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The Biomechanical Evaluation of the Vitamin K_2Effect on Bone Response around Orthodontic Mini-Screw Implants

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ABSTRACT

Aims: This study was introduced to show the effect of vitamin K_2 on the bone response around temporary anchorage devices via the biomechanical evaluation.

Materials and Methods: In the conducted study, eight white mature healthy male rabbits were taken as experimental animals equally divided into two main groups in regard to the time length of the vitamin K_2 administration to the experimental groups after 2 and 4 weeks, means every group was included four rabbits two control and two vitamin K_2 given as each experimental group has its specific control. All the steps of the surgical procedure were achieved in the Animal Experimental Room in the College of Dentistry of University of Mosul. Twenty-four temporary anchorage devices 6 mm in length and 1.4 mm in diameter were installed into the lateral surface of the right femur, 3 mini-implants in each. The vitamin K_2 daily dose of the two experimental groups of 2 and 4 weeks was a 900 µg administered by the loading technique. The biomechanical estimation was performed by the Periotest M device and two times; firstly at the time of temporary anchorage devices installations for the measures of the primary stability and secondly after the end of every period of 2 and 4 weeks for the measures of the secondary stability.

Results: The biomechanical estimation showed a highly significant increase of the secondary stability over the primary one of the vitamin K_2 for the two experimental groups of 2 and 4 weeks, while a significant decrease in the secondary stability over the primary of the control 2 weeks and a non-significant difference between the secondary and primary stability for the control 4 weeks. Moreover, for the 2 weeks group there was a highly significant increase of the vitamin K_2 secondary stability over the same stability of its control and for the 4 weeks group there was a significant increase of the vitamin K_2 secondary stability over the same stability of its control.

Conclusion: The two timings experimental vitamin K_2 groupsof 2 and 4 weeksshowed higher temporary anchorage devices secondary stabilities over their controls.

Key Words: vitamin K_2, temporary anchorage device, Periotest, primary stability and secondary stability.

INTRODUCTION

Anchorage in orthodontics defined as; "the resistance to unwanted tooth movement"[1].In the majority of orthodontic cases, a correct anchorage is crucial for the treatment success [2]. Nowadays, the skeletal anchorage or the temporary anchorage device (TAD) [3]or the mini-screw [4]becomes an important novel way to support the orthodontic anchorage [5]. The importance of the mini-screw came from its ability to offer a variety of benefits [6]in treating even challenging orthodontic cases[7], so it gains the glossary orthodontic name the "Orthognathic like Orthodontics." [8]

The success rate of the mini-screws is 75% - 94% [9,10] or 95%[11], this ratio is not satisfactory[12, 13]and means that from 1 to 3 out of 10 inserted anchorage devices would be lost due to missing their stabilities[14]. One of the factors that showed role in the anchorage's retention is the density of bone around this device.[15]

Studies on vitamin K_2suggested that this molecule may stimulate multiple vitamin K-dependent proteins (VKDPs) and converts them from the inactive uncarboxylated form to the active carboxylated one to confer functioning [16], an example of these proteins is the osteocalcin (OC) that is needed to build strong bones.[17, 18]

This study was introduced in order to biomechanically estimate the effect of vitamin K_2 as a natural substance for inducing bone response around a temporary anchorage device in order to increase the mini-screw stability and success rate.

MATERIALS AND METHODS

The Experimental Animals

The experimental animals utilized in the study were eight healthy mature male white rabbits, divided equally into two main groups in regard to the time length of the vitamin K_2 administration to the experimental groups after 2 and 4 weeks, means every group was included four rabbits two control and two vitamin K_2 given as each experimental group has its specific control. The weights of them were 1.8-2.3 Kg, while the ages were 8-12 months. The rabbits were obtained from the local market with the help of a veterinarian, an individual cage for each rabbit, so that they could be supervised by the veterinarian 4 weeks before the experiment and to ensure that the rabbits' behaviors were been well. Each rabbit was given a standard eating protocol including; the quantity and quality of the feeding and drinking. Also their weights, defecations, and activities were been monitored.

The Surgical Procedure

The surgery was performed in the experimental animal room in the College of Dentistry / Mosul University. Twenty-four mini-screws made of Ti-6Al-4V alloy (GSSEM / South Korea) and of 1.4 mm in diameter and 6 mm long were used in the current study. Each rabbit received 3mini-screwsper femur. The Periotest M device was used to measure the primary stability of the mini-screws by the assist of the veterinarian.

Aseptic operation was achieved to prevent wound infection, which occurs due to a self-inflicted wound trauma, this could be prevented by the surgical area gentle preparation, a traumatic tissue manipulation, correct with no tension wound edges proximation during closure, suture materials with less reaction, and a local anesthesia all around the operational site [19]. Also the temporary anchorage devices installations were done according to the standard work including; the presence of operating room and instruments sterilization and the using of disposable kits for surgery [20]. The feeding protocol of the experimental rabbits (diet and drink) was not changed and continued at the whole day before the surgery. Also the rabbits' weights were monitored through the whole experimental period.

1. Anesthesia

An intramuscular anesthetic solution (0.2 ml/kg B.W. of 10% Ketamine) and a muscle relaxant solution (0.15 mg/kg B.W. of 2% Xylazine) were injected to each rabbit before surgery. Each animal under the experiment within 10 minutes was been anesthetized and the anesthesia effect would be continued for 1hour.

2. Temporary Anchorage Devices Installation

The right leg of each rabbit was sprayed with a (10% Povidone Iodine) after confirming the anesthesia, then the medial surface of the right femur of each rabbit in the opposite side of the temporary anchorage device insertion (the temporary anchorage device was inserted at the lateral side of right femur) was also with a (2% Xylocaine-1ml) be anesthetized locally [21] for good homeostasis. Then the surgical site hair were shaved off and again with a (10% Povidone Iodine) disinfecting the naked skin with a sterile surgical gauze. An incision of about 30mmin length was performed at the skin parallel to the femur long axis, at the femur lateral outer side, as shown in (Figure 1).

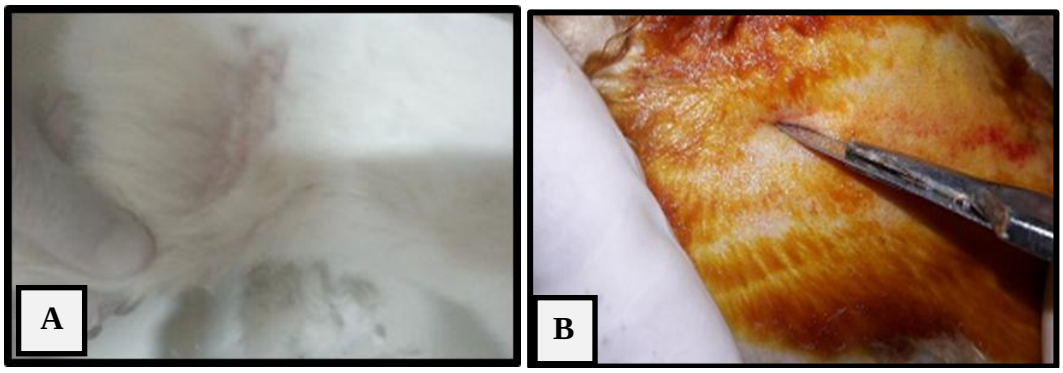

Figure 1:A. Shaving off the hair of the lateral side of right femur B. The incision was done parallel to the femur long axis.

Then muscle and fascia blunt dissections were done. After that the periosteum was elevated and the bone was naked at the lateral surface of the femur using a flap reflector and by a gentle reflection. The room temperature at about 25°C was kept to perform good results. The distance between every two temporary anchorage devices of the three self-drilling mini-screws that were been installed at the right femur (cortical bone) is about a 10 mm, as shown in (Figure 2).

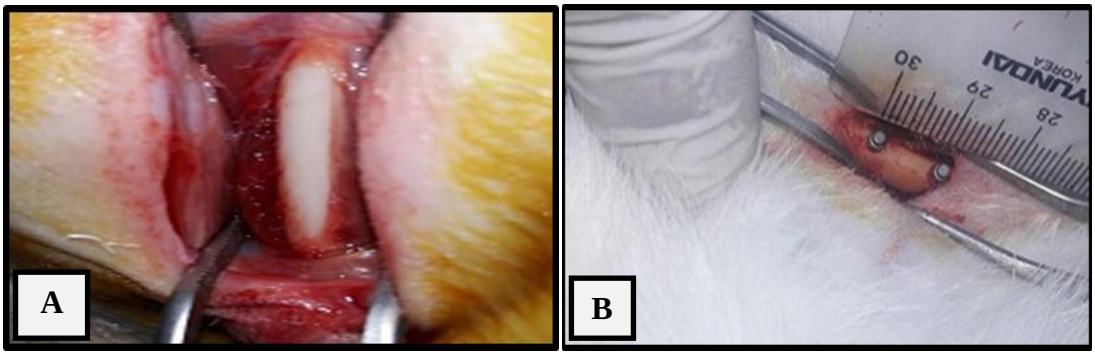

Figure 2:A. Flap reflection and the femoral bone was naked B. The 10 mm distance between two self-drilling mini-screws.

The mini-screws were inserted with their manual screw driver. The sterile self-drilling screw was carefully taken out from its storage container using its special manual screw driver that is usually used for the mini-screw installation, the screw should be installed in a direction perpendicular to the femoral cortical bone, and the insertion continued gradually until the whole screw body be inside the bone and its neck be in contact with the cortical bone. The other two screws were installed in the same way and they been paralleled to each other and to the first one. All mini-screws were installed in same manner, by the same operator, in a manual way, in the same room and usually at the morning day, as shown in (Figure 3).

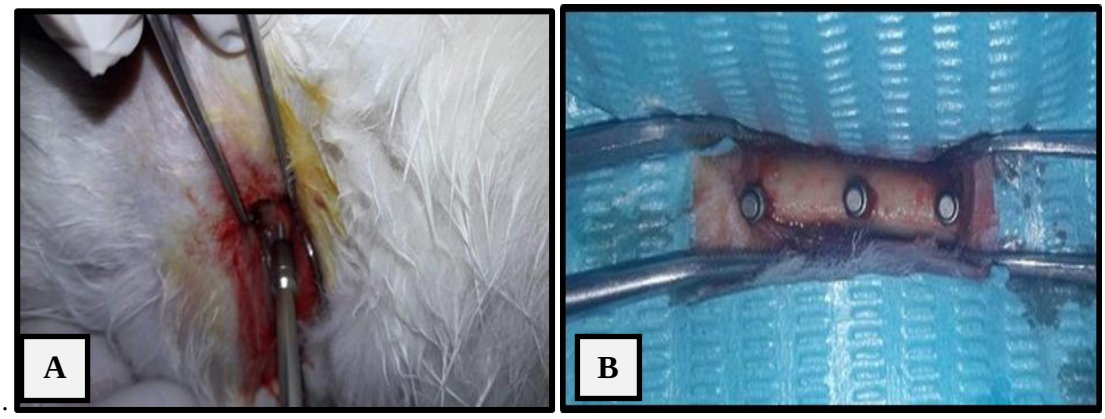

Figure 3: A. Inserting the mini-screw with its screw driver B. The three mini-screws were installed in their places.

3. Primary Stability Evaluation

The initial stability was estimated by the Periotest M device after the temporary anchorage devices were been in their places. According to the device manufacturer instructions, the measuring would be taken by holding the Periotest M hand piece perpendicular to the temporary anchorage devices heads [22] with a distance of 2-3mm between the hand piece tip and the mini-screw head [23], then by pressing the start button that included in the hand piece, the measuring would be started and showed on the device display as a Periotest value (PTV), as shown in (Figure 4). The mini-screw final value was considered by taking the mean of every three readings of every mini-screw.[24]

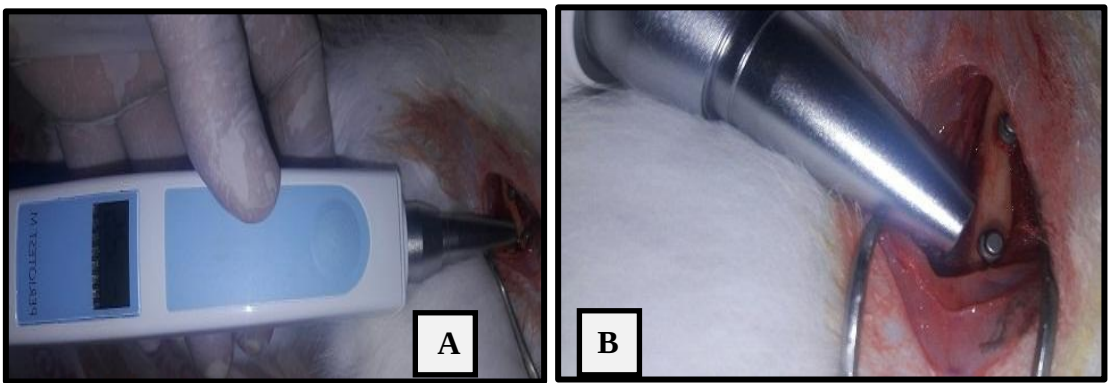

Figure 4: A. Periotest M device B. Its hand piece tip direction and distance from the mini-screw head.

4. Wound Suturing and Dressing

After measuring the mini-screws initial stabilities, skin tissue and fascia suturing were performed by using a non-absorbable silk suture-Iran, as shown in (Figure 5). Local antiseptic antibiotic (oxy-tetracycline) was then be sprayed at the operation region and then it covered with a piece of a sterile bandage without putting a pressure on the temporary anchorage devices.

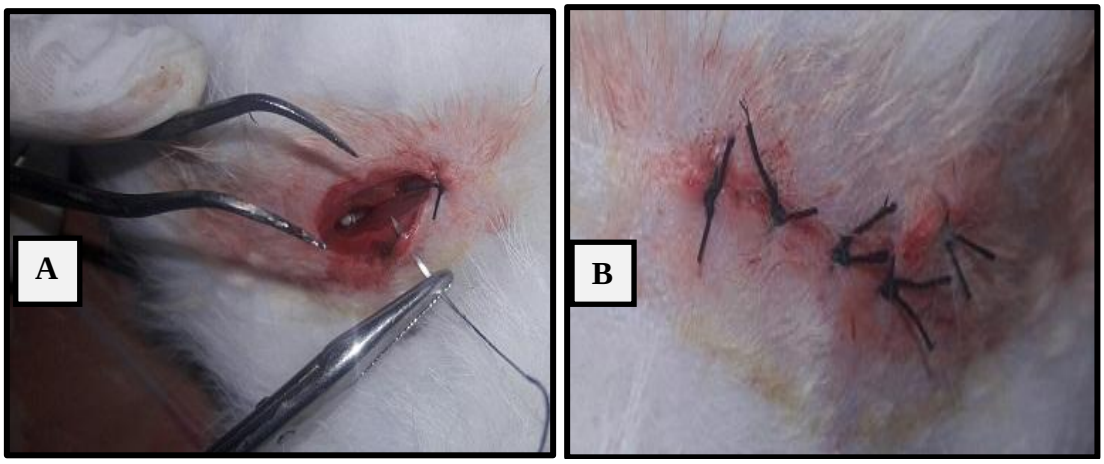

Figure 5: A. Skin and Fascia layers suturing, B. The suturing was completed and the stiches were in their places.

Recovery and Post-operative Animal Care

A special care of the rabbits was accomplished before the anesthesia, during it and post-operatively. The animals should be restraint in order to support their operations till healing was completed. After healing was been achieved with no complications, the animals were then been transferred to their own individual cages, so that every animal was able to take its recommended experimental diet. These cages were included the animal healthy environmental requirements; good illumination and temperature, air exchange, food, water, and cleaning access facilities.[25]

In a separate room (quiet and clean) the animal was allowed to recover and was kept maintaining its body temperature. The unconscious rabbit was not placed in a cage with the conscious one. Also soft bedding materials (lint-free fabrics or paper) were been planned to the animal instead of the usual animal bedding to prevent particles from entering the wound site. Interventions of the animal food (fruits and vegetables of small pieces, baby food, soaked diet and trans-gel) were been introduced to its standard food before surgery to promote feeding and drinking assistants after operation. In addition, the non-absorbable silk sutures were been removed through 7-10 days after surgery. [26]

The post-operative antibiotic was a Pen Vet 300 0.1ml/rabbit (Procaine Benzyl penicillin, Alfasan International B.V., Woerden, Netherlands) a single dose in a day for three days that was injected intramuscularly. Daily substitution of the wound dressing for the first week, and disinfection of the wound daily and continuously till healing be finished by a (10% Povidone Iodine) spray were also been accomplished.

The animal appetite (eating and drinking) and pain signs (body posture abnormality, motion disability and aggressiveness) were evaluated daily. In addition, the rabbit's body weight was also been recorded daily for the first one week post-surgery. The animal appearance, general health, activity, temperament, response, surgical wound condition and the limping degree of it were also been monitored every day [27, 28].Also the animal defecation and the consumption of the established rabbit experimental vitamin K_2were monitored daily.

Rabbit Vitamin K_2 Consuming Program

As there were different examining periods of the experimental animal, the vitamin K_2consumingprogram time length was also different, for the first experimental group the program lasted for two weeks and for the second group continued for four weeks, but the beginning of the program was the same for the two groups, which was at the day of the surgery. The consuming way of the animal dependent on the way that the intended food for the experiment been available in the markets (i.e. capsules, tablets, powder, or injections).

According to previous studies concerning with the calculation of the drugs and vitamins doses given to the animals, the animal dose in units of (mg/kg) can be measured from the human equivalent dose (HED) also in units of (mg/kg) of the drug or vitamin and the conversion factor (Human *km*/Animal *km*) which is in turn dependent on the body surface area (BSA) of the human and animal, as shown from the following equation: Animal dose (mg/kg) = HED (mg/kg) × Conversion factor. The *km* factor was measured from a series of body weight: $km = 100/K \times W^{0.33}$, K: a unique value to every species [29], as shown in (Table 1).

Table 1: Conversion of Human Doses to Animal Doses Based on the Body Surface Area (BSA) [29].

Species	Body Weight (kg)	Body Surface Area (m^2)	*Km* Factor	Conversion Factor
Human (Adult)	60	1.6	37	1.00
Human (Child)	20	0.8	25	1.48
Baboon	12	0.6	20	1.85
Dog	10	0.5	20	1.85
Monkey	3	0.24	12	3.08
Rabbit	1.8	0.15	12	3.08
Guinea Pig	0.4	0.05	8	4.63
Rat	0.15	0.025	6	6.17
Hamster	0.08	0.02	5	7.40
Mouse	0.02	0.007	3	12.33

The safety rabbit daily requirement of vitamin K_2 (MK-7) was a 900µg/day, which was nearly to the average of the normal range of it a 555-1110µg/day, as the HED of this vitamin was a 180-360µg/day or a 0.180-0.360 mg/day[17, 30]. Animal dose (mg/kg) was calculated as the following; 0.180-0.360 × 37/12 = 0.555-1.11. Then every limit dose × 1000 = 555-1110 µg/day was the normal range of the rabbit daily vitamin K_2 requirement in a µg/day. [29, 31]

The recommended dose of 900µg vitamin K_2 (MK-7) that was produced from the vitamin companies was derived from the fermented soybeans, available as vegetarian capsules and each capsule was with the concentration of 300µg, as shown in (Figure 6). So each animal was given equally three capsules per day to be a 900µg, this dose considered as a safety animal dose from the normal dose ranges, the capsules were given to the rabbit at an early morning for good absorption at an empty stomach.

Figure 6: Vitamin K_2 (MK-7) Capsules.

The procedure of administering the capsules to every rabbit was through an oral gavage tube connected to the usually used disposable syringe for injection, and by the loading way, the capsules were been administered. These capsules were mixed with 5 ml distilled water for each animal.

Secondary Stability Evaluation

Every animal of the two different timing groups at the end of its experimental healing period of 2 weeks and 4 weeks was sacrificed with an over dose of ketamine hydrochloride [32]. To measure the secondary stability, a re-opening of the surgical site was performed by reflecting the whole flap with a gentle incision along the femoral bone long axis, then by a careful entry to the healed wound, the mini-screws were been reached and the secondary stabilities of them were been recorded by the same way that was used to measure their primary stabilities, biological stability measurements were considered as indicators for the degree of an implant-bone integration.

RESULTS

Periotest Values of the Primary Stability and Secondary Stability of the Two Weeks Group

The results of the ANOVA descriptive statistics including; mean, standard error, standard deviation, minimum and maximum values of the PrC1, PrK_21, SeC1 and SeK_21 showed; a highly significant difference between PrC1and PrK_21 (P= 0.008), the PrK_21 stability mean was higher than PrC1 mean, a highly significant difference between SeC1 and SeK_21 (P= 0.000), the SeK_21 stability mean was higher than SeC1 mean, a significant difference between PrC1 and

SeC1 (P= 0.014), the PrC1 stability mean was higher than SeC1 mean, and a highly significant difference between PrK_21 and SeK_21 (P= 0.007), the SeK_21 stability mean was higher than PrK_21 mean, as shown in (Table 2).

Table 2: Descriptive analysis of the primary stability and secondary stability for the control and vitamin K_2 two weeks group

Group	No.	Mean	SE	Min.	Max.	t-value	Sig.
PrC1	6	-0.833	0.472	-2.2	+1.0	3.250	0.008 (HS)*
PrK_21	6	-3.017	0.477	-4.0	-1.1		
SeC1	6	+0.667	0.186	0.0	+1.4	13.360	0.000 (HS)*
SeK_21	6	-5.010	0.390	-6.1	-3.8		
PrC1	6	-0.833	0.472	-2.2	+1.0	2.960	0.014 (S)**
SeC1	6	+0.667	0.186	0.0	+1.4		
PrK_21	6	-3.017	0.477	-4.0	-1.1	3.380	0.007* (HS)
SeK_21	6	-5.010	0.390	-6.1	-3.8		

PrC1: Primary stability of the control 2 weeks period; PrK_21: Primary stability of the vitamin K_2 2 weeks period; SeC1: Secondary stability of the control 2 weeks period; SeK_21: Secondary stability of the vitamin K_2 2 weeks period;*HS: Significant Difference at $P \leq 0.01$; **S: Significant Difference at $P \leq 0.05$; All the measurements in a Periotest value (PTV).

Periotest Values of the Primary Stability and Secondary Stability of the Four Weeks Group

The results of the ANOVA descriptive statistics including; mean, standard error, standard deviation, minimum and maximum values of the PrC2, PrK_22, SeC2 and SeK_22 showed; a non-significant difference between PrC2 and PrK_22 (P= 0.780), the PrC2 stability mean was higher than PrK_22 mean, a significant difference between SeC2 and SeK_22 (P= 0.014), the SeK_22 stability mean was higher than SeC2 mean, a non-significant difference between PrC2 and SeC2 (P= 0.180), the SeC2 stability mean was higher than PrC2 mean, and a highly significant difference between PrK_22 and SeK_22 (P= 0.000), the SeK_22 stability mean was higher than PrK_22 mean, as shown in (Table 3).

Table 3: Descriptive analysis of the primary stability and secondary stability for the control and vitamin K_2 four weeks group

Group	No.	Mean	SE	Min.	Max.	t-value	Sig.
PrC2	6	-1.150	0.682	-3.9	0.4	0.280	0.780 (NS)*
PrK_22	6	-0.850	0.818	-3.2	+1.4		
SeC2	6	-3.100	1.163	-6.0	+0.4	2.960	0.014* (S)**
SeK_22	6	-6.783	0.439	-7.9	-5.0		
PrC2	6	-1.150	0.682	-3.9	0.4	1.450	0.180 (NS)*
SeC2	6	-3.100	1.163	-6.0	+0.4		
PrK_22	6	-0.850	0.818	-3.2	+1.4	6.390	0.000 (HS)***
SeK_22	6	-6.783	0.439	-7.9	-5.0		

PrC2: Primary stability of the control 4 weeks period; PrK_22: Primary stability of the vitamin $K_2$4 weeks period; SeC2: Secondary stability of the control 4 weeks period; SeK_22: Secondary stability of the vitamin $K_2$4 weeks period;*NS: Not Significant; **S: Significant Difference at $P \leq 0.05$; ***HS: Significant Difference at $P \leq 0.01$; All the measurements in a Periotest value (PTV).

DISCUSSION

In the conducted study, the lowest mini-screw stability was the secondary stability mean of the control 2 weeks group (SeC1) which has the highest PTV of +0.667 mean value, while the highest mini-screw stability was the secondary stability mean of the vitamin $K_2$4 weeks group (SeK_22) which has the lowest PTV of -6.783 mean value, all the other values of the two periods groups were in between the above mentioned mean values of mini-screws stabilities.

Sachdeva*et al*. in (2016)[33] and Al-Ohali in (2017) [34]suggested that a mini-screw of a PTV of -8 to 0 was considered as a well-integrated mini-screw and can be loaded. Yano *et al*. in (2014)[35]mentioned that a stable mini-screw is that one that has a lower PTV. Also Crum *et al*. in (2014) [36]estimated a highly stable mini-screw measured by the Periotest M device and concluded that this mini-screw was obtained the lowest PTV of a -7 or -8.

The PTV of the primary stability mean for the control 2 weeks (PrC1) was -0.833, for the vitamin (K_2) 2 weeks (Pr$K_2$1) was -3.017, for the control 4 weeks (PrC2) was -1.150, for the vitamin K_2 4 weeks (Pr$K_2$2) was -0.850. Chen *et al*. in (2017) [37] mentioned that a PTV of +2.5can still describe a stable mini-screw. Sachdeva*et al*. in (2016) [33] and Al-Ohali in (2017) [34] mentioned the opposite that a mini-screw with this value cannot be loaded.

The mini-screw mechanical retention is a determining factor for its primary stability [38] which depends on the implant-bone contact which in turn relies on multiple factors such as; implant geometry [39], bone density [15], cortical bone thickness [40] and surgical technique that includes the insertion method as mentioned by Tepedino*et al*. in (2017) [41]that a self-drilling technique showed a higher maximum insertion torque and a higher dislocation resistance than a self-tapping one, so gives a good primary stability.

Between primary stability mean for the control 2 weeks and the same stability mean for the vitamin (K_2) 2 weeks there was a highly significant difference in the stability mean of the mini-screws as measured by the Periotest with the mini-screws of the vitamin K_2 group have the lowest PTV (more stable), this may be due to sample size differences or variations [11] as the vitamin K_2 groupmay has a more dense bone because a higher density gives a more stability [15]. While between the primary stability mean for the control 4 weeks and the same stability mean for the vitamin $K_2$4 weeks there was a non-significant difference in the stability mean of the mini-screws.

The PTV of the secondary stability for the control 2 weeks (SeC1) was +0.667, for the vitamin (K_2) 2 weeks (Se$K_2$1) was -5.010, for the control 4 weeks (SeC2) was -3.100 and for the vitamin K_2 4 weeks (Se$K_2$2) was -6.783. So there was a significant difference in the stability mean of the mini-screws between the control and vitamin K_2 after the end of every period, with the mini-screws of the vitamin K_2 were more stable and the end period of 4 weeks has the highest secondary stability.

These results were due to the effect of vitamin K_2[42, 43]as it causes an increase in the bone density as suggested by van Ballegooijen and Beulens in (2017)[16]and so increases the mini-screw stability [15]. This conclusion was confirmed in the current study by the significant difference between the primary stability value for the control 2 weeks of -0.833 and its secondary stability value of +0.667 as the secondary stability showed a decrease in the stability mean. Zhang *et al*. in (2013)[44] suggested the presence of cartilage islands in the bone defect of a rabbit model histologically at the 2 weeks period. Alsothe outcomes agreed with the non-significant difference between the PTV mean of the primary stability value for the control 4 weeks of -1.150 and its secondary stability of -3.100, although the secondary stability for the control 4 weeks hasa stability mean slightly higher than its primary one, but the healing period was not completed at the two period groups of the controls as suggested by Sohn *et al*. in (2010) [45]and Truong, in (2014) [46]that the earliest bone healing phase would be started after 2-4 weeks in a rabbit model. Roberts *et al*. in (1984) [47]and Truong in (2014) [46]suggested that a mature secondary osteons would be formed at 6-16 weeks healing phase at the posterior leg in a rabbit model.

The relation was not the same between the primary stability mean for the vitamin (K_2) 2 weeks of -3.017 and its secondary stability of -5.010 and between the primary stability mean for the vitamin $K_2$4 weeks of -0.850 and its secondary stability of -6.783 that the two period groups showed a highly significant difference in the stability mean with the secondary stabilities were higher than the primary one and that the highest stability was the secondary stability for the vitamin $K_2$4 weeks as this is due the effect of vitamin K_2 [17, 30]in addition to the effect of healing as vitamin K_2 would adjunct the process of healing by increasing the activation of osteocalcin [18, 48, 49] so lowering the rate of bone loss. [50, 51, 52]

CONCLUSION

Vitamin K_2can be considered as a brilliant material in the orthodontic therapy by its function in the elevation of the amount of active osteocalcin and bone density around the mini-screws, so elevating their stabilities and success rates, although further studies are needed to confirm this conclusion.

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