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Original article

The Effect of Initial Periodontal Therapy on RANKL/OPG Ratio in Gingival Crevicular Fluid

Kholoud Ftis[1], Walid Ftis[2], Samira Buzinin[1]*

[1]*Department of Periodontology, Faculty of Dentistry, University of Tripoli, Tripoli, Libya*
[2]*Department of Oral Medicine, Faculty of Dentistry, University of Tripoli, Tripoli, Libya*
Corresponding Email. *samirabuzinin@gmail.com*

ABSTRACT

Background and objectives: *Alveolar bone resorption an event characterizing periodontitis, induced by osteoclast activity which is regulated by ratio between receptor activator of NF-κB ligand (RANKL) and Osteoprotegerin (OPG). This study investigated the changes of RANKL/OPG ratio in Gingival Crevicular Fluid (GCF) the performing the initial periodontal therapy, on a total of 12 patients with periodontitis.* ***Methods***. *GCF samples were collected from an area with active periodontitis and a healthy area. RANKL and OPG levels were measured before and after initial periodontal therapy. A group of 10 subjects without periodontitis were included as control group for the comparison.* ***Results***. *The current findings showed decreased RANKL level in areas exhibited active periodontitis after initial periodontal therapy with no changes involving OPG levels, denoting that periodontal treatment resulted in decreased RANKL/OPG ratio in sites with destructive periodontal activity.* ***Conclusion***. *In view of the obtained results, it can be concluded that periodontal therapy acts on the RANKL/OPG ratio, through inhibition of osteoclastogenesis. These, may encourage periodontists to apply these molecules in the procedure of proper periodontal diagnosis as well in monitoring the response and efficacy of periodontal treatment.*

Keywords: Periodontal disease, RANKL, OPG, GCF.

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INTRODUCTION

Periodontal disease is one of the most common chronic human diseases initiated by bacterial infections, which affects the teeth supporting tissues that can result in tooth loss in adults [1]. This disease results in the progressive degradation of periodontal ligament (PDL) and alveolar bone together with formation of periodontal pockets and / or gingival recession [2]. Periodontal pathogens induce immune-inflammatory responses characterized by release of inflammatory mediators [3]. Alveolar bone destruction is the main cause of tooth loss. The height of the alveolar bone depends on the recession and formation of the bone and the balance between these two processes and is sustained steadily [4]. The alveolar bone resorption is a mechanism underlying pathogenesis of periodontitis, and is caused by an increase in and a preponderance of osteoclast activity [5-7]. It has been postulated that, osteoclast activation is regulated by interplay of three molecules that constitute RANK/RANKL/OPG axis [8-10]. RANKL is a protein of the tumor necrosis factor family that activates RANK receptor, and Osteoprotegerin ligand (OPG-L) is a soluble protein that functions as a decoy receptor for RANKL [10,11]. Thus, OPG-L is an inhibitor of osteoclast formation. The normal

metabolic activity of the bone and the stability of the bone mass depend on the balance between RANKL and OPG [12].

There is a wide body of evidence in the literature declaring that the alveolar bone destruction is associated with an imbalance in RANKL and OPG [13]. Previous studies reported that the expression of OPG was found to be decreased, while RANKL was increased in periodontal diseases [14-18]. Studies reported higher RANKL levels in patients with periodontitis compared to healthy subjects [19-21]. In contrast, lower OPG levels were found in subjects with periodontitis than in healthy subjects [22-25]. These results may indicate that RANKL/OPG ratio is higher at sites with periodontal activity.

Concerning the effects of initial periodontal therapy including scaling and root planning, it seems likely that there is deficient information regarding the levels of these molecular markers in the GCF. Hence, the present study was designed and carried out to investigate the possible variations involving the RANKL and OPG levels in the GCF following the completion of initial periodontal therapy

METHODS

Patients

The sample size was determined in relation to the main variable (RANKL & OPG) concentrations. Twenty-two eligible participants in this cross sectional study were recruited from routine dental patients attending the Periodontal clinic at Faculty of Dentistry, University of Tripoli in Tripoli, Libya. Permission to carry out the study was obtained and approved by the scientific committee of Faculty of Dentistry, University of Tripoli.

Twelve patients with chronic periodontitis, their age ranged between 48–59 years, and control group of 10 periodontal health subjects without signs or symptoms of periodontal disease, such as attachment loss, probing depth greater than 3 mm, bleeding on probing, in any teeth except third molars, as well as no radiographic evidence of bone loss. Patients of the study group diagnosed with mild or advanced chronic periodontitis according to criteria of Armittage [26]. Subjects with aggressive periodontitis; systemic diseases or consumption of drugs affecting bone metabolism (osteoporosis, arthritis, hormonal treatment, bisphosphonates, anti-inflammatory drugs); antibiotic, anti-inflammatory or contraceptive treatment for the last three months prior to study initiation; antiplatelet therapy for the last 7 days prior to study onset; primary or secondary occlusal trauma; periapical or periodontal abscess in some of the teeth included in the study; any type of periodontal treatment within the last 6 months; under orthodontic treatment; smoking; and pregnant or breastfeeding, were not included.

Periodontal Clinical Examination & Treatment

The probing depth, recession, attachment loss, Gingival Bleeding Index" andSilness and Loe index [27] assessed dental plaques were used.In the study group, periodontal scaling and root planning treatment of the 4 quadrants was performed for two consecutive weeks without the use of antiseptics or antibiotics, and the patients were instructed in oral hygiene, including both brushing techniques and proximal hygiene. Four weeks after the completion of initial periodontal therapy, the patients were examined clinically, and samples of GCF were collected again.

Collection of Gingival GCF and Sample Processing:

This was carried out according to previously mention method [28]; supra-gingival plaque was removed with a sterile curette; sulcus was isolated with cotton rolls and dried with air to avoid contamination with saliva. Tip of the Periopaper was inserted into the sulcus or periodontal pocket until resistance was found and maintained for 30 seconds; tips dyed with blood were discarded. Subsequently, the GCF volume was measured by the Periotron 8000. Each collected sample was allocated into a sterilized Eppendorf tube with 100 microliters of buffer containing phosphate-buffered saline (PBS) with protease inhibitors (C.N. P8340 Sigma, MO, USA). Eppendorf tubes were centrifuged at 15,000 rpm for 5 min; then, another 100 microliters of buffer were added, and the tubes were centrifuged for 5 min at 15,000 rpm. Finally, 200 microliters of each sample was stored at –80°C prior to processing. RANKL and OPG were quantified using an ELISA technique; technical specifications of

RANKL (total Human ELISA, BioVendor) and OPG (Bender MedSystems) kits were followed according to manufacture instructions; total RANKL and OPG concentrations were assessed.

Statistical analysis

The collected data were analyzed statistically; Mann-Whitney test was used to compare the distribution of a parameter between the control group and the study group in an area of the mouth and at a particular time point. Significance level used in the analysis was 5% ($\alpha = 0.05$).

RESULTS

The patient's group consisted of 7 men and 5 women in the study group, while control group were 5 women and 5 men; mean age was 46.6±3.5 in the study group and 47.3±3.7 in control group. The mean probing depth of the location with periodontitis in the study group decreased significantly after treatment ($p<0.001$); a trend towards a decrease in healthy location was observed, although the difference was not significant ($p >0.05$). Despite decreased probing depth at the diseased location after initial periodontal treatment, however the depth did not reach the level of the healthy location after the treatment; this difference was significant ($p<0.001$). The comparison between the patient's group with the control group, showed that mean probing depth of the healthy location of the study group did not differ from that of the control group either before or after the treatment. At the diseased location of the patient's group, although the decreased probing depth from baseline to that recorded after 6 months was significant, and these values at 6 months' post-treatment were not equal to those found for the control group; therefore, the differences were significant ($p<0.001$).

The clinical attachment gains of the patient's group increased significantly after treatment ($p<0.001$); a trend towards a gain was observed in the healthy location but was not significant ($p > 0.05$). After treatment, the clinical attachment gain was higher than at the healthy location, but diseased location value did not reach the value of the healthy location after treatment ($p<0.001$). Healthy and diseased locations showed differences in CAL before initial periodontal treatment compared to the control group. After periodontal treatment, only the healthy location showed values similar to those found for the control group. Initially, bleeding upon probing in all diseased locations of the patient's group (100%), whereas bleeding only occurred in 6.7% of the locations after initial periodontal treatment ($p<0.001$). The control group did not record bleeding, while the patient's group showed values of 45.33% before treatment (T1) and 7.2% after initial periodontal treatment (T2); significant decreased % of bleeding between T1 and T2 ($p<0.001$). However, bleeding decreased significantly with treatment, it remained higher at T2 than values in subjects with periodontal health (Control group). The mean Plaque (PI) in control group was 0 because it was one of the inclusion criteria, however patient's group PI showed values of 2, 48 ± 0, 42 in T1 and 0, 38 ± 0,39 after periodontal treatment (T2); significant decreased PI between T1 and T2 in the study group ($p<0.001$). Mean GCF volume in control group was 0.10 ± 0.04 µl, while in patient's group before treatment (T1); it was 0.14 ± 0.1 µl in healthy location and 0.78 ± 0.35µl in diseased location. Following initial periodontal treatment (T2), GCF values were 0.17 ± 0.01 µl in healthy location and 0.39 ± 0.21µlin diseased location. A significant decrease was observed in the volume between T1 to T2 in the pathologic location ($p <0.001$).

Significant differences in RANKL concentration were found in the patient's group ($p<0.01$) between the healthy location and the diseased location, before the periodontal treatment (T1), and highest values found in periodontal disease location. After the periodontal treatment, RANKL concentration in patients group was almost unchanged from T1 (pretreatment) to T2 (post-treatment) at the healthy location; however, RANKL concentration decreased significantly at the pathologic location. Thus, RANKL values significantly decreased from T1 to T2 at the pathologic location ($p < 0.05$). No difference in RANKL concentration between the both diseased and healthy locations post-treatment. The initial periodontal treatment in diseased locations led to a decreased RANKL level until values equal to healthy locations were obtained (pre- or post-treatment). RANKL levels were significantly higher at diseased locations in

patients with periodontitis before treatment ($p<0.01$). Comparing of RANKL in control group with healthy location of the patient group showed no significant difference.

A significant decreased RANKL concentration at diseased locations in periodontal patients was found after periodontal treatment, although no significant differences were observed in healthy locations ($p > 0.05$); may indicate that RANKL levels in healthy subjects did not differ from subjects with periodontitis after periodontal treatment. After initial periodontal treatment (T2), an increased OPG levels at periodontitis locations in the patient's group, and this change was significant ($p < 0.05$). In contrast, no significant changes in OPG levels were found at the healthy locations. Thus, there was homogeneity of post-treatment OPG levels between the diseased and healthy locations; OPG levels were higher in the control group than in the patient's group regardless of periodontal treatment performance.

Changes in RANKL/OPG Ratio

There was a significant difference involving the RANKL/OPG ratio between the healthy and pathologic locations of the study group before periodontal treatment, with higher ratios in the pathologic locations. A significant decreased ratio was found from pretreatment (T1) to post-treatment (T2) at the pathologic locations of the study group, but no changes were found in the ratio at healthy locations. In the patients group, no differences were found between the healthy and pathologic locations after initial periodontal treatment. The comparison between the control group and patient groups showed that RANKL/OPG was always significantly lower in control study than the ratio of patient group.

DISCUSSION

There is overwhelming evidence that, there is a role for RANKL–OPG system in periodontitis, which can be applied in diagnostic or therapeutic purposes [8], [28]. Molecular and immunological analyses are now essential to verify the effect of different biomarkers associated with periodontal diseases [29]. Although GCF has several diagnostic advantages, however there are some difficulties including following: time consuming, exhaustive, and requiring multiple sampling of sites and sample contamination. Since diverse mediators involved in alveolar bone remodeling are continuously washed into saliva by GCF, hence, collection and analysis of salivary / GCF biomarkers constitutes a reliable sampling method [30-31].

It was evident from the results of the present study that, OPG values were higher in the healthy patients than in the treated or untreated patients in the study group. These results suggest that local inflammation may have a low influence on the OPG levels of the patients in the study group, as the values of the different samples were similar. These values show that subjects suffering from periodontitis have lower OPG levels than healthy patients; that is, subjects susceptible to periodontitis have lower basal OPG levels. The hypothesis that anti-osteoclastogenic levels are higher in the control group is consistent with the results of the different studies [34]. However, one factor that should be taken into account is that the subjects in the control group of our study were younger than the subjects in the study group; thus, age may be a factor that influences the basal OPG level.

Regarding the RANKL levels, higher values were found in the untreated location with the periodontal condition in the study group than in the other locations in both the study and control groups. In addition, except for the untreated affected locations, similar RANKL levels were found at all locations, with no significant differences among them. This finding confirms that the RANKL levels are higher in locations with destructive periodontal activity, which results in higher osteoclast activity; these findings were consistent with that of previous studies [35-38]. However, when this local inflammation is absent, such as in the control group, at the healthy locations in the study group before treatment and at both locations (healthy and pathologic) post-treatment, the RANKL levels are lower and similar. This result confirms the significance of T cells as RANKL producers [39-41].

CONCLUSION

In conclusion, RANKL/OPG levels were low in control group, in treated healthy locations and in treated

diseased locations of patients suffering from periodontitis. The latter two levels were very similar but were not as low as the levels observed in the control group, which had the lowest levels. RANKL/OPG ratio was high at the locations with untreated periodontitis. This study assessed the changes of these biomarkers after performing the initial periodontal therapy, and did not have predetermined values of RANKL or OPG that mark the limit between periodontitis and periodontal health. Therefore, larger sample size studies are required that may an attempt to clarify this aspect toward obtaining clearer information in this regard.

Disclaimer

The article has not been previously presented or published.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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