Nidec-Shimpo, Itasca, Ill) that was perpendicular to cantilever (Fig 1, *B*). No other forces were applied to the teeth before or during this phase. The experimental teeth were randomly selected. If a patient had 4 premolars to be extracted, pairs of teeth were allocated to 2 time points. The patients were instructed about proper oral hygiene. After mechanical stimuli, the teeth were extracted at these time points: 1, 3, 7, 14, 21, or 28 days. Just before the extractions, the force magnitude was measured again. The experimental and control teeth were extracted at the same time. Several time points were chosen to allow the evaluation of cytokine fluctuation after mechanical stimuli concomitantly in the PDL and GCF. After the experimental period, the patients were referred to start orthodontic treatment.

GCF samples were collected using periopaper strips (gingival fluid collection strips; Oraflow, Smithtown, NY) at the mesiobuccal and distobuccal sites of the control and experimental premolars. The teeth were previously isolated with cotton rolls, cleaned of plaque deposits, and dried gently with air before the paper strips were carefully inserted 1 mm into the gingival crevice for 30 seconds. Samples of GCF absorbed by the periopapers were measured using a calibrated Periotron 8000 (model 400 8000 precision gingival crevice fluid meter; Oraflow). Readings from the Periotron were converted to an actual volume (microliters) by reference to a standard curve calibrated with human serum.[13,14] The PDL of each extracted tooth was taken from all root surfaces and immediately scraped using a 13/14 Gracey curette (Maximus, Contagem, Minas Gerais, Brazil) (Fig 1, *C*). All tooth extractions and sample collections were performed by 1 professional (D.F.M.).

Samples of GCF and PDL were separately placed in sterile tubes that were immediately immersed in liquid nitrogen, and then kept frozen at −80°C for further analysis. Afterward, the GCF and PDL samples were separately homogenized in phosphate-buffered saline solution (0.4 mmol/L of sodium chloride and 10 mmol/L of sodium phosphate containing protease inhibitors [0.1 mmol/L of phenylmethylsulfonyl fluoride, 0.1 mmol/L of benzethonium chloride, 10 mmol/L of ethylenediaminetetraacetic acid, 0.01 mg/mL of aprotinin A, and 0.05% Tween-20 at 1 mg/mL]). The mixture was agitated on vortex for 30 seconds and then centrifuged (12.000 g) for 10 minutes at 4°C. Supernatants were collected and assayed with the cytometric bead array (CBA). Analyses of PDL and GCF were determined using a BD CBA Human Th1/Th2/Th17 Cytokine Kit (Becton, Dickinson and Company, BD Biosciences, San Diego, Calif) and analyzed on a BD FACSCalibur flow cytometer (Becton, Dickinson and Company). The concentrations of analytes (interleukin [IL]-2, IL-4, IL-6, IL-10, IL-17A, interferon-gamma [IFN-γ], and tumor necrosis factor-alpha [TNF-α]) were measured using a standard curve according to the manufacturer's instructions. The sensitivity of the assays was 20 to 5000 picograms per milliliter for all