Effects of vitamin D deficiency on the rate of orthodontic tooth movement: An animal study

Rawan M. Khalaf a,1,*, Abdullazez A. Almudhi b,2

a Postgraduate Student for the degree of Doctor of Science in Dentistry (DScD), Department of Pediatric Dentistry and Orthodontics, College of Dentistry, King Saud University, Riyadh, Saudi Arabia
b Assistant Professor, Division of Orthodontics, Department of Pediatric Dentistry and Orthodontics, College of Dentistry, King Saud University, Riyadh, Saudi Arabia

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Abstract Bone remodeling and orthodontic tooth movement (OTM) are controlled by certain essential molecules, one of which is vitamin D. Increased levels of vitamin D have been associated with increased rates of OTM. The purpose of this study was to determine the effect of vitamin D deficiency on the rate of OTM, and to determine their association after applying orthodontic forces.

Materials and Methods: Wistar rats were divided into two groups: control group with average vitamin D levels and experimental group with induced vitamin D deficiency. Orthodontic appliances were fixed to initiate tooth movement. Distance between the reference teeth were measured in millimeters on day zero, and repeated every 7 days, till day 21.

Results: A significant difference within the experimental group was found; as well as within the control group, there was also no significant interaction between time and the type of group.

Conclusion: The rate of Orthodontic tooth movement was not affected by induced vitamin D deficiency in rats.

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1. Introduction:

Vitamin D deficiency is a health issue seen throughout the world and Saudi Arabia is no exception (Al-Zoughool et al., 2015; Ardawi et al., 2012; Elsammak et al., 2010). Vitamin D concentration is lowered in the body when sunlight exposure vitamin D intake is decreased, thereby leading to certain health problems such as suboptimal calcium absorption and secondary hyperparathyroidism (Haines and Park, 2012).

Alteration in the levels of vitamin D prohormone is known to stimulate or inhibit bone resorption (Boyce and Xing, 2007). Specific molecular and cellular responses in the ligament of periodontium will cause remodeling in the alveolus bone (emkataramana et al., 2012a) the rate of bone resorption is determined by Osteoclasts, and therefore, the tooth movement rate and remodeling (Alansari et al., 2015).

Bone remodeling and orthodontic tooth movement (OTM) (has been found to be controlled by some fundamental molecules, including vitamin D (Nimeri et al., 2013a) locally injected vitamin D3 increased release of calcium through osteoclastic bone resorption thus accelerating OTM in humans hence reducing treatment time and cost (Al-Hasani et al., 2011). Clinical trials on humans are limited since they must be administered occasionally by local injections that can cause pain and discomfort (Nimeri et al., 2013b). Some studies have tried to alter OTM rate via external Vitamin D3 doses, which showed an increase in OTM rate (Bartzela et al., 2009; Gameiro et al., 2007). Vitamin D can affect bone resorption at very low doses, making it an important hormone in the normal turnover of bone. Doses as low as 0.2 ng/g can produce an increase in the rate of bone resorption in normal young mice (Reynolds et al., 1973).

There are numerous studies on the vitamin D influence on OTM. However, none of the reviewed scientific evidence, whether experimental or clinical studies, have reported the effect of vitamin D deficiency on the rate of OTM in the clinical practice of orthodontics. Therefore, the aim of this experiment was to evaluate the effect of vitamin D deficiency on the rate of OTM.

2. Material and Methods

2.1. Experimental Animals

An ethical clearance was obtained from the University board Ethical committee. Sixteen male Wistar rats, aging 8 to 9 weeks old and weighing 300 to 330 g (g) were used. The sample power was calculated using the G power sample power calculator (Universtat Keil) using protocols for the calculation of power in animal experiments (Charan and Kantharia, 2013). It was estimated that for an effect size of 0.8 and power of 0.95 would need 8 rats per group to perform a repeated measures ANOVA.

The six cages that were used to store the rats comprised of transparent type-IV polycarbonate with metal grid tops and were placed within an animal holding cabinet in a noise-free environment. Three cages each were labelled according to the two groups; Group C (Control): Normal Rats and Group E (Experimental): Rats with induced vitamin D deficiency. The rats were randomly assigned to the cages in groups of three or two; the maximum capacity of a single cage being three rats.

All the rats were sheltered and maintained under conventional housing conditions, constant room temperature (24–25 °C), relative humidity (55%), and 12 h of light and dark cycle, with free access to food and water. Distilled water was provided in plastic bottles and was renewed twice per week. The rats were given one week to acclimatize to the animal facility’s new environment before the experiment.

2.2. Experimental vitamin D deficiency induction in the experimental group

Vitamin D deficiency was induced using the novel technique introduced by Stavenuiter et al., where they used paricalcitol injections (Zemplar, Abbvie Limited, United Kingdom), which took them 2 weeks to establish vitamin D deficiency in the E group (Stavenuiter et al., 2015). The injection of 0.1 ml was a dilution of Saline and paricalcitol which was injected 3 times per week for each rat.

An additional week of inactivity was observed, totaling the time to three weeks prior to commencement of the OTM procedures, where serum vitamin D was analyzed and compared between E and C groups at the end of the third week to acknowledge the successful vitamin D deficiency establishment in the E group. During this week of inactivity, all other conditions were maintained as per the protocol, with the omission of vitamin D deficiency induction.

2.3. Installation of the orthodontic appliance

Orthodontic forces were applied to all the animals on Day Zero; three weeks after commencement of vitamin D deficiency induction in Group E. The appliance comprised of a closed Nitinol coil spring (Light 9 mm, 3 M Unitek- Monrovia, CA USA) (Fig. 1). The active coil spring placed between the upper incisor and the upper first molar on the left side was fixed in place with a 0.010” diameter stainless ligature wire around both teeth. This appliance was described previously in many animal studies and is considered a standardized technique that meets the clinical setting (Bakathir et al., 2016; Brudvik and Rygh, 1993; Franzon Frigotto et al., 2015; Kobayashi et al., 2000; Leiker et al., 1995; Seifi et al., 2015).

The animals were sedated using Sevoflurane Inhalation Anesthetic (BAXTER HEALTHCARE CORPORATION, Puerto Rico, Arabian Health Care Co.). A sectioning microscope was used with an optical lighting system (Leica inc. MS5) to better visualize intraorally throughout the procedure. Prior to ligating the appliance into place, shallow notches were created using a small round bur mounted on an air rotor handpiece in conjunction with a suction device (Kobayashi et al., 2000). The notches were created at the gingival level on the distal surface of the left first molar and the mesial aspects of both central incisors at the same level. Care was taken not to nick the second molar. A self-etching primer (3 M Unitek, Monrovia CA, USA) was used in the notches created on the molars and incisors.

The ligature wire was passed interdentally between the first and second molars, wrapped around the first molar, coil spring attached, and tightened by twisting it using a ligature wire for-
ceps till it fit snugly around the tooth. The excess wire was then cut using a ligature cutter. The coils were checked, wherein a force of 60 g was measured using a tension gauge (accuracy of 0.01 gms, Correx 6th edition, HAAG-STREIT AG, Koeniz, Switzerland) (Taddei et al., 2012; Venkataramana et al., 2012b). Another ligature wire was tied around the incisor after inserting it into the previously created notches, coil spring attached, and the wire was fit tightly around the surface of the left central incisor. All ligature wires were bent closely towards teeth surfaces to avoid injuries.

A thin coat of composite resin (3 M Unitek Transbond supreme LV, Monrovia, USA) was applied around the notches and ligatures and light-cured to avoid dislodgement of the appliance (Venkataramana et al., 2012b). Care was taken to avoid composite contacting the second molar. Composite resin packed in the grooves between the two central incisors were cured as one unit in a way to unite both teeth to prevent further eruption, anchorage loss and loss of connective tissue in the marginal periodontium following eruption blockage.

No reactivation was performed during the entire experimental period.

OTM was measured directly in the anesthetized animals’ dental arch with a digital caliper, gauged with an accuracy of 0.01 mm (Bakathir et al., 2016; Franzon Frigotto et al., 2015; Leiker et al., 1995; Peron et al., 2017; Seifi et al., 2015). All the measurements were performed by one person, to avoid potential rater error. Intra rater reliability was confirmed by measuring the same distance 2 times with a one-hour time gap in between. The landmarks used were the most mesial point on the maxillary left first molar and the center of the palatal surface of the ipsilateral maxillary central incisor. Four such measurements were taken on day 0, before placing the orthodontic device; day 7; day 14; and day 21, just before sacrificing the animals. All the animals were then sacrificed by overdosing them with an inhalation anesthetic, Sevorane and cervical dislocation (Peron et al., 2017; Takano-Yamamoto et al., 1992). The orthodontic appliance protocol and the force used were validated through a pilot study, which was done on two wistar rats aging 8 weeks old and weighing 300 g (g), measurements being taken twice, 7 days apart from each other.

2.4. Statistical analysis

Normality of the sample was estimated using the Shapiro Wilk test and was found to be normally distributed (p = 0.851). The difference between groups at each time period was calculated using the independent samples \( t \) test. The difference in means over each time period was calculated using the repeated measures ANOVA with pair-wise comparisons calculated using the paired \( t \)-test for post-hoc comparisons. The level of significance for all tests was set at \( p < 0.05 \) and all statistical analyses were performed using the SPSS ver. 25 data processing software (IBM-SPSS, Armonk NY, USA).

3. Results

When the overall OTM was compared between the C and E groups the C group showed greater values at each of the three intervals. However, these differences were not statistically significant at 0–7 days (p = 0.709), 0–14 days (p = 0.313) or 0–21 days (p = 0.359) (Table 1).

When the OTM over time was compared separately for the C and E groups significant changes in the OTM were observed over time. In the E group the pairwise comparisons found significant differences between 0 days – 7 days and 0 days – 14 days (p = 0.013), 0 days – 7 days, and 0 days – 21 days (p < 0.001). Similar results were found in the C group with significant differences between 0 days – 7 days and 0 days – 14 days (p = 0.014), 0 days – 7 days, and 0 days – 21 days (p = 0.001). The repeated measures ANOVA found the differences to be significant when considered for time (\( F = 29.9, p < 0.001 \)) (Table 2).

There was significant drop in the OTM over time in both groups (Fig. 2).

4. Discussion

The results presented in this in vivo study provide initial evidence to the fact that vitamin D deficiency may not be significantly harmful to the OTM rate.

The coiled spring was found to be durable and easy to insert. The appliances remained intact and induced tooth movement in all animals during the study period, the animals were examined periodically for any loose or broken appliances. Rodent-study data must be understood and interpreted with caution, wariness, and carefulness before ascertaining conclusions for humans although rats have the similarity of the PDL with humans (Curl et al., 2014).
The safety and convenience of operation to the animals and examiner was a primarily consideration to use general anesthesia for most of the procedures on the animals, regardless of the complexity that required the anesthesia. The risk of excessively exposing the animals to anesthesia, limited the duration of the experiment to 21 days.

To obtain OTM in rats, the present study used a standardized technique previously described by Brudvik and Rygh (Brudvik and Rygh, 1993). This technique has been commonly used in a rat model, since it mimics OTM in humans, and comprises of an orthodontic appliance consisting of a closed Niti-nol coil spring. However, this method to quantify the amount of tooth movement can be affected by many variables, such as dental tipping, and a greater movement of central incisors, among other variables. (Bakathir et al., 2016; Brudvik and Rygh, 1993; Franzon Frigotto et al., 2015; Kobayashi et al., 2000; Leiker et al., 1995; Seifi et al., 2015) Composite was added on the edges to lessen the irritation of any ligatures and sharp projections, and to ensure no dislodgement of the appliance occurred.

To protect the appliances from dislodging owing to the space developed distal to the first molar or due to the continuous physiologic elongation of the upper central incisors, and to minimize animal discomfort, the rats received orthodontic treatment for a period of three weeks only; thus preventing unnecessary animal suffering during the experiment, which is why these four measurements were chosen. Also, to give enough time for the active appliance to create a distance that can be measured with convenience, and to have a comparable result to other similar studies. These complications usually take time to present themselves and could differ between animals and should be considered in studies with longer treatment periods. They were not evident in the current study owing to the short study period of three weeks.

| Table 1 Differences in OTM between Experimental and Control Groups. |
|---------------|---|---|---|---|---|---|
| **Group**     | **N** | **Mean** | **Std. Deviation** | **Std. Error Mean** | **T** | **Sig.** |
| 0 Day-7 Days   |     |    |    |    |    |    |
| Experimental  | 8   | 0.6387 | 0.29152 | 0.10307 | −0.381 | 0.709 |
| Control       | 8   | 0.71  | 0.44088 | 0.15587 |          |    |
| 0 Day-14 Days  |     |    |    |    |    |    |
| Experimental  | 8   | 1.1937 | 0.65249 | 0.23069 | −1.046 | 0.313 |
| Control       | 8   | 1.58  | 0.81541 | 0.28829 |          |    |
| 0 Day-21 Days  |     |    |    |    |    |    |
| Experimental  | 8   | 1.7675 | 0.64855 | 0.2293  | −0.948 | 0.359 |
| Control       | 8   | 2.115 | 0.8089  | 0.28599 |          |    |

*Calculated using the independent t test. Differences are not statistically significant.

| Table 2 Change in OTM over time. |
|---------------|---|---|---|---|---|---|
| **Group**     | **Time Period** | **Mean** | **Std. Deviation** | **95% Confidence Interval** | **Time** | **F** | **Sig.** |
|               |               |         |                | Lower Bound | Upper Bound |
| Experimental  | Rate between 0 Day & 7 Days* | 95.4522 | 2.01791 | 93.765 | 97.139 | 29.9 | 0.00** |
|               | Rate between 0 Day & 14 Daysb | 91.4537 | 4.68636 | 87.536 | 95.372 |      |      |
|               | Rate between 0 Day & 21 Daysc | 87.3955 | 4.49952 | 83.634 | 91.157 |      |      |
| Control       | Rate between 0 Day & 7 Days* | 94.7943 | 3.1931 | 92.125 | 97.464 | 18.543 | 0.00*** |
|               | Rate between 0 Day & 14 Daysb | 88.3908 | 6.04181 | 83.34 | 93.442 |      |      |
|               | Rate between 0 Day & 21 Daysc | 84.4161 | 6.09616 | 79.32 | 89.513 |      |      |

*Calculated using the repeated measures ANOVA. ** Differences are significant at p < 0.05. a,b,c Differences in superscript suggest difference significant at p < 0.05 when compared using the paired t test.
This appliance was repeatable with a substantial degree of standardization. The technique was uncomplicated, and it was reported to be comparable to those seen in monkeys or dogs using more complex appliances (Waldo and Rothblatt, 1954).

In contrast to our study, it has been reported that the local administration of vitamin D doses can accelerate OTM effectively in vivo. A study that compared the amount and rate of OTM between young and adult Wistar rats with a local administration of vitamin D injections, noticed an increase in OTM with simultaneous increase in vitamin D administration, measured every three days (Takano-Yamamoto et al., 1992).

Similarly to the amount of force used to initiate OTM in the present study, Seifi et al. conducted a 21-day experiment on Wistar rats with an orthodontic force of 60 g; with local injections of prostaglandin E2 (PGE2) alone, and with calcium gluconate. They reported a non-significant increase in OTM (Seifi et al., 2003).

In the present study and for the first time in literature, it was demonstrated that vitamin D deficiency does not significantly inhibit OTM in rats. However, the fact that there is some alteration in the rates of OTM, leaves room for further investigations on the effects of vitamin D deficiency, orthodontists may need to consider its detection during treatment planning.

Nevertheless, the roles of other factors such as RANKL/OPG ratio, serum calcium, phosphorus, and parathyroid hormone on OTM cannot be ruled out, as they may have potential impact on the physiologic bone response and are regulated by systemic hormones and local factors that affect bone cells and their replication.

It is also recommended to use this experimental OTM model combined with a histological approach and serum blood analyses for other variables to provide valuable insights in understanding the mechanism of osteoclasts from the bone surface after OTM, and to better explore the mechanism in which vitamin D acts during OTM (Kobayashi et al., 2000).

The sacrifice was performed because these rats cannot be used for any other future research. The animals’ jaws are being preserved for future histomorphometrical, histopathological and biochemical analysis.

Currently, there are no reviewed studies on the effect of vitamin D deficiency on OTM. Owing to the increased demand for orthodontic treatment among patients who might suffer from vitamin D deficiency, it is compulsory to understand the underlining biologic process, thus, such information will help prevent the prolonged treatment time by considering the well-being and overall health of the patient.

5. Conclusion

The results of the present in vivo experiment concluded that, vitamin D deficiency induced in Wistar rats did not affect rate of OTM when comparing E and C groups with each other, but a statistical significance was seen within each of E and C groups, at every seventh day interval.

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Ethical Approval and IRB

Institutional Review Board (IRB) Ethical approval was obtained from the Scientific Research Ethics Committee, King Saud University, Riyadh, Saudi Arabia (IRB # 4/67/398137), (reference #: KSU- SE1810). Another ethical approval was obtained from the College of Dentistry Research Center (CDRC), King Saud University, Riyadh, Saudi Arabia (Reference #: PR0080). Furthermore, the animals experiment research procedures were reviewed and approved by the Experimental Surgery and Animal Laboratory Center, where the experiment took place.

CRediT authorship contribution statement

Rawan M. Khalaf: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft. Abdullazez A. Almudhi: Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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