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Research Article

Do Changes of Osteoprotegrin and Matrix Metalloproteinase-8 Levels in Gingival Fluid may Influence Orthodontic Tooth Movement Procedure?

Laila Abdel-Fattah Amer[1]*, Hisham A Abozeid[2] and Mostafa M Hosny[3]

[1]Lecturer of Orthodontics, Faculty of Oral and Dental Medicine, MTI University, Cairo, Egypt

[2]Lecturer of Periodntology and Oral Medicine, Faculty of Dental Medicine, Al-Azhar University, Egypt

[3]Assistant. Professor of Periodntology and Oral Medicine, Faculty of Dental Medicine, Al-Azhar University, Egypt

***Corresponding Author:** Laila Abdel-Fattah Amer, Lecturer of Orthodontics, Faculty of Oral and Dental Medicine, MTI University, Cairo, Egypt.

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Abstract

Background: The ratio between RANKL and Osteoprotegerin (OPG) regulated the Osteoclasts activity, while MMPs activity accounts for matrix turnover/destruction, and modulation of the immune response. So, are they can influence the teeth movement during orthodontic treatment?

Aim: The present study was designed and undertaken to evaluate the possible reflection of changes in GCF-OPG and GCF-MMP-8 levels on orthodontic tooth movement procedure, and significance of clinical health status in their levels.

Material and Methods: Ten patients were included; gingival health status was assessed with Gingival index scores, and GCF samples were collected to measure the OPG and MMP-8 levels at baseline, 2 weeks, 6 weeks and 3 months later after orthodontic tooth movement initiation, applying ELISA technique to measure their levels in GCF according manufacturer's instruction.

Results: Clinical healthy gingival status showed changes at 2, 6 weeks, and 3 months, according to criteria of Gingival Index, compared to those recorded at baseline; but did not reach to significance, except between recorded scores at 6 weeks with baseline ($p < 0.05$). The GCF level of OPG was higher at baseline than those at 2 weeks, 6 weeks, and 3 months later; values were decreased at all intervals compared to baseline value. MMP-8 in GCF at baseline showed higher level compared to that measured at 2 weeks, 6 weeks, and 3 months. The measured levels at 2 weeks, 6 weeks and 3 months did not significantly differ ($p > 0.05$).

Conclusion: MMP-8 and Osteoprotegin GCF level changes can be used as valid biomarkers assessing the bony changes induced by orthodontic teeth movement and reflecting the associated periodontal tissues events.

Keywords: Osteoprotegrin; Matrix Metalloproteinase-8 Levels; Orthodontic Tooth Movement; GCF

Introduction

Orthodontic tooth movement is a complex procedure that includes interactions between the alveolar bone cells and periodontal ligament (PDL) cells, along with multiple intercellular actions. It includes bone resorption on compression side and bone formation on tension side, which result in changing tooth position within the alveolar bone [1]. Mechanical stress from orthodontic appliances induces PDL cells to produce biologically active substances as cytokines and enzymes responsible for connective tissue remodeling [2]. Exerted forces exerted on the tooth is associated with PDL's changes that may modify the flow rate and composition of gingival crevicular fluid (GCF) [3]. Orthodontic movement promotes remodeling of alveolar bone mediated by inflammatory-like reactions characterized by vascular changes and infiltration of leukocytes [4]. These substances can be monitored through non-invasive procedures by measuring changes in composition of GCF and saliva during orthodontic treatment. RANKL is a ligand of osteoprotegerin/osteoclast genesis-inhibitory factor (OPG/OCIF) and is expressed in plasma membranes of osteoblasts/stromal cells, [5] it induces osteoclast differentiation from hemopoietic precursors and stimulates their bone resorption. OPG is a novel soluble decoy receptor termed "bone protector" as it protects the skeleton from excessive bone resorption [6] Cellular sources of OPG include osteoblasts, endothelial cells, fibroblasts, and vascular/smooth muscle cells [7]. OPG induce apoptosis of mature osteoclasts, and alveolar bone destruction is associated with an imbalance in RANKL and OPG [8-10]. Thus, signaling and regulation of RANKL/OPG expressions may play critical roles in bone remodeling during orthodontic tooth movement.

Tooth movement initiation by orthodontic force is a type of tissue injury that leads to an inflammatory response. Orthodontic force application causes disruption of homeostasis within periodontal microenvironment, and microcirculation of periodontal ligament (PDL) is disturbed as well [11]. Mechanic-stress-induced interleukin-8 from periodontal ligament cells regulate osteoclast genesis and play role in efficient orthodontic tooth movement. Biologic effects of OPG on bone cells include inhibition of terminal stages of osteoclast differentiation, suppression of activation of matrix osteoclasts, and induction of apoptosis. RANKL and OPG were detected in human GCF; RANKL was elevated whereas OPG was decreased in periodontitis [12]. OPG concentrations are high in developing bone and its expression is increased by bone morphogenetic protein, IL- 1, TNF, TGF and estrogen [13,14]. Subsequently, OPG levels found to be decreased with PGE2, Vitamin D3 and parathormones [15]. RANKL and OPG not only stimulate osteoclast differentiation, but also play a major role in osteoblastic proliferation [16]. Osteoclasts cause bone resorption by demineralization of inorganic portion of bone by acid and degradation of organic component of bone by cathepsin K and MMPs [17,18]. Collagenase-1 (MMP-1) and collagenase-2 (MMP-8) are matrix metalloproteinases that initiate tissue remodeling by cleaving native triplehelical interstitial collagen. Thus, sequence of events regarding biomarkers and their role in orthodontic tooth movement is of importance; for example OPG levels in GCF during canine retraction were significantly higher on mesial side of retraction; this lend support to idea that OPG may considered as the biomarker of choice and its changes may be linked to bone resorption in response to compression force.

Matrix metalloproteinases *(MMPs*) represent a family of human zinc-dependent endopeptidases and involved in a wide variety of physiological and pathological processes, as skeletal growth and remodeling, wound healing, cancer as well as inflammatory diseases [19]. Several enzymes degrading connective tissue include the proteases (MMP-8) that breaks down collagen. Matrix metalloproteinase activity accounts for the rate of matrix turnover or destruction, and modulation of the immune response in a more direct fashion [20]. MMP-8 (Collagenase-2) is produced by a wide range of resident and inflammatory cells, but its main source is neutrophil, and it represents the major collagenase in gingival tissue and GCF, accounting for about 80% of collagenases, followed by MMP-13 (~18%), while MMP-1 is seldom detected [21]. Elevated MMP-8 levels in GCF, saliva and oral rinse differentiate periodontitis from gingivitis and healthy sites. Total MMP-8 levels and MMP-8 active forms from neutrophils and mesenchymal cells in sites from progressive periodontitis subjects were reported [22]. Based on these findings; active sites might have persistently high MMP-8 levels and activation via oxidative pathway.

The GCF level of MMPs and their inhibitors has been generally shown to peak at an average of 1 to 2 days after the application of the stimulus and return to baseline values after approximately 1 week [23-25]. The role of RANKL and its inhibitor OPG in inducing alveolar bone remodeling during orthodontic tooth movement has been demonstrated [26-28]. Thus, analysis of GCF mediators

reflects the biological activity that occurs in the periodontium during orthodontic tooth movement. The presence of MMP-1 and -8 was measured daily for 1 month in GCF of patients treated with orthodontic fixed appliances. GCF samples were collected before fixed appliance activation and every 24 hours for 1 month thereafter. It was found that, MMP-1 and -8 in GCF were elevated and fibroblast-type MMP-8 reflects the periodontal remodeling during orthodontic tooth movement.[4] A randomized split-mouth study examined the levels of MMPs -1, -2, -3, -7, -8, -12, and -13 in GCF at different time points during orthodontic tooth movement. It was concluded that MMPs are released in sufficient quantities such that tooth movement occurs but with no significant increase in GCF levels [29]. During orthodontic tooth movement, collagenous extracellular matrix of PDL and alveolar bone is remodeled. Bone resorption by osteoclasts involves demineralization of the bone inorganic matrix by acid and degradation of bone organic matrix by cathepsin K and MMPs; although the precise role of MMPs in osteoclastic bone resorption is not fully understood [30,31] Collagenase-1 (MMP-1) and collagenase-2 (MMP-8) initiate this tissue remodeling due to their unique ability to cleave native triple-helical interstitial collagen. Experimental study showed that during orthodontic force application, MMP-1 gene expression was increased, and later decreased after force removal [32]. However, detection of MMP-1 in GCF of patients undergoing orthodontic treatment was inconclusive [33]. In view of these, GCF biomarkers reflect biological activity of periodontal tissues that could serve as valuable diagnostic tool to monitor the orthodontic tooth movement procedure in clinical practice. Hence, the present study was designed and performed to investigate the levels of MMP-8 and OPG in GCF during orthodontic tooth movement.

Patients and Method

Patient selection

Ten patients ranging in age between 14-23 years (mean age of 17.63 ± 3.35) were recruited from those attending at Outpatient clinic, Faculty of Dental Medicine, Al-Azhar University, Cairo, Egypt. Patients were selected according to following criteria: (i) free from any systemic diseases (ii) no history of antimicrobial therapy or anti-inflammatory drugs in the last 3 months prior to study, (iii) healthy periodontal tissues with no probing depths exceeding 3 mm in whole dentition and no radiographic evidence of bone loss. Subjects with systemic diseases, or using of drugs affecting bone metabolism (osteoporosis, arthritis, hormonal treatment, bisphosphonates, immunosuppressant); primary or secondary occlusal trauma; any type of periodontal treatment within the last 6 months; smoking; and pregnant or breastfeeding were not included. Nature of the study was explained to patients and informed consent was obtained from each patient at the first visit.

Pre-Orthodontic mouth preparation and clinical evaluation of gingival status

Before insertion of orthodontic appliance all participants underwent a session of supra-gingival scaling, teeth polishing and received oral hygiene instructions to reach a level of meticulous plaque control. Clinical health status of the gingiva was assessed with criteria of Gingival index of *Loe and Silness* [34]. This assessment was carried out initially (baseline), then at 2 weeks, 6 weeks and 3 months later during the orthodontic tooth movement.

Orthodontic treatment

The included patients showed malalignment of upper teeth and require proper position alignment. Hence, the goal of orthodontic treatment is to bring the teeth into alignment and correct vertical discrepancies by leveling out the arches. This goal was achieved through a combination of labiolingual and mesio-distal tipping guided by 0.014/0.016-inch NiTi (nickel-titanium) arch wire. The 0.014/0.016-inch NiTi arch wire provided light continuous force of approximately 50 grams. Orthodontic therapy with fixed appliance for treatment of malalignment of upper and lower teeth was started, and patients were followed every 2 weeks and motivation was performed during the period of the study, when necessary.

Collection of GCF samples and quantitation of MMP-8 and OPG

The GCF samples were collected from the labial surfaces of bonded upper incisors at baseline before initiation of orthodontic therapy, 2 weeks, 6 weeks, and 1 month after initiation of orthodontic therapy. Supra-gingival plaque was removed, isolated with cotton rolls and dried with air to avoid contamination with saliva. GCF was collected with paper strips, placed into the sulci until mild resistance was felt and left in place for 30 seconds; strips contaminated by saliva or blood were excluded. After GCF collection, strips were placed in Eppendorf vial and kept under -20º C, till the assaying time.

GCF samples were analyzed for OPG and MMP-8 using commercially available human ELISA kit (BioVendor Research and Diagnos-

tic Product European Union), according to previously mentioned method, [35] and following manufacturer's instructions. ELISA determinations were performed in duplicate; a sandwich-type ELISA where a monoclonal anti-human OPG, adsorbed onto micro-wells, binds to OPG in the sample, respectively. Results were calculated using the standard curves included in each assay kit. The intensity of the color was measured at 450 nm, concentration of OPG was determined in pictograms/milliliter (p g/mL).

Statistical analysis

Data were processed and analyzed using SPSS 16.8 (Statistical Package for Scientific Studies) for Windows. The changes in GCF levels of MMP-8 and OPG within the study group at different time intervals were compared using ANOVA. Differences between GCF level of MMP-8 and OPG at baseline, 2 weeks and 1 month interval was analyzed using Bonferroni post hoc test was conducted. Spearman`s correlation coefficient was used to determine significant correlation between MMP-8 and OPG at different intervals, significance was set at $P \leq 0.05$.

Results

All patients were able to comply with study protocol and showed cooperation with the procedure with no complains; their oral health status was maintained in an acceptable condition. Assessment of gingival condition showed slightly increased values of Gingival index scores (GI) at 2 and 6 weeks compared to those recorded at baseline; GI showed mean of 0.84 SD 0.16 at 2 weeks, and 0.92 SD 0.14 at 6 weeks, compared to baseline mean (0.46 SD 0.08). However, the GI recorded decreased mean value at 3 months (0.48 SD 0.09). The comparison between these values did not reach to the significance, except when comparing between the recorded scores at 6 weeks with that recorded at baseline ($p < 0.05$). These findings are illustrated in figure 1.

Results of OPG Levels in GCF

Baseline GCF OPG showed Mean 198.2±31.15 which was higher than those measured at 2 weeks (138.2±48.19), 6 weeks (105.2±33.79), and 3 months later (83.20±24.19). OPG-GCF values showed decreased levels at all intervals compared to the level recorded at baseline. There was a statistically significant increase in the OPG-GCF levels at 2 weeks, 6 weeks, and 3 months following beginning of orthodontic tooth movement compared to the baseline values ($P < 0.05$); figure 2 illustrating these findings. Regarding the differences and % change in mean OPG levels measured at the different study intervals: there was a significant difference in the level of OPG (pmol/l) between the baseline and 6 weeks (59%, $P < 0.001$) and 6 weeks (48%, $P < 0.05$) following the application of leveling orthodontic force. In addition, the comparison between the OPG-GCF levels at baseline and 3 months showed significant difference (39%, $P < 0.05$).

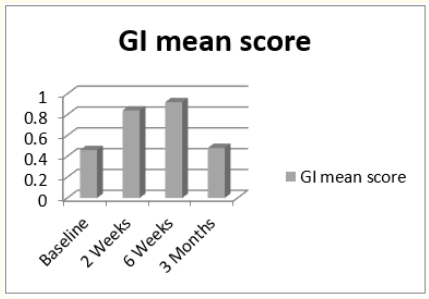

Figure 1: The recorded Gingival Index scores as a clinical measurement of the healthy gingival condition of the included patients at different times during the orthodontic tooth movement procedure.

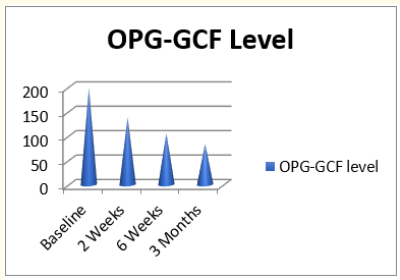

Figure 2: The measured levels of OPG in the GCF of patients treated with fixed orthodontic appliances at starting (baseline), 2 weeks, 6 weeks and 3 months later.

Mean MMP-8 levels in GCF measured at different periods of orthodontic tooth movement:

The MMP-8 in GCF at baseline showed higher level (Mean 147.8 ng/µl, SD 43.68), $p < 0.001$) compared to that at 2 weeks later (118.64 ng/µl, SD 37.35); at 6weeks (Mean 108.65 ng/µl, SD 28.42, $p < 0.001$), and 3 months (Mean 97.78 ng/µl, SD 26.80, $p < 0.001$). The levels at 2 weeks, 6 weeks and 3 months did not significantly different, $p > 0.05$); (Figure 3).

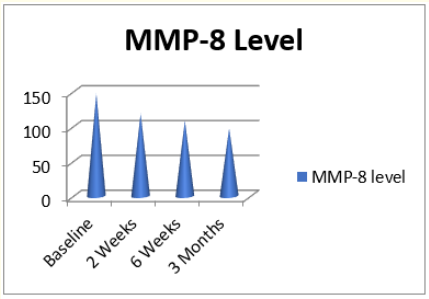

Figure 3: The measured levels of MMP-8 in the GCF of patients treated with fixed orthodontic appliances at starting (baseline), 2 weeks, 6 weeks and 3 months later.

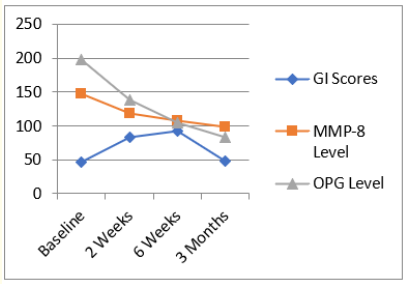

Figure 5: Correlation between the GI scores, MMP-8 level, and OPG level recorded at different times of orthodontic tooth movement.

Correlation between clinical health status of gingiva and levels of MMP-8 and OPG in GCF

The measured levels of MMP-8 and OPG in the GCF were strongly correlated with each other as well as with the clinical healthy status measured with criteria of Gingival Index; this relation between these biomarkers as well as between them with healthy gingival status were markedly noted starting from the second week till the end of follow up period of 3 months (Figures 4 and 5).

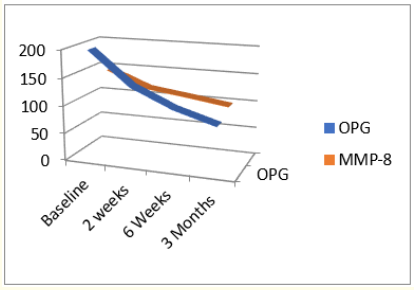

Figure 4: The measured levels of both MMP-8 and OPG in the GCF of patients treated with fixed orthodontic appliances at starting (baseline), 2 weeks, 6 weeks and 3 months later.

Discussion

The GCF is a mixture of substances derived from serum, host inflammatory cells, structural cell of periodontium, and oral bacteria [36]. analysis of GCF provide useful diagnostic tool in periodontics and orthodontic field [37]. Biomarkers related to bone deposition (bone alkaline phosphatase and OPG) represent a new and interesting era regarding understanding of bone growth/remodeling; [38] thus monitoring of these markers during orthodontic movement might be a useful procedure for clinicians to analyze degree of bone remodeling process. During orthodontic movement, RANKL is responsible for generation and maintenance of osteoclasts by binding RANK [36], while OPG acts as a decoy receptor that binds to RANKL and blocks osteoclastogenesis [27]. OPG functions to impede differentiation process for both osteoclasts and osteoclastic activity within the cells. In this respect, it was found that RANKL levels were increased during the treatment and in contrast, the OPG levels decreased [39] The changes in these cytokines may be involved in bone resorption as a response to compression force. Soft tissue is also remodeled following orthodontic tooth movement; these tissues are metabolized by various enzymes, including MMPs and tissue inhibitors of TIMPs. Collagenases, MMP-1 and 1MMP-8, degrade collagen fibers, whereas gelatinases (MMP-2 and MMP-9) degrade denatured collagen, complementing collagenases [40]. Bone remodeling is a process which is controlled by RANKL and OPG system since they are considered primary key factor for bone metabolism [41]. OPG, and RANKL have a decisive role in regulating the degeneration of periodontal hard and soft supporting tissue. Studies evaluating RANKL, OPG levels in GCF interpreted that high RANKL, and low OPG protein levels participate in the underlying process of periodontal tissue breakdown [42-44]. This study was carried out to determine the possible effects of orthodontic tooth movement on the levels of MMP-8 and OPG in Gingival fluid (GCF).

The results of the present study revealed significant decrease of OPG levels in GCF after 2 weeks from orthodontic force application followed by subsequent decrease at 6 weeks as well as 3 months

later. These findings are similar to a study found that compressive force application to PDL resulted in a decrease of GCF levels of OPG in compressed sites of distalizing teeth [45]. Additionally, OPG expression decreased in GCF samples collected from areas adjacent to teeth undergoing orthodontic tooth movement at 24hours after applying a mechanical compression to PDL [46] Levels of OPG in GCF samples taken from juvenile and adult patients during orthodontic tooth movement showed significantly lower OPG values for the experimental teeth after 24hours in both groups, but a greater decrease was reported in the juvenile patients [47]. However, a study reported that, OPG level increased to approximately baseline levels by 168 hours which was not the case in the present study where at the end of the follow up period of the present study (3 months), as the level OPG remained below baseline values. This could be attributed to the different experimental design used in those studies. Compressed PDL fibroblasts increased osteoclast genesis in peripheral blood mononuclear cell cultures by upregulating RANKL but OPG expression did not change regardless of the amount of compression force or the duration of compression [48]. A study indicated that compression forces significantly decreased the secretion of OPG in a time and magnitude-dependent manner in pre-cultured human PDL cells [48]. On the other hand, emerging evidence suggests that RANKL and OPG play an important role in regulating periodontal tissue turnover during orthodontic tooth movement [49]. Cell-cell signaling by RANKL is essential for the induction of osteoblasts differentiation while OPG acts as an inhibitor of osteoclasts function by competing with RANKL for the membrane receptor RANK [50]. These facts denote that periodontal tissue remodeling resulting from orthodontic force application is a complex process involving many signals and pathways working simultaneously.

Kusumi., *et al.* [46] found an increase in OPG synthesis following application of tensile stress to human osteoblasts. Although another study [47] found decreasing OPG levels immediately after mechanical loading (24-48 hours) and attributed this to ischemia and hypoxia induced by mechanical compression of microvasculature. In this particular study, the lag phase corresponded to decrease in OPG levels following 2 weeks of appliance insertion. The pressure site demonstrates decreased cellular activity and tension site shows increased cellular activity [48]. During lag phase, due to presence of hyalinized zone the cellular activity even at the tension site comes to a standstill correlating to decreased levels of OPG following 2 weeks when compared to 48 hours of appliance insertion. Thus, at 5 weeks of appliance insertion, OPG levels gained a sharp increase, which correlate and corresponds with previous findings that stretching of periodontal ligament fibers causes upregulation of OPG levels during various stages of orthodontic tooth movement [49].

The MMP-8 is a predominant collagenase mostly identified in GCF and associated with periodontitis severity, especially in activated/active form (aMMP-8), and has been regarded by several researches as one of the most promising biomarkers for periodontitis in oral fluids [50]. Regarding the level of MMP-8 in GCF measured in the present study, results showed decreased values at all times compared with the measured level at baseline. Unfortunately, this finding did not agree with a study reported consistently enhanced levels of MMP-8, in GCF from orthodontic treated teeth at 4-8h after force application relative to baseline. Reason for this may be attributed, at least in part; to the measurement time as in the present measuring MMP-8 level was done two weeks from baseline, which was not the case in a study [33], measured MMP-8 level after very short time following force application. Again, this finding did not lend support to that of another study reported that, GCF levels of MMP-8 measured over 28 days of orthodontic movement were significantly elevated around orthodontic moved teeth in comparison with control teeth [4]. In fact, we have no reason for such discrepancy, except that they compared site to site, which did not performed here, hence this require further clarification.

Conclusion

GCF levels of MMP-8 and Osteoprotegrin showed changes related to tooth movement changes; hence assessment of GCF biomarkers may be of value in this aspect. Further studies of several biomarkers in GCF are needed toward better clarification of relationship between these mediators' production and tooth movement activation. This will yield, no doubt, a clearer data of potentiality of GCF as a diagnostic tool to monitor clinical outcome in orthodontics.

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