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Survey of Changes in Salivary Level of sRANKL and Osteoprotegerin through Orthodontic Tooth Movement

Atefeh Ataie, Fariba shahri, Majid Sokhtanloo, Majid Azizi*

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Abstract

Aim: The aim of the present study was to evaluate the salivary level of sRANKL and OPG and sRANKL/OPG ratio during the clinical orthodontic tooth movement**. Materials and Methods:** 20 female patients with age range of 14-20 years with mild crowding and class I malocclusion participated in this study. Unstimulated saliva collected to evaluate the level of sRANKL and OPG prior to orthodontic treatment, 24 hours, 2, 5, and 8 weeks after device activation. ELISA test was performed to measure the concentration of biomarkers and data were analyzed by SPSS version 11.5 software with significance level set at <0.05. **Results:** Concentration of sRANKL and OPG and also sRANKL/OPG ratio was significantly different between time points. Salivary level of sRANKL decreased after 24 hours and then increased. OPG was decreased after 24 hours and then increased in next follow up measurements except in the 5th week in which it was decreased. SRANKL/OPG ratio increased after 24 hours, decreased after 2 weeks, and then increased after 5 and 8 weeks. Salivary level of both markers and their ratio was significantly higher in the 8th week measurement in comparison to other time points (P-value < 0.05). **Conclusion:** Based on the results of the present study, evaluation of sRANKL, OPG, and sRANKL/OPG ratio in patients under orthodontic treatment could lead to identification of tooth movement phases after applying orthodontic forces. Clinical Significance: Our study proposed the sRANKL and OPG salivary levels as the molecular markers of different phases of orthodontic tooth movement. These markers could be use for assessment of bone remodeling during orthodontic movement and the response of individuals to treatment.

Keywords: Orthodontic Tooth Movement, Salivary Biomarker, Tooth Movement Phase, Enzyme-linked immunosorbent assay (ELISA)

Introduction

Remodeling of periodontal ligament (PDL) and alveolar bone following mechanical forces result in orthodontic tooth movement (Kanzaki et al., 2004; Kanzaki et al., 2006; Tan et al., 2009; Krishnan & Davidovitch, 2006) Orthodontic tooth movement occurs in 3 phases including initial, lag and post lag phases. Initial phase characterized by an immediate and rapid movement seen in the first 24-48 hours of force application (Krishnan & Davidovitch, 2006; Burstone, 1962; Baumrind, 1969). The lag phase occurs in 4-20 days. In this phase the tooth is stable due to the formation of hyalinisation tissue. The hyalinization tissue resorption in the post lag phase causes the rapid tooth movement (Krishnan & Davidovitch, 2006; Burstone, 1962). Animal studies proposed the presence of linear phase which is seen in 40 days after force application. The direct bone resorption is the probable cause of tooth movement in this phase. (Van Leeuwen et al.,1999; von Böhl et al., 2004). RANKL and OPG are key regulators of bone remodeling which reflect the cellular activity during tooth movement (Tan et al., 2009; Kawasaki et al., 2006; Dunn et al., 2007; Yamaguchi, 2009; Nakano et al., 2010; Favus, 2006; Pellegrini et al., 2008; Mulcahy et al., 2011) . RANKL causes the formation and activation of osteoclasts through the binding to RANK receptors on osteoclast progenitor cells (Theoleyre et al., 2004). RANKL produced in 2 isoforms of sRANKL and mRANKL. mRANKL which is found on the cell membrane of the osteoclasts produced by osteoblastic progenitor cells, while sRANKL is a soluble molecule secreted by T lymphocytes (Schoppe et al., 2002; Nakashima et al., 2000). OPG prevents the differentiation of osteoclasts and also induces the apaptosis of these cells. The bone remodeling is controlled by a balance between RANKL and OPG (Theoleyre et al., 2004;

Atefeh Ataie
Assistant professor, Department of Periodontics, Faculty of Dentistry, Birjand University of Medical Sciences, Birjand, Iran.

Fariba shahri
Associated professor of orthodontics, Department of Orthodontics, Faculty of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran.

Majid Sokhtanloo
Associated professor of biochemistry, Department of biochemistry, Faculty of Medicine, Mashhad University of Medical sciences, Mashhad, Iran.

Majid Azizi*
Assistant professor, Department of Orthodontics, Faculty of Dentistry, Birjand University of Medical Sciences, Birjand, Iran.

***Email:** majidazizi1386@gmail.com

Burgess et al., 1999; Lacey et al., 2000). Although many human and animal studies evaluated the relationship between the amount of RANKL and OPG with bone remodeling and root resorption (Kanzaki et al., 2004; Tan et al., 2009; Kawasaki et al., 2006; Yamaguchi, 2009; Shiotani et al., 2001; Brooks et al., 2009; Nishijima et al., 2006) but there is still debate about the RANKL and OPG changes during the different phases of tooth movement (Flórez-Moreno et al., 2013). Both GCF and saliva samples were used in the studies (Kawasaki et al., 2006; Yamaguchi, 2009; Nishijima et al., 2006; Barbieri et al., 2012; Grant et al., 2012) to evaluate the changes of suspected molecular and cellular markers of remodeling (McGehee & Johnson, 2004; Frodge et al., 2008). Our study aimed to evaluate the amount of RANKL, OPG and sRANKL/OPG ratio during different phases of tooth movement in unstimulated saliva.

Material and methods

20 female patients without systemic disease who needed fixed orthodontic treatment entered this study. All the patients were treated by non-extraction method. Damon 3 self-ligating system with 0.022 inch slot was used for orthodontic treatment. The alignment was done with cupper NiTi 0.014 inch arch wire. The unstimulated salivary samples were gathered for RANKL and OPG assessment. To collect unstimulated saliva, the patients open her mouth and secreted saliva were sampled. 5 mL of saliva sample was collected in a sterile plastic centrifugation tube (Bio-one, Frickenhausen, Germany). All the patients were fasted and also had not perform any hygiene procedure 8 hours before the sample collection. The samples were stored in a portable refrigerator and were transferred to the laboratory in less than 10 minutes. In the laboratory the saliva samples were centrifuged for 5 minutes in 4 º C and under 800 g force. (IEC Centra CL2 centrifuge, Thermo Electron Milford, Mass). The superficial layer of each tube was extracted and divided into 50 µL volume containing. Before further process these samples were stored in – 75 º C. Sample collected in 5 different time points including before and 24 hours, 2, 5 and 8 weeks after beginning of the treatment. sRANKL and OPG were measured by ELISA using Ampli sRANKL human ELISA and OPG ELISA commercial kit. According to the manufacture instruction, the standard solution of samples were prepared and added to the plates containing (50 µL Biotene and 10 µL OPG or sRANKL antibody). These plates were incubated in 37 º C for 60 minutes. Then the plates were washed 5 times and 50 µl of chromogen solution was added to the plates and incubated in a dark environment for 15-20 minutes for OPG and 10 minutes for sRANKL measurement. 50 µL stop solution was added to the plates and after 15 minutes, microplate reader which was set to 450 nm was used to measure the amount of biomarkers. Analysis of the samples and the concentration was measured by ChroMate Manager Software. The total levels of both free forms and also the sRANKL-OPG complex form of these proteins were measured.

Results

In our study 20 female subjects with the mean age of 17/69 ± 2/18 entered the study. All the subjects had Cl.I malocclusion and mild crowding. 100 salivary samples were collected and the concentration of different markers was measured. Table 1 shows the mean concentration of sRANKL in different time points. The concentration of sRANKL decreased 24 hours after the activation of device and increased gradually. The peak of the sRANKL concentration was seen in the 8^{th} week. The statistical analysis showed that the reduction of sRANKL 24 hours after surgery and increasement of sRANKL between 5^{th} to 8^{th} week were statistically significant. The mean concentration of OPG was shown in table 4-2. Also table 2 showed the changes of sRANKL concentration. The concentration of OPG decreased in the 1^{st} day after the device activation but it increased in the following 2 weeks. A negligible reduction was found in the 5^{th} week and this value increased at 8^{th} week follow up. Repeated measure of analysis showed that there was a significant difference of concentration of OPG between different time points. According to the pair wise comparison, the difference of concentration of OPG between all time points was significantly different except the difference between 2^{nd} and 5^{th} week. The ratio of sRANKL/OPG was shown in table 3. This value increased 24 hours after the device activation and decreased in the following 2 weeks. After that it increased until it reached to its peak in the 8^{th} week. The sRANKL/OPG ratio in the 8^{th} week was significantly greater than the other time points. Also the difference of the values between baseline and 2^{nd} week and also between 24 hours and 5^{th} week was statistically significant.

Disscusion

The majority of studies evaluated the amount of remodeling biomarkers during single tooth movement (Nishijima et al., 2006; Grant et al., 2012; Bergamo, 2014; Perinetti et al., 2005). In our study we evaluated the biomarkers after bimaxillary set up by self-ligated damon system and cupper Niti 0-14 wire which was a friction-less system and decreased the unfavorable forces and resulted in the production of a continuous force. As bone remodeling mediators in saliva originates from GCF, we used the unstimulated saliva as biologic sample and the timing of sampling was relevant to the different phases of tooth movement. The result of our study was similar to Florez-Monero et al. and Barbier et al. study, which showed that sRANKL and OPG significantly decreased in the initial phase. While in Grant et al. and Nishijima et al. study, the increasement of sRANKL was seen. The use of extraction method and also the time of sampling (few months after the beginning of orthodontic treatment) in Grant et al. and Nishijima et al. study were the probable explanation for controversial results. In-vitro studies showed that the immediate reduction of sRANKL and OPG in the beginning of treatment is the result of alveolar bone bending, compression of PDL and the ischemia following the vessels closure (Brooks et al., 2009; Sprogar et al., 2010).

sRANKL/OPG ratio increased in the initial phase which was similar to the results. In the lag phase sRANKL and OPG were increased while the ratio was decreased. Revascularization of PDL, improvement of cellular activity and healing of periodontal attachment explains the significant increasement of OPG and significant reduction of the ratio in the lag phase (Buduneli et al., 2008). The non-significant changes of sRANKL may be due to lower activity of osteoclasts in the lag phase (Krishnan & Davidovitch, 2006; Burstone, 1962; Yamaguchi, 2009; Sakellari et al., 2008; Buduneli et al., 2009). During the post lag phase the salivary levels of sRANKL and ratio increased while reduction of OPG was seen. These changes were the result of increased activity of osteoclasts and rapid tooth movement (Burstone, 1962; Kim, 2018). The reduction of OPG in Florez-Monero et al. study in 5th week may be due to compression of PDL caused by rapid tooth movement. In our study, the increased level of sRANKL in 5th week was the result of the reactivation of wire after 1 month. But in Florez-Monero et al. study that such an activation was not done, the increasement of sRANKL was not found in the 5th week. Significant increased levels of sRANKL and OPG during the linear phase could be the result of both osteoblasatic and osteoclastic activity in compression and tensile sites respectively (Burstone, 1962; Van Leeuwen et al.,1999; von Böhl et al., 2004; Kim, 2018 ; Domon et al.,1999). Despite the increased level of OPG in the last phase, it was still lower the base line level. Although the increasing level of OPG was a sign of bone formation but it was not reached to the base line level because the bone maturation occurs after 3 months (Pellegrini et al., 2008; Flórez-Moreno et al., 2013).

Conclusion

Based on the results of the present study, evaluation of sRANKL, OPG, and sRANKL/OPG ratio in patients under orthodontic treatment could led to the identification of tooth movement phases after applying orthodontic forces.

Clinical significance

Our study proposed the sRANKL and OPG salivary levels as the molecular markers of different phases of orthodontic tooth movement. These markers could be used for assessment of bone remodeling during orthodontic movement and also the response of individuals to orthodontic treatment.

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Table 1. Mean concentration and standard deviation of sRANKL in different time points.

Follow ups	number	Concentration of OPG(pg/mL)	
		mean value	standard deviation
Before activation	20	7.85	1.37
24 hours after activation	20	6.38	0.90
2 weeks after activation	20	7.39	1.13
5 weeks after activation	20	7.84	1.34
8 weeks after activation	20	16.42	7.19

Table 2. mean concentration and standard deviation of OPG in different time points.

Follow ups	number	Concentration of OPG(pg/mL)	
		mean value	standard deviation
Before activation	20	88.18	15.77
24 hours after activation	20	60.89	9.04
2 weeks after activation	20	75.94	12.60
5 weeks after activation	20	73.39	12.01
8 weeks after activation	20	82.79	13.14

Table 3. Mean concentration and standard deviation of sRANKL/OPG ratio in different time points.

Follow ups	number	sRANKL/OPG ratio	
		mean value	standard deviation
Before activation	20	0.093	0.030
24 hours after activation	20	0.107	0.024
2 weeks after activation	20	0.100	0.023
5 weeks after activation	20	0.110	0.028
8 weeks after activation	20	0.207	0.120