ORIGINAL RESEARCH

Evaluating the Effect of Denosumab in Preventing Anchorage Loss: A Split-mouth Randomized Controlled Trial

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Abstract

Aim: The trial was focused on assessing the effect of Denosumab in preventing anchorage loss during en-masse anterior retraction and evaluating its effect on the retraction.

Materials and methods: This was a split-mouth randomized controlled trial. Ten subjects were randomly allocated with equal probability for Denosumab and control interventions in the contralateral quadrants using computer-generated randomization sequence. During the start of retraction, Denosumab (5 mg/0.2 mL) and injectable sterile water were administered locally on the intervention and control sides, respectively. Lateral cephalograms taken during the start of retraction and later in the 3rd and 6th months into retraction were used to evaluate anchorage loss and retraction. Independent sample *t*-test and Mann-Whitney *U* test compared anchorage loss and retraction between the two groups in the maxilla and mandible. Paired *t*-test and Wilcoxon signed-rank test assessed the anchorage loss and retraction during the first and the second 3 months of retraction.

Results: In the maxilla, Denosumab was effective in preventing anchorage loss with a *p*-value of 0.001 whereas it was not effective in the mandible (*p*-value—0.172). A significant reduction in anchorage loss was observed with Denosumab in the second 3 months of retraction compared to the first 3 months. There was no significant difference in the retraction among both groups.

Conclusion: Denosumab was effective in minimizing the anchorage loss in the maxilla without affecting the anterior retraction.

Clinical significance: Denosumab can be effectively used for reinforcing anchorage in the maxilla during en-masse anterior retraction.

Keywords: Anchorage, Denosumab, RANKL inhibitor, Tooth movement.

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Introduction

Anchorage, the resistance to undesired tooth movement, is one of the indispensable aspects of orthodontic treatment to achieve the expected treatment outcomes.[1] Various techniques have been designed and are in practice to control the anchorage loss, namely, temporary anchorage devices (TADs), transpalatal arch, Nance palatal arch, lingual arch, intermaxillary elastics, and headgear.[2,3] However, there are disadvantages associated with these techniques such as patient compliance, patient apprehension, unwanted tooth movements, safety, and stability of the TADs.[4,5] Thus pharmacological agents might be an alternative way to control anchorage.

Orthodontic tooth movement depends on both bone resorption and deposition.[6] The receptor activator of nuclear factor kappa B ligand (RANKL) is a member of the tumor necrosis factor (TNF) family and has an important part in the process of osteoclastogenesis.[7] In the bone, RANKL is expressed on the osteoblasts and it gets activated by binding to the RANK receptor present on the osteoclasts. The activated RANKL leads to the formation of mature osteoclasts.[8] Whereas, osteoprotegerin (OPG) acts as a decoy receptor of RANKL and competes with RANK for binding to RANKL. The inhibition of RANK-RANKL interaction eliminates the terminal stages of osteoclast maturation and prevents osteoclastogenesis and in turn bone resorption.[9,10] A group of animal studies using the local injection of osteoprotegerin and local gene transfer of OPG to inhibit tooth movement have proved the efficiency of OPG in preventing osteoclastogenesis.[11–16]

Denosumab, a humanized monoclonal antibody against RANKL, mimics OPG by binding to the main activator site of RANKL.[10,17] Denosumab is used in humans to treat osteoporosis, bone loss due to prostate and breast cancers, and various bone tumors.[18–20] The use of Denosumab in preventing tooth movement in humans has not been evaluated to date. Therefore, the study aimed to evaluate the effect of Denosumab in preventing anchorage loss during

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en-masse anterior retraction along with its effect on the rate of anterior retraction. The null hypothesis of the study was that there is no significant difference in the amount of anchorage loss in the Denosumab and control group during en-masse anterior retraction.

Materials and Methods

Trial Design and Setting

This was a prospective split-mouth randomized controlled clinical trial conducted in the Department of Orthodontics and Dentofacial Orthopedics, Saveetha Dental College and Hospitals, Chennai. The study design was approved by the Institutional Review Board and the Institution Human Ethical Committee (SRB/SDC-ORTHO-1801/19/03). The trial was also registered in the Clinical Trial Registry—India (CTRI/2019/09/021140).

Sample Size Calculation, Study Population, and Selection Criteria

Sample size calculation was done for a power of 95% based on the results of the anchorage study by Thiruvenkatachari et al.[21] using G power analysis software. The mean and standard deviation of the anchorage loss in the maxilla were used for the calculation. 10 subjects undergoing orthodontic treatment were selected for the study based on the following selection criteria and consent was signed by all the subjects.

The inclusion criteria were:

- The age-group of 15–30 years
- Patients who require maximum or absolute anchorage
- Crowding is less than or equal to 4 mm
- Dentoalveolar proclination
- Angle's Class I dental malocclusion

The exclusion criteria were:

- Immunocompromised patients
- Hypocalcemic patients, as hypocalcemia can be worsened by the administration of the drug Denosumab
- Patients with active carious lesions or periodontitis
- Patients with systemic diseases
- Patients with a history of hypersensitivity reactions to any drug
- Pregnant patients

Randomization Sequence

Participants were allocated with equal probability for Denosumab (experiment group) and the control group in contralateral quadrants. Randomization was done using a computer-generated pseudo-random code and stratification was included within the randomization based on gender to ensure equal sexes in each group.

Allocation Sequence

When a patient consented to take part in the study, the operator contacted the central trial coordinator who was independent of the trial. Details of the patients, which only included patients' names and gender, were taken. The central trial coordinator assigned a specific trial number for each patient and then randomly allocated the patients to either:

- Denosumab Right Upper (RU) and Left Lower (LL) quadrants and control Left Upper (LU) and Right Lower (RL) quadrants or
- Denosumab LU and RL and control RU and LL quadrants

Using the computer-generated randomization method as mentioned above.

Blinding

The subjects and the investigator who made all the measurements were blinded on the type of intervention.

Derivation of Drug Dosage

The dosage of denosumab was decided based on the pharmacokinetics of the drug and conversion of the animal dosage value obtained from the study by Dunn and coworkers.[11] Animal dose to human dosage conversion was done using the formula given below.[22]

Human equivalent dose (HED) (mg/kg) = Animal dose × (Animal weight/Human weight) (0.33)

The HED value was divided by a factor value of 10 for the first dose in entry to human studies. The calculated dosage of the drug was formulated by diluting the commercially available 60 mg of Denosumab (Olimab, Intas Pharmaceuticals, India) to 5 mg (0.2 mL) using sterile water for injection.

Intervention

Routine blood investigations along with serum calcium level and hypersensitivity test were done for all subjects before the administration of the drug. Serum calcium level was investigated as hypocalcemia induced due to vitamin D deficiency is highly prevalent among the Asian Indian population and it might be worsened by the administration of Denosumab.[23,24] A hypersensitivity test was done by administering an intradermal injection of 0.05 mL of the drug diluted to 1:100 concentration using water for injection and observing the patient for 30 minutes for any hypersensitivity reactions.[25,26] Subjects were advised to report if they consume analgesics for orthodontic pain or other medications for any systemic ailments that arose during the trial. Subjects who consumed other medications during the trial were decided to be removed from the trial. This was done to eliminate any chance of drug interactions and the possible effect on tooth movement.

Local injection of Denosumab 5 mg (0.2 mL) was given on the buccal aspect of upper and lower first molar submucosally using insulin syringe on intervention sides whereas injectable sterile water was administered on the buccal aspect of the upper and lower the first molar on the control side during the start of retraction stage of the treatment. No other anchorage reinforcement techniques were used and en-masse anterior retraction was done using nickel-titanium closed coil springs exerting a force of 200 g on each side. The force was measured using a dontrix gauge. The Ni-Ti coil spring was extended from the first molar to the retraction hook crimped between the canine and lateral incisor on a 0.019 × 0.025 inch stainless steel (SS) archwire ligated using SS ligature wire to 0.022″ slot MBT metal brackets. The crimpable hook was also welded to the archwire to ensure stability. The subjects were followed up for seven reviews spaced at 1-month intervals each. Lateral cephalograms were obtained during the start of retraction (T0), third month (T1), and sixth month (T2) after the start of retraction. The amount of anchorage loss and anterior retraction was evaluated in these appointments to determine the maximum effect of the drug and also to evaluate the need for a second dose of injection in future studies.

Outcomes

The primary and the secondary outcomes of the study were to assess the amount of loss of anchorage by measuring the mesial

movement of the first molar and to evaluate the amount of anterior retraction, respectively.

Evaluation of Anchorage Loss

The loss of anchorage was evaluated by superimposing the lateral cephalograms taken during T0, T1, and T2.

To identify the right and left side molars on the lateral cephalogram, a 0.017 × 0.025-inch SS wire bent in an L shape was placed in the buccal tube of the first molars. The horizontal portion was inserted from the mesial side of the buccal tube and cinched distally on the right side (Fig. 1A). On the left side, the wire was inserted from the distal surface of the buccal tube and cinched mesially (Fig. 1B).[21]

Maxillary anchorage loss was measured by superimposing the cephalograms along the palatal plane on the anterior nasal spine according to McNamara's method. The horizontal distance between the vertical arm of the L wire on the superimposed image of molars indicated the anchorage loss in the maxilla.[27]

The tracings were superimposed on the anterior superior border chin border, the inner cortical plate of the symphysis on its lower surface, and the mandibular canal to assess the mandibular anchorage loss.[28] The distance between the vertical arm of L wire on the superimposed molars indicated the loss of anchorage in the mandible.

Evaluation of Anterior Retraction

On the radiograph, the left and right retraction hooks were distinguished by locating them along the archwire from the respective molars.[29] Superimpositions for maxilla and mandible were done according to McNamara's method[27] and Bjork's method[28] as described previously for anchorage loss assessment. The distance between the retraction hooks traced at various time intervals indicated the amount of anterior retraction in both the upper and lower arches.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics software version 20.0 for Windows. Intraexaminer reliability was examined by repeating the measurements after 5 weeks for five samples that were randomly selected and calculating the intraclass correlation coefficient.

Descriptive statistics were done for the obtained data. The normality distribution of the data was calculated using the Shapiro Wilk test. Independent sample *t*-test/Mann-Whitney *U* test was used to compare the mean anchorage loss and mean retraction between the two groups in the maxilla and mandible. Paired *t*-test/Wilcoxon signed-rank test was done to see the difference in anchorage loss and rate of retraction during the third month and the sixth month into retraction on the intervention and control sides in maxilla and mandible separately. The significance value (*p*-value) and confidence interval were set as 0.05 and 95%, respectively, for all the comparison tests.

Results

Participant Flow and Baseline Data

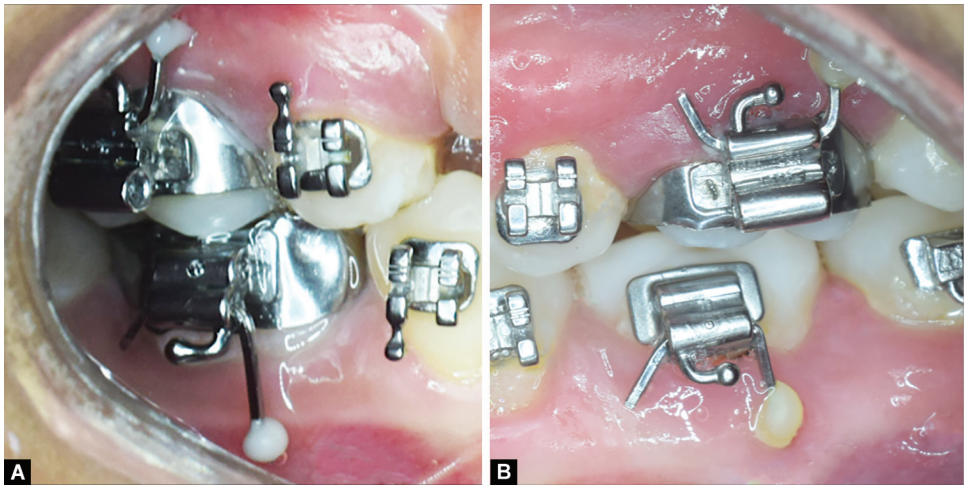
Eligibility assessment was done for a total of 26 subjects, out of which three subjects did not meet the eligibility criteria and 13 refused to participate. Flowchart 1 represents the progress of the trial. Eight female and two male subjects with a mean age of 20.60 ± 4.11 years were involved in the study. All the subjects were systemically healthy during the entire course of the trial and there was no attrition of samples.

Analyses

The results showed that the data for anchorage loss were nonparametrically distributed whereas the data for the amount of retraction were normally distributed. Descriptive statistics for anchorage loss and retraction is represented in Table 1.

Anchorage Loss

The results of the Mann–Whitney *U* test showed that during the first 3 months of retraction, i.e., from T0 to T1, there was no significant difference in anchorage loss between the Denosumab and the control side in both maxilla (*p*-value—0.543) and mandible (*p*-value—0.97). In the next 3 months of retraction (T1–T2), there was a significant difference in anchorage loss in the Denosumab and control side in the maxilla (*p*-value—0.001) while there was no significant difference in the mandible (*p*-value—0.052). The amount of anchorage loss during the entire 6 months of retraction period (T0–T2) showed that in maxilla Denosumab was effective in preventing loss of anchorage with a *p*-value of 0.001 while it



Figs 1A and B: (A) Right side molars with the vertical arm of the L-shaped wire on the mesial side of the buccal tube; (B) Left side molars with the vertical arm of the L-shaped wire on the distal side of the buccal tube

Flowchart 1: The consort flow chart

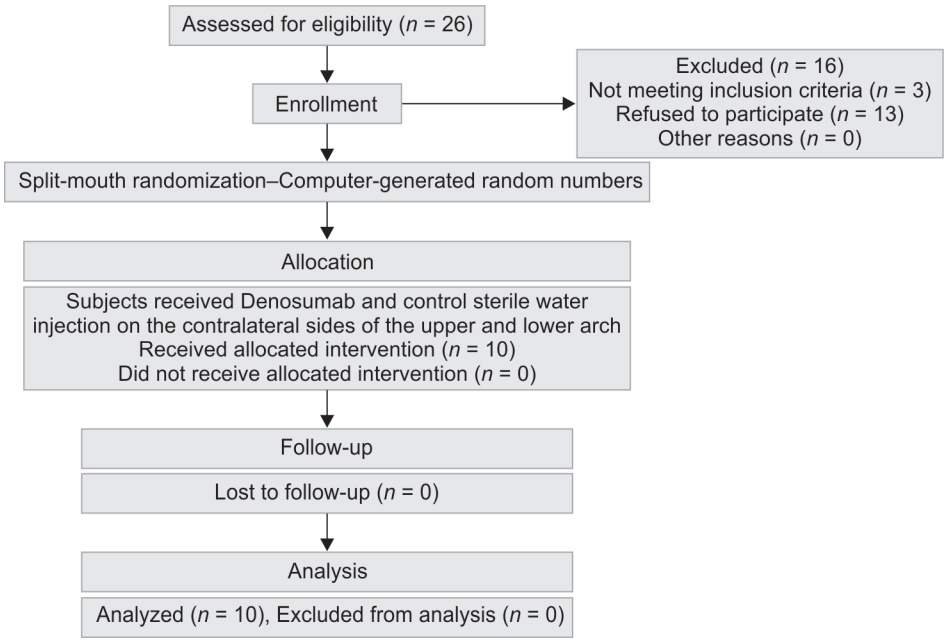


Table 1: Mean and standard deviation for the amount of anchorage loss and retraction on Denosumab and control sides in maxilla and mandible

	Amount of anchorage loss (mm)			*Amount of retraction (mm)*		
	T0–T1	*T1–T2*	*T0–T2*	*T0–T1*	*T1–T2*	*T0–T2*
Maxilla						
Denosumab	0.93 ± 0.48	0.21 ± 0.34	1.14 ± 0.44	1.83 ± 0.15	1.97 ± 0.68	3.92 ± 0.52
Control	1.11 ± 0.53	1.04 ± 0.53	2.18 ± 0.73	1.93 ± 0.22	2.25 ± 0.55	4.17 ± 0.47
Mandible						
Denosumab	0.90 ± 0/43	0.29 ± 0.44	1.19 ± 0.67	1.97 ± 0.2	1.67 ± 0.44	3.64 ± 0.36
Control	0.91 ± 0.62	0.60 ± 0.40	1.52 ± 0.57	1.81 ± 0.15	1.89 ± 0.39	3.72 ± 0.36

Table 2: Results of statistical analyses comparing the anchorage loss and retraction among Denosumab and control sides at T0–T1, T1–T2, and T0–T2 in maxilla and mandible

	Anchorage loss		*Anterior retraction*	
	Mean difference (mm)	*p value*	*Mean difference (mm)*	*p value*
Denosumab—control (maxilla)				
T0–T1	−0.18	0.543	−0.1	0.252
T1–T2	−0.83	0.001*	−0.28	0.328
T0–T2	−1.04	0.001*	−0.25	0.275
Denosumab–control (mandible)				
T0–T1	−0.01	0.970	0.16	0.060
T1–T2	−0.31	0.052	−0.22	0.253
T0–T2	−0.33	0.172	−0.80	0.629

*p value <0.05—statistically significant

was not significantly effective in preventing anchorage loss in the mandible (p-value—0.172) (Table 2).

According to Wilcoxon signed-rank test, in the Denosumab side, there was a significant reduction in the anchorage loss during the second 3 months of retraction compared to the first 3 months with a p-value of 0.028 and 0.014 for maxilla and mandible, respectively, whereas there was no difference in anchorage loss in the control side between T0–T1 and T1–T2 with a p-value of 0.683 for maxilla and 0.202 for mandible (Table 3).

Anterior Retraction

The results of the independent t-test indicated that there was an insignificant difference in the amount of retraction between the Denosumab and control sides at all the three-time intervals (p-value >0.05) (Table 2). Similarly, the paired t-test showed an insignificant difference (p-value >0.05) in the amount of retraction seen in the first 3 months and the second 3 months of retraction in both the Denosumab and the control sides (Table 3).

Intraexaminer Reliability

Intraclass correlation coefficient value of 0.094–0.98 was obtained indicating excellent intraexaminer reliability.

From the results obtained, it can be inferred that the null hypothesis was rejected for maxilla whereas it was not for the mandible, i.e., the drug Denosumab was effective in reinforcing anchorage in the maxilla but not in the mandible. Also, Denosumab did not have any effect on anterior retraction.

Discussion

The present trial was aimed to evaluate the effect of Denosumab in locally inhibiting bone resorption and preventing molar anchorage

Table 3: Results of statistical analyses comparing the anchorage loss and anterior retraction seen during first 3 months and second 3 months of retraction in Denosumab and control sides in maxilla and mandible

	Anchorage loss		*Anterior retraction*	
	Mean difference ± SD (mm)	*p value*	*Mean difference ± SD (mm)*	*p value*
(T0–T1)–(T1–T2) maxilla				
Denosumab	0.72 ± 0.68	0.028*	−0.14 ± 0.71	0.507
Control	0.07 ± 0.75	0.683	−0.32 ± 0.72	0.192
(T0–T1)–(T1–T2) mandible				
Denosumab	0.61 ± 0.55	0.014*	0.3 ± 0.58	0.137
Control	0.31 ± 0.87	0.202	−0.08 ± 0.46	0.595

*p value <0.05—statistically significant

loss during en-masse anterior retraction and also to evaluate its effect on the anterior teeth. The results of the trial showed that Denosumab was effective in controlling anchorage loss by decreasing the mesial movement of the molar in the maxillary arch. Even though the amount of anchorage loss during the first 3 months of the retraction was similar in both the experimental and the control groups, Denosumab significantly reduced the mesial movement of the molar and enhanced anchorage control during the second 3 months of the retraction. The time taken by the drug to prevent osteoclast formation and increase the bone mineral density could be the reason for the very minimal mesial movement of molars seen in the latter stage of retraction.[30,31] During the initial days after the injection, it takes time to inhibit the RANKL preventing osteoclast formation and eventually contributing to increasing the bone mineral density. As the bone mineral density increases, the rate of bone resorption decreases and prevents tooth movement. Hence we suggest the use of this drug 1 or 2 months before the start of retraction to utilize the maximum benefit. In the mandibular arch, there was an insignificant reduction in the loss of anchorage in the Denosumab side compared to the control side. This can be attributed to the less tendency for anchorage loss in the mandible due to the thick cortical bone present in the mandible which is more resistant to resorption compared to the maxillary bone.[32]

The localized action of the Denosumab was also evident from the fact that it did not inhibit or reduce the amount of anterior retraction in both jaws. The action of the drug was restricted to the area of the injection and the teeth away from the site of injection were not affected.

The inference of our study was similar to that of the animal studies that used the local injection of osteoprotegerin to enhance anchorage control.[11–15] In an anchorage study done on Sprague Dawley rats, 5 mg of OPG twice weekly was effective in reducing the forward movement of molar to 0.20 ± 0.03 mm for incisor retraction of 1.05 ± 0.03 mm at the end of 21 days of retraction. The ratio of anterior retraction to the posterior movement was 5.2:1.[11] Fernandez et al., in two of his animal studies, showed similar results with subcutaneous injection of 5 mg OPG twice weekly. Mesial molar movement of 0.2 mm was noted in the OPG side whereas molar movement of 0.99 mm was noted in the control side.[12,13] Ten milligrams of OPG subcutaneous injections administered daily up to 8 days on C57Bl/6 mice reduced the molar movement to 0.02 mm. The same study also highlighted that OPG was effective in reducing mesial molar movement compared to Pamidronate.[15] In a similar study by Sydorak et al., it was shown that the action of OPG could be localized by microsphere encapsulation of the drug.[14] In all the above animal studies the amount of mesial molar movement seen was less than that seen in our study. This might be because of the variations in the density of the murine and human alveolar bone and also the murine molars were anchored to retract only two incisors whereas in our study the molars were anchored to retract six anterior teeth.

Bisphosphonates, simvastatin, aspirin, relaxin, chemically modified tetracycline 3, and other related molecules are some of the other pharmacological agents known to inhibit tooth movement and are studied *in-vivo* in animals.[33,34] Of these bisphosphonates is one of the most commonly studied drugs to inhibit tooth movement in animals[35–38] but it has not been used for clinical trials due to the adverse effects of osteonecrosis and irreversible binding to the marrow spaces. Unlike bisphosphonates, RANKL inhibitors such as OPG and Denosumab do not stay in the bone and their effects fade away as the drug is eliminated from the body.[39]

Denosumab has a bioavailability of 61% after subcutaneous injection. It takes 10 days for 60 mg of s.c. injection to reach peak plasma concentration. Its plasma half-life is 25–38 days. Metabolism of the drug is unknown whereas it is eliminated by the reticuloendothelial system.[40]

In children, it is to be used with caution as it causes postnatal developmental defects of the teeth and adequate information is not available regarding the safety of the drug in pregnant women. Therefore, children below 15 years and elderly subjects above 30 years were not included in the study considering the effect of Denosumab on the growth of children and the possible systemic disorders seen in the elderly. It is contraindicated in patients with hypersensitivity, hypocalcemia, and those who are in chronic kidney failure stage IV or V with creatinine clearance of less than 30 mL/minute.[40]

The adverse effects include nausea, fatigue, asthenia, dyspnea, cataract, eczema, and flu-like syndrome. Severe hypocalcemia of serum calcium (Ca) level less than 7 mg/dL is one of the serious adverse effects of Denosumab. This can be controlled by intake of Ca and vitamin D supplementation. Other rare but serious adverse effects include hypophosphatemia (serum phosphorus level less than 2 mg/dL) and osteonecrosis of the jaw. These adverse effects are reported only in a very less percentage of cases who are under high doses of the drug, about 120 mg 3 months once.[40] None of these ill-effects was observed in the subjects of the current study. Considering the currently available literature this is the first clinical trial evaluating the anchorage control potential of the drug Denosumab and it has proved to be effective in controlling the same.

Generalizability

Denosumab reduces the anchorage loss by inhibiting the movement of the molars when it is injected locally near the

target teeth. Considering the reversible effects of the drug and its potential to inhibit the tooth movement in humans, it can be considered for preventing anchorage loss in patients who are apprehensive about placing implants or in those cases where the bone thickness and density are compromised and stability of the implant is questionable. The results of this study can be applied to systemically healthy patients of the age-group 15–30 years.

Limitations

The limitation of the study was that the applicability of the drug to age-groups below 15 years and above 30 years was not assessed. Also, further clinical trials need to be done with different dosages of the drug and varied timing of administration of the drug. As suggested before, it can be administered 1 or 2 months before the start of the retraction since a more significant reduction in tooth movement was noted 3 months after the administration of the drug. The effect of the drug on postorthodontic stability has not been covered in this study.

Conclusion

Local injection of Denosumab was efficient in reinforcing anchorage in the maxilla but not mandible. Positively, it also did not affect the rate of anterior retraction in both the maxilla and mandible.

Clinical Significance

Denosumab can be effectively used for reinforcing anchorage in the maxilla during en-masse anterior retraction.

Acknowledgments

The clinical trial was conducted in the Department of Orthodontics and Dentofacial Orthopedics, Saveetha Dental College and Hospitals, Chennai. The study design was approved by the Institutional Review Board and the Institution Human Ethical Committee (SRB/SDC-ORTHO-1801/19/03). The trial was also registered in the Clinical Trial Registry—India (CTRI/2019/09/021140).

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