Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 796- 807
Received 18April2020; accepted 23June2020

Evaluation Of Rankl And Opg Levels In Orthodontic Patients Treated With Andwithout Micro-Osteoperforation- A Randomized Controlled Trial

N**. Thilaka Sre, K.S. Nagachandran, R. Devaki Vijayalakshmi**

Faculty of Dentistry, Meenakshi academy of higher education and research, Meenakshi Ammal dental college and hospital, Alapakkam main road, Maduravoyal, Chennai, Tamilnadu, India.

ABSTRACT

OBJECTIVE: The present study aimed to evaluate and compare the levels of RANKL and OPG in orthodontic patients with and without micro-osteoperforation. **MATERIALS AND METHODS:** Twenty subjects of either sex in the adolescent to adult age group (18-30 years) who seek orthodontic treatment were randomly divided into two groups. Group I consisted of ten subjects for whom micro-osteoperforation was done before the commencement of enmasse retraction. Group II consisted of ten subjects without micro-osteoperforation procedure. GCF samples were collected distal to the canines/first premolars in both groups at four different time intervals namely T0- before the start of retraction, T1- 1st day of retraction, T2- 45th day of retraction and T3- 90th day of retraction. Samples were analyzed for the levels of RANKL and OPG**. RESULTS:** RANKL levels were found to be increased at T1 and T2 but reduced at T3. The levels of OPG was reduced at T1, then increased at T2 and further increase at T3. The OPG/RANKL ratio was the highest at T1 with reduction of the ratio at T2 and failing down further at T3. **CONCLUSION:** RANKL levels was increased in the experimental group and continued to remain raised till 45 days of retraction and met a decline at 90 days of retraction. OPG levels reduced after 1 day of retraction later followed by a gradual rise up to 90 days of retraction. Thus, MOPs need to be performed at regular intervals in order to maintain the higher levels of RANKL to accelerate tooth movement.

KEY WORDS: Micro-osteoperforation, minimally invasive, RANKL, OPG, Enmasse retraction.

INTRODUCTION

Whenever a patient comes for an orthodontic treatment, he/ she might have an esthetic or functional demand for initiating an orthodontic treatment. But most patients take into consideration, the third factor as well which is the duration of the treatment. In order to meet the demands of the patients, so many techniques in accelerating orthodontic treatment were considered and analyzed for its efficiency.

Such techniques can be broadly divided into pharmacological and non-pharmacological techniques[1]. The non-pharmacological methods include corticotomy, piezocision, robotic prefabricated wires, indirect bonding technique, low-level laser therapy, electrical currents stimulation, pulsed electromagnetic fields, piezoelectricity, low-level mechanical vibration. The pharmacological techniques include the injection of prostaglandins, relaxin and platelet-rich plasma.

Micro-osteoperforation is a minimally invasive technique where minute perforations are drilled into the bone with the help of Propel device[1]. The perforations can be performed either

in linear or triangular pattern. The perforations should penetrate through the cortical plate long enough to cause the desired effect. This can be performed by the Orthodontists themselves and patient compliance was found to be better with this procedure.

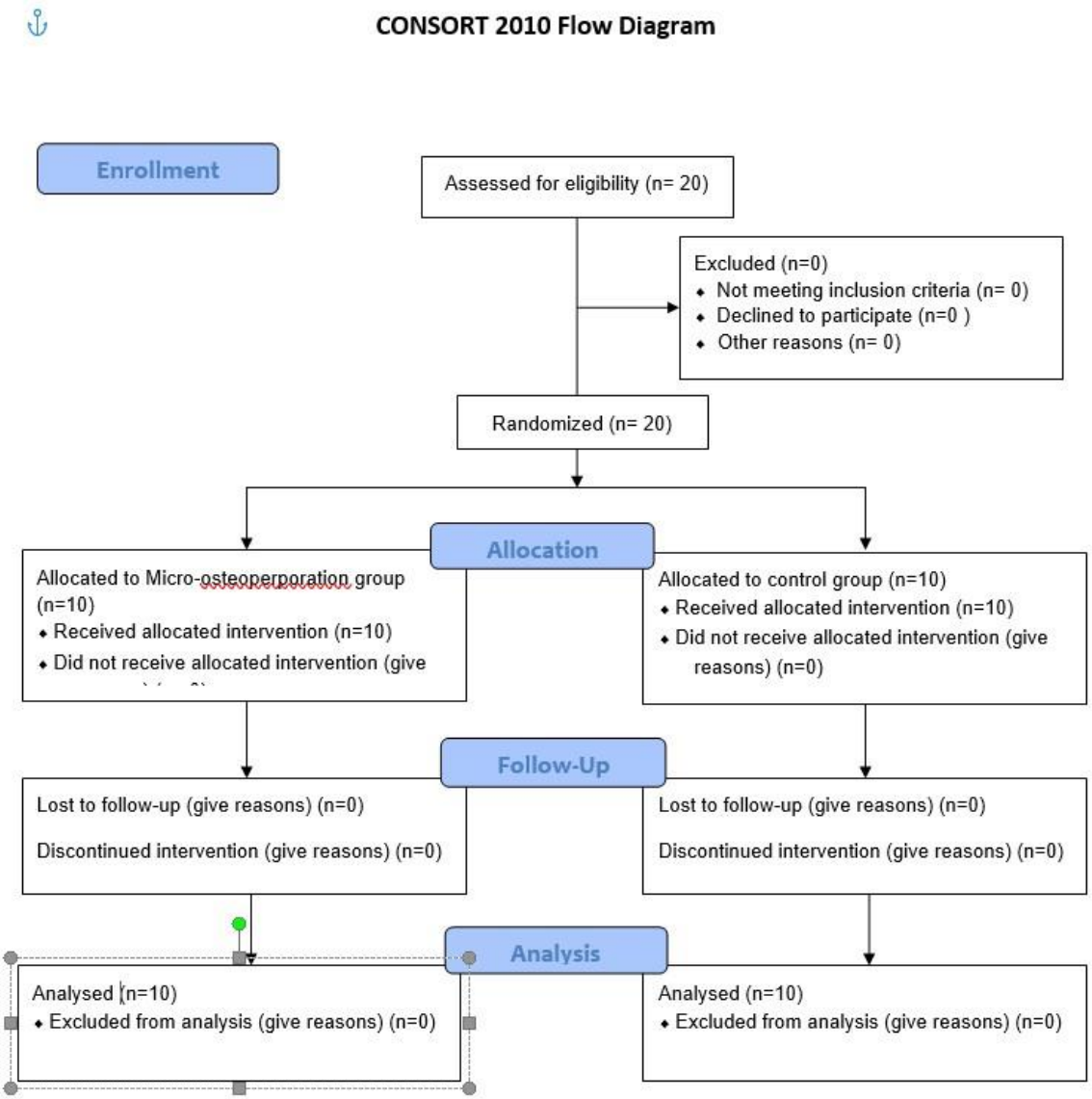
Mani Alikani(2013) found a statistically significant increase in the biomarkers such as IL 1α, IL 1β, CCL 3, CCL 5, TNF α, IL 6, IL8 when estimated at 24 hrs after micro-osteoperforation[2]. Many authors have done researches on micro-osteoperforation and all these studies extended for a maximum time period of 28 days[2,3,4,5,6,7,8,910,11,12]. But the range of action of micro-osteoperforation still remains as a question. This question still exists which indicates a need for a study which should include the evaluation of the efficiency of the procedure for a longer period of time. Thus, the current study focussed on investigating the effectiveness of the micro-osteoperforation procedure for a prolonged period of time ie, for 3 months. Also, As the basic elements needed to proceed with the orthodontic tooth movement, RANKL and OPG are the basic bone biomarkers needed for the maintenance of the balance between the bone formation and resorption. Our study has included the evaluation of these biomarkers for assessing the range of action of micro-osteoperforation.

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 796- 807
Received 18April2020; accepted 23June2020

The aims of this study were to evaluate the levels of inflammatory markers, RANKL (Receptor activator of nuclear factor-kB ligand) and OPG(Osteoprotegerin) in the gingival crevicular fluid during tooth movement in orthodontic patients and compare the RANKL and OPG levels in patients with and without micro-osteoperforation technique.

MATERIALS AND METHODS

Figure 1: CONSORT flow-diagram



The current study was approved by the Institutional Review Board of Meenakshi Ammal dental college. Twenty subjects (12 boys and 8 girls) who came for the orthodontic treatment and underwent either upper first or second premolar extraction were included in the study. The subjects were randomly allotted into one of the two groups. Group I consisted of

experimental subjects who were given micro-osteoperforations in the buccal cortex of the extraction space and Group II consisted of the control subjects who were given the conventional treatment without perforations (Figure 1).

Once the selection was made, twenty subjects were given the conventional orthodontic treatment with initial aligning and levelling. After aligning and levelling, the subjects were randomly allotted to one of the two groups according to the randomization protocol. For both the groups, at the start of Retraction treatment (T0), GCF samples were collected with the help of micropipettes from the papilla distal to maxillary canines in patients with first premolar extraction and distal to first premolars in patients with second premolar extraction.

In the experimental subjects (Group I), three linear micro perforations were made along the long axis of canine with micro implants of dimension 1.5mm diameter and 8mm in length in the maxillary extraction space on both the quadrants as shown in figure 4. Three perforations were made in vertical fashion with uniform depth of 5mm and an inter-distance of 2mm between perforations. Following this, the conventional enmasse retraction procedure was initiated either with friction or frictionless mechanics. In the control subjects (Group II), only the retraction procedure was carried out without any micro perforations. GCF samples were again collected at 1st day(T1), 45th day of retraction (T2) and 90th day of retraction(T3).

After collection of each sample, it was transferred to Eppendorf tube containing 400µL of Phosphate Buffer Solution (PBS) and stored at a temperature of -80°C. Once all the samples were collected, they were analyzed for the levels of RANKL and OPG using ELISA kits. The person who evaluated the biomarker levels was blinded regarding the patient characteristics and intervention groups.

INCLUSION CRITERIA

- Subjects of either sex in adult age group of 21 to 30 years with the mean age of 25.5±3.7 years.
- Subjects with either class I, or ClassII division 1 or div 2 malocclusion and advised extraction of upper first premolars were considered.

EXCLUSION CRITERIA

- Subjects who had Previous orthodontic treatment
- Subjects with class III malocclusion
- Subjects who didn't have extraction included in their treatment protocol
- Subjects who were periodontal compromised
- Subjects with NSAIDS therapy

STATISTICAL ANALYSIS

The SPSS software (Version 16.0) was used to perform statistical analysis. The Shapiro-Wilk's test was used to check normality of the distribution and data was found to be skewed. The comparison within groups at different time intervals were analyzed using non-parametric

Analysis of Variance(ANOVA). Mann-Whitney U test was used to compare the significance between the two groups and Wilcoxan signed rank test was used to compare significance between the two time periods within a particular group.

RESULTS

ANALYSIS OF RANKL:

The levels of RANKL were found to be increased significantly in both the groups at T1 (146.81 ± 16.27pmol/L) and T2(102.76 ± 8.81pmol/L) when compared with the levels before the start of retraction (P value < 0.001). At T3, the levels of RANKL were found to be reduced to the pre-retraction range in the experimental group(73.94 ± 9.14pmol/L) and increased in the control group(73.18 ± 10.13pmol/L) but the increase was not statistically significant (P- 0.2). Thus, from the values (Table 1), it clearly indicated that micro-osteoperforation found to have an effect in increasing the levels of RANKL at T1 and T2 and not at T3 (Figure 2).

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 796- 807
Received 18April2020; accepted 23June2020

		Concentration of OPG (pg/µL)			Concentration of RANKL(pmol/L)	
		Experimental	Control		Experimental	Control
T0	Min	15.82	14.4		60.3	53.8
	Median	17.43	16.37		74.71	68.43
	Max	19.65	18.31		91.3	80.6
	P	NS			NS	
T1	Min	11.41	12.56		124.6	79.5
	Median	13.75	15.07		143.61	94.95
	Max	15.7	16.41		175.8	109.7
	P	NS			*	
T2	Min	12.63	12.83		89.6	67
	Median	16.22	15.47		101.05	84.2
	Max	18.4	16.87		118.59	97.6
	P	NS			*	
T3	Min	17.02	13.81		61.68	60.1
	Median	18.07	16.03		74.94	73.65
	Max	19.8	17.43		86.8	88.9
	P	*			NS	

Table 1: Inter group and intra group comparison of the RANKL and OPG levels using Mann Whitney U test and Wilcoxan signed rank test.

Figure 2: Graphs showing levels of RANKL in group 1 and group 2 at different time intervals

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 796- 807
Received 18April2020; accepted 23June2020

ANALYSIS OF OPG

After analysis of the levels of OPG, it has been found that the lev els found to be reduced in both experimental (13.75 ± 1.64 pg/µL)and control (15.07 ± 1.52 pg/µL) groups at T1 but was not statistically significant (Figure 4). At T2, the OPG levels found to be increased (16.22 ± 1.94 pg/µL) in the experimental group when compared to the control group. Further there was a rise in the levels of OPG at T3 where more increase noted in the experimental group (18.07 ± 0.81 pg/µL) than in the control group (16.03 ± 1.30 pg/µL) but with no statistically significant difference (Figure 3).

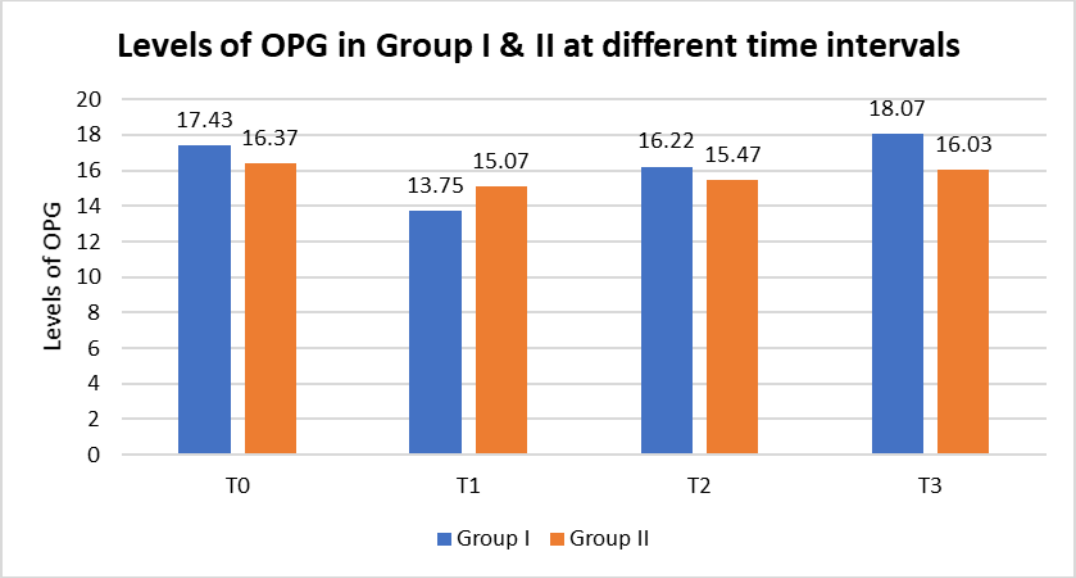
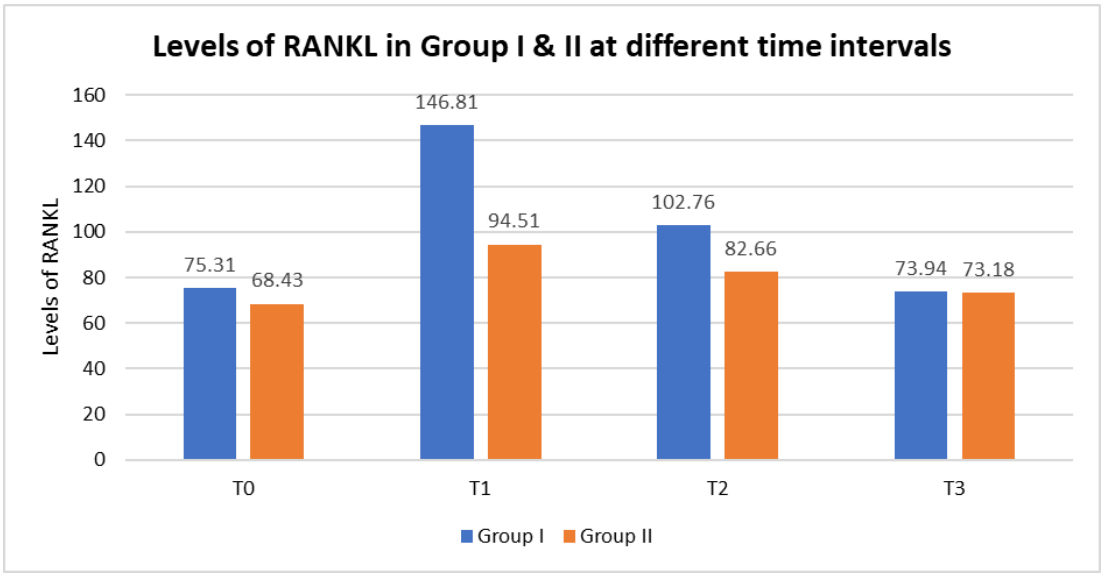


Figure 3: Graphs showing levels of OPG in group 1 and group 2 at different time intervals

ANALYSIS OF RANKL/OPG RATIO

When a graph is plotted with the RANKL/OPG ratios in both experimental and control groups at all the time intervals, it has been noted that there was a peak elevation noted at T1



and declining at T2 and further declining at T3 (Figure 4).

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 796- 807
Received 18April2020; accepted 23June2020

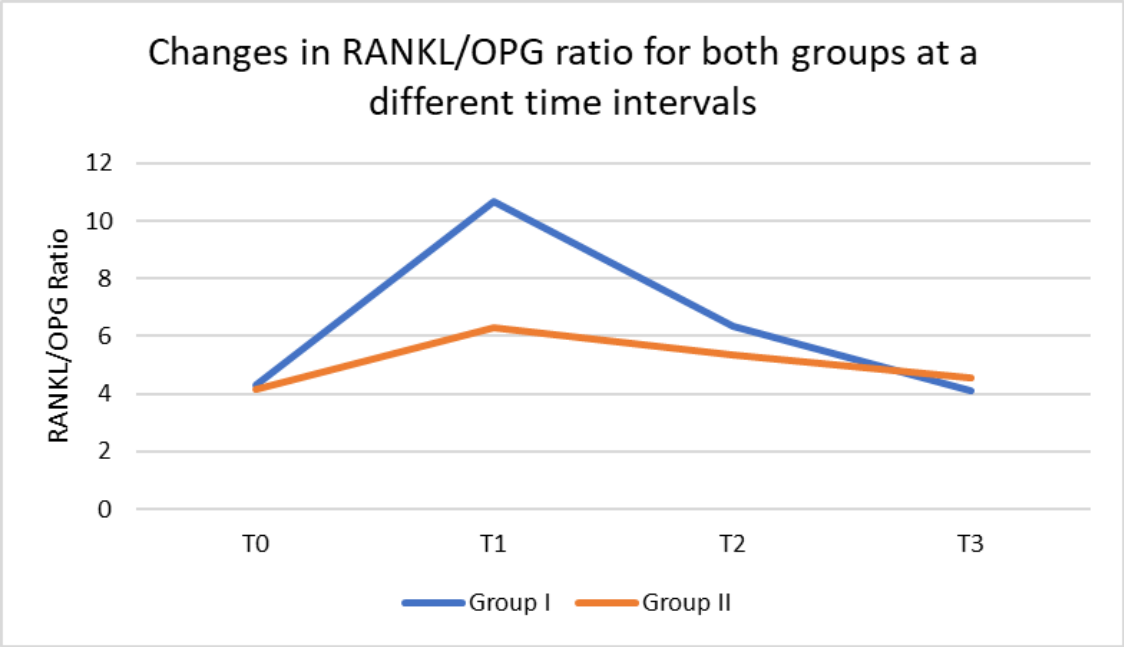


Figure 4: Graphs showing changes in RANKL/OPG ratio between two groups at two different time intervals

DISCUSSION

Tooth movement is a coupled reaction which is initiated with bone resorption and followed by bone deposition occurring as a chain of reactions involving the role of certain inflammatory mediators. Over the decades' numerous studies have been reported regarding the role of these inflammatory cytokines and the rate tooth movement. Amila vujacic evaluated the role of different cytokines in orthodontic tooth movement and found that IL 1β, IL- 6 and TNF-α act as mutual activators and inhibitors of tooth movement[13].

Although patients often prefer orthodontic treatment for an improved esthetics, they do desire a lesser duration of the orthodontic treatment as well. In an effort to improve the esthetics of the patients and reduce the treatment duration simultaneously, many techniques were introduced and tried to prove its real significance.

Regional Acceleratory Phenomenon (RAP) is a reaction of the tissues against a noxious stimulus and assists in healing of the affected tissue. In Accelerated Orthodontics, when these techniques were performed, this in turn activates or increases RAP in the particular area thereby resulting in faster tooth movement. Nita Viwattanatipa in their systematic review investigated the effectiveness of both corticotomy and piezocision in increasing the rate of canine retraction which proved that both the procedures can be considered as a predominant technique in the field of accelerated orthodontics[14].

A less invasive technique, micro-osteoperforation was carried out using specially designed commercially available device namely Propel. This particular device consists of disposable micro implant tips which are used to drill minute perforations into the bone where an increased tooth movement is required. The greatest advantage of this minimally invasive

technique is that this micro-osteoperforation can be performed by Orthodontists themselves without any elaborative surgical procedure.

Mani Alikhani et al has performed series of research on the effect and effectiveness of micro-osteoperforation on accelerating the rate of tooth movement[2]. It was considered that about 2 to 4 perforations per site would be ideal regarding the number of perforations and about 3 to 7mm depth of penetrations into the bone should be sufficient to achieve catabolic processes in order to accelerate tooth movement. They also concluded that three perforations distal to canines increased the rate of orthodontic tooth movement by 2.3 fold.

As RANKL and OPG remain as important components essential to switch over the process of bone resorption and bone deposition, the present study included the estimation of levels of RANKL and OPG in patients treated with micro-osteoperforation.

Parichehr Ghalayani evaluated the effects of diclofenac and celecoxib on the process of osteoclastogenesis and found that both the drugs directly affected the process of osteoclastogenesis with its effects on RANKL/ OPG gene expression[15]. Thus, the patients under any anti-inflammatory drug therapy were excluded from the present study.

Also, GCF samples were collected on the day of start of retraction, 1st, 45th and 90thday of retraction. Earlier study by Mani Alikhani evaluated the levels of different cytokines on 1stday, 7th day, 14th day and 28thday of retraction[2]. It was found that a significant rise in the levels at day 1 with a gradual decline from 7th day to 28thday. Moreover, their study estimated the levels only for a maximum period of 28 days. But the long-term effect of MOPs still remains unclear and hence the present study aimed to estimate the OPG & RANKL levels at the time intervals of 45 days and 90 days after retraction.

Thus, in the present study during orthodontic tooth movement coupled with micro-osteoperforation, RANKL levels were significantly increased at 24 hours after the start of retraction in the experimental group when compared to the control group which constituted about 35%. This indicated that there was increased amount of tooth movement at 24 hours of retraction, though the levels of RANKL were declining at T2, it still had a statistical significance when compared to the control group which constituted about 19%. Hence it can be mentioned that three microosteoperforations for accelerating the orthodontic tooth movement had its effect on maintaining increased levels of RANKL atleast for a period of 45 days. Later at T3, the RANKL levels met a decline which suggested that MOPs were not effective at 90th day of retraction. Hence, we can postulate that repeated MOPs at intervals can maintain the elevated levels of RANKL inducing localized bone resorption constantly enhancing the orthodontic tooth movement. However, as a regulatory mechanism of bone remodelling, OPG acts as an inhibitory receptor for bone resorption and thus by estimating these levels could give proper insight into the role of RANKL/OPG ratio in maintaining the balance during orthodontic tooth movement.

The present study results showed that there was a significant decline in the values of OPG at T1 when compared to T0 which constituted about 9.5%. This indicated that there would have been active bone resorption taking place at 24 hrs of orthodontic tooth movement which was mediated by the RANKL. Later at T2, the OPG values were increased than T1 and noted a significance decrease when compared to the control group at T2 which constituted about 4.6%. Probably we can assume that this downregulation of OPG facilitated the RANKL to promote the burst of osteoclastogenesis enhancing bone resorption even at 45 days after retraction. Nonetheless in the experimental group at T3 the OPG levels showed an upregulation thereby indicating the regulatory role of OPG in controlling the bone resorption.

Thus, the balance between the OPG and RANKL is essential in maintaining normal bone turn over. When the RANKL/OPG ratio was analyzed, it was found that this ratio was increased at day 1. Later there was a gradual decrease in this RANKL/OPG ratio upto 90 days revealing that both the biomarkers compete with each other to maintain the bone remodelling during orthodontic tooth movement.

As we all know that the biology of tooth movement involves an orchestration of chemical mediators, these bone specific biomarkers OPG and RANKL should always be present at the bone remodelling site even during regular as well as accelerated tooth movement. Thus in order to accelerate the tooth movement, bone resorbing factor RANKL need to be higher to bring about acceleration and hence MOPs were assumed to have an effective range of action upto a period of 45 days in maintaining the higher levels of RANKL. Also, it is a well-known fact that retraction phase of tooth movement requires a regular activation once in 4 to 6 weeks. If we extrapolate this concept in accelerated tooth movement, MOPs should also be performed at regular intervals in order to enhance the rate of tooth movement and reduce the treatment time.

Future studies might include to prove the effect of micro-osteoperforation in the lower arch as the bone is very dense in the mandible. Also, studies can be done involving more time intervals to determine the levels of RANKL and OPG accurately.

CONCLUSION:

The following conclusions were derived from the present study:

- Three micro-osteoperforations was effective in accelerating orthodontic tooth movement.
- RANKL levels was increased in the experimental group and continued to remain raised till 45 days of retraction.
- OPG levels reduced after 1 day of retraction later followed by a gradual rise up to 90 days of retraction.
- RANKL/OPG ratio was gradually decreased from 24 hours to 90 days of retraction to maintain the bone remodelling

- Micro-osteoperforation reserved the RANKL levels higher up to 45 days to enhance bone resorption indicating repetition of the procedure at regular intervals in order to accelerate tooth movement during retraction.

ACKNOWLEDGEMENTS: None

FUNDING
This review was not funded by any funding agencies.

CONFLICT OF INTEREST
The reviewers declare that there was no conflict of interest.

REFERENCES

[1] M. Alikhani, A clinical guide to accelerated Orthodontics: With a focus on microosteoperforations, 1st edition, 2017.
[2] M. Alikani and M. Raptis, "Effect of microosteoperforations on the rate of tooth movement," Am Orthod Dentofacial Orthop, vol. 144, no. 5, pp. 639-648, 2013.
[3] C. C. Teixeira and E. Khoo, "Cytokine expression and accelerated tooth movement," J Dent Res, vol. 89, no. 10, pp. 1135-1141, 2010.
[4] X. Yang, Y. Wang and D. Bai, "Effects of TGF- β1 on OPG/RANKL expression of cementoblasts and osteoblasts are simi lar wi thout stress but different with mechanical compressive stress," The scientific World Journal, p. 12, 2016.
[5] E. Chan, O. Dalci, P. Petocz, A. K. Papadopoulou and M. A. Darendeliler, "Physical properties of root cementum: Part 26. Effects of micro-osteoperforations on orthodontic root resorption: A microcomputed tomography study," Am J Orthod Dentofacial Orthop, vol. 153, no. 2, pp. 204-213, 2018.
[6] M. Feizbakhsh and D. Zandian, "The use of microosteoperforation concept for accelerating differential tooth movement," Journal of the World Federation of orthodontics, vol. 7, pp. 56-60, 2018.
[7] A. Alkebsi and E. A. Maaitah, "Three-dimensional assessment of the effect of microosteoperforations on the rate of tooth movement during canine retraction in adults with class II malocclusion: A randomized control led clinical trial," Am J Orthod Dentofacial Orthop, vol. 153, no. 6, pp. 771-785, 2018.
[8] T. Sugimori and M. Yamaguchi, "Micro-osteoperforations accelerate orthodontic tooth movement by stimulating periodontal ligament cell cycles," Am J Orthod Dentofacial Orthop, vol. 154, no. 6, pp. 788-796, 2018.
[9] S. Sivarajan, J. G. Doss, S. N. Papageorgiou, M. T. Cobourne and M. C. Wey, "Mini-implant supported canine retraction with micro-osteoperforation: A split-mouth randomized clinical trial," Angle Orthod, vol. 89, no. 2, pp. 183-189, 2019.
[10] B. Alqadasi, K. Aldhorae, . E. Halboub, N. Mahgoub, A. Alnasri, A. Assiry and H. Y. Xia, "The Effectiveness of Micro-osteoperforations during Canine Retraction: A Three-

dimensional Randomized Clinical Trial," J Int Soc Prev Community Dent, vol. 9, no. 6, pp. 637-645, 2019.

[11] A. A. Aboalnaga, . M. M. S. Fayed, N. .. El-Ashmawi and . S. A. Soliman, "Effect of micro-osteoperforation on the rate of canine retraction: a split-mouth randomized controlled trial," Prog Orthod, vol. 20, 2019.

[12] M. Shahabee, H. Shafaee, . M. Abtahi, A. Rangrazi and E. Bardideh, "Effect of micro-osteoperforation on the rate of orthodontic tooth movement-a systematic review and a meta-analysis," Eur J Orthod., vol. 42, no. 2, pp. 211-221, 2020.

[13] A. Vujacic, J. Pavlovic and A. Konic-Ristic, "The role of cytokines in orthodontic tooth movement," Current approaches in Orthodontics, vol. 24, no. 12, pp. 35-49, 2018.

[14] N. Viwattanatipa and S. Charnchairerk, "The effectiveness of corticotomy and piezocision on canine retraction: A systematic review," Korean J Orthod, vol. 48, no. 3, pp. 200-211, 2018.

[15] P. Ghalayani, M. Minaiyan, S. M. Razavi, S. Hajisadeghi, N. Naghsh and M. S. Abuie, "Effects of diclofenac and celecoxib on osteoclastogenesis during alveolar bone healing, in vivo," Dent Res J (Isfahan), vol. 11, no. 3, pp. 357-363, 2014.